REF 201902 NeuMoDx™ Strep A/C/G Vantage Test Strip

℞ only

CAUTION: For US Export Only

IVD For *in vitro* diagnostic use with the NeuMoDx 288 and NeuMoDx 96 Molecular Systems

For insert updates, go to: www.qiagen.com/neumodx-ifu
For detailed instructions, refer to the NeuMoDx 288 Molecular System Operator's Manual; P/N 40600108
For detailed instructions, refer to the NeuMoDx 96 Molecular System Operator's Manual; P/N 40600317

INTENDED USE

The NeuMoDx Strep A/C/G Vantage Assay, as performed on the NeuMoDx 96 Molecular System and NeuMoDx 288 Molecular System, is a rapid, automated, qualitative *in vitro* nucleic acid amplification test for the direct detection and differentiation of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus* [GAS]) and *Streptococcus dysgalactiae* (pyogenic Group C and G β-hemolytic Streptococcus, including subsp*. dysgalactiae group C, and Streptococcus dysgalactiae* subsp*. equilisimilis* Group C and G [GCS/GGS]) in throat swab specimens obtained from patients with signs and symptoms of pharyngitis. The assay utilizes real-time Polymerase Chain Reaction (PCR) for the separate detection of *Streptococcus pyogenes* and *Streptococus dysgalactiae* DNA in throat swab specimens. The NeuMoDx Strep A/C/G Vantage Assay is intended to be used as an aid in the diagnosis of GAS and GCS/GGS infections in symptomatic patients, but not to guide or monitor treatment for GAS or GCS/GGS infections. Concomitant cultures may be necessary to recover organisms for epidemiological typing or for further susceptibility testing.

SUMMARY AND EXPLANATION

NeuMoDx Strep A/C/G Vantage Assay is designed to detect and differentiate GAS and GCS/GGS DNA simultaneously. The assay targets the region for the LPXTG-motif cell wall anchor domain-containing protein in the GAS genome and the sequence for the Nisin resistance protein present in GCS/GGS genomes. For detection of GAS and/or GCS/GGS DNA using the NeuMoDx Strep A/C/G Vantage Assay, a throat swab sample is collected in liquid Amies transport medium. To prepare for testing, the liquid Amies transport medium tube is placed in designated sample carriers and loaded onto the NeuMoDx System to begin processing. For each sample, the NeuMoDx System mixes a 50 µL aliquot of the liquid Amies transport medium with NeuMoDx Lysis Buffer 6 and automatically performs all steps required to extract the target nucleic acid, prepare the isolated DNA for real-time PCR amplification, and if present, amplify and detect the products of amplification (sections of the *targeted* gene sequences of the GAS, GCS or GGS genomes).

The NeuMoDx Strep A/C/G Vantage Assay includes a DNA Sample Process Control (SPC1) to monitor for the presence of potential inhibitory substances and NeuMoDx System or reagent failures that may be encountered during the extraction and amplification processes.

Infection with *Streptococcus pyogenes*, a beta-hemolytic bacterium that belongs to Lancefield serogroup A, also known as the group A streptococci (GAS), causes a wide variety of diseases in humans. A ubiquitous organism, *S pyogenes* is the most common bacterial etiology of acute pharyngitis, or inflammation of the pharynx, commonly called "strep throat." Strep throat is more common in children, about 20 – 30% of pharyngitis episodes. In comparison, it causes approximately 5 – 15% of pharyngitis infections in adults.[1,2] Purulent complications of pharyngitis usually occur in patients not treated with antimicrobial agents and include otitis media, sinusitis, peritonsillar or retropharyngeal abscesses, and suppurative cervical adenitis. Nonsuppurative complications include acute rheumatic fever (ARF) and acute glomerulonephritis.[3]

Streptococcus dysgalactiae subsp. *equisimilis* (GGS/GCS) are normal commensal flora of the human upper airway and are frequently asymptomatic colonizers of the skin, gastrointestinal tract, and female genital tract. This often causes an underappreciation of their role in streptococcal disease burden, as GCS/GGS are associated with the same spectrum of illnesses caused by *S. pyogenes*. In children, these organisms are implicated most commonly in respiratory tract infections, particularly pharyngitis. The true incidence of pharyngitis caused by groups C and G streptococci is difficult to determine because of the frequency at which asymptomatic colonization occurs. Nevertheless, compelling evidence implicates group C and G streptococci as true causes of pharyngitis.[2–4] GCS/GGS of human origin are now considered to constitute a single subspecies, *Streptococcus dysgalactiae* subsp*. equisimilis*. A comparison of the complete genome sequence of a clinical isolate of GGS, *S. dysgalactiae* subsp*. equisimilis,* with that of other streptococcal species demonstrated it is most closely related to *S. pyogenes*, with 72 percent sequence similarity.[5] *S. dysgalactiae* subsp*. equisimilis* shares many virulence factors with *S. pyogenes*, including the antiphagocytic M protein, streptolysin O, streptolysin S, streptokinase, and one or more pyrogenic exotoxins similar to those implicated in streptococcal toxic shock.[5]

Although pharyngitis caused by streptococci is usually self-limiting, rapid and accurate detection is important, as early treatment with appropriate antibiotics is known to reduce symptom severity and duration, decrease transmission of the organism, and reduce the risk of acute rheumatic fever.[3] As most pharyngitis is viral in origin, accurate diagnosis can reduce the unnecessary use of antibiotics and potential development of antibiotic resistance. Still, diagnosis based on clinical features alone is difficult since GAS symptoms overlap with those of viral pharyngitis. The "gold standard" for detecting GAS in the pediatric population is culturing a throat swab on blood agar. However, the relatively long lag time between collecting the specimen and final microbiological diagnosis—approximately 48 hours—limits the utility of this method for routine use in outpatient settings. Since the 1980s, commercial rapid antigen detection tests (RADTs) have been available as a means of GAS detection.[6,7] The advantage of RADTs is that they can be quickly performed in the physician's office. Yet despite having good specificity (> 95%), RADTs often have reduced sensitivity (~86%) compared to culture.[6] The persistent need for highly sensitive and rapid assays to compete against culture methods paved the way for the development of molecular assays. Nucleic acid amplification test (NAAT) methods have been developed for the detection of GAS typically has higher sensitivity (>90%) and good specificity (>95%).[8–10]

The NeuMoDx Strep A/C/G Vantage Assay allows for the rapid, accurate detection of group A streptococci and pyogenic group C and G streptococci.

PRINCIPLES OF THE PROCEDURE

The NeuMoDx Strep A/C/G Vantage Assay combines the technologies of DNA extraction and amplification/detection by real-time PCR. Throat swab samples are collected in liquid Amies transport medium collection tubes. The NeuMoDx System automatically aspirates an aliquot of the liquid Amies swab specimen to mix with NeuMoDx Lysis Buffer 6 and the extraction reagents contained in the NeuMoDx Extraction Plate to begin processing. The NeuMoDx System automates and integrates DNA extraction and concentration, reagent preparation, and nucleic acid amplification and detection of the target sequence using real-time PCR. The included Sample Process Control (SPC1) helps monitor for the presence of potential inhibitory substances as well as system, process, or reagent failures. No operator intervention is necessary once the specimen is loaded onto the NeuMoDx System.

The NeuMoDx Systems use a combination of heat, lytic enzyme, and extraction reagents to perform cell lysis, DNA extraction and removal of inhibitors. The released nucleic acids are captured by paramagnetic particles. The microspheres, with the bound nucleic acids, are loaded into the NeuMoDx Cartridge where the unbound, non-DNA components are further washed away with NeuMoDx Wash Reagent and the bound DNA is eluted using NeuMoDx Release Reagent. The NeuMoDx System then uses the eluted DNA to rehydrate proprietary NeuDry™ amplification reagents containing all the elements necessary for amplification of the GAS and GCS/GGS targets as well as a section of the SPC1 sequence. This enables simultaneous amplification and detection of the target(s) and control DNA sequences. After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target (if present) DNA sequences occur in the PCR chamber. The NeuMoDx Cartridge, including the PCR chamber, is designed to contain the amplicon following real-time PCR, thereby essentially eliminating contamination risk post-amplification.

The amplified targets are detected in real time using hydrolysis probe chemistry (commonly referred to as TaqMan® chemistry) using fluorogenic oligonucleotide probe molecules specific to the amplicons for their respective targets.

TaqMan probes consist of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end. While the probe is intact, the fluorophore and the quencher are in proximity, resulting in the quencher molecule quenching the fluorescence emitted by the fluorophore via FRET (Förster Resonance Energy Transfer).

TaqMan probes are designed to anneal within a DNA region amplified by a specific set of primers. As the Taq DNA polymerase extends the primer and synthesizes the new strand, the 5' to 3' exonuclease activity of the Taq DNA polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore from it and breaks the close proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing an increase in fluorescence.

A TaqMan probe labeled with a fluorophore (Excitation: 470 nm & Emission: 510 nm) at the 5' end, and a dark quencher at the 3' end, is used to detect GAS DNA and a TaqMan probe labeled with a fluorophore (Excitation: 585 nm & Emission: 610 nm) at the 5' end, and a dark quencher at the 3' end, is used to detect GCS/GGS DNA. For detection of the Sample Process Control, the TaqMan probe is labeled with an alternate fluorescent dye (Excitation: 530 nm & Emission: 555 nm) at the 5' end, and a dark quencher at the 3' end. The NeuMoDx System monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When amplification is complete, the NeuMoDx System analyzes the data and reports a final qualitative result (POSITIVE / NEGATIVE / INDETERMINATE / UNRESOLVED).

REAGENTS/CONSUMABLES

Material Provided

REF	Contents	Tests per unit	Tests per package
209102	**NeuMoDx Strep A/C/G Vantage Test Strip** *Dried real-time PCR reagents containing GAS- and GCS/GGS-specific TaqMan probes and primers along with Sample Process Control-specific TaqMan probe and primers.*	16	96

Reagents and Consumables Required but Not Provided (Available Separately from NeuMoDx)

REF	Contents
100200	**NeuMoDx Extraction Plate** *Dried paramagnetic particles, lytic enzyme, and sample process controls*
401700	**NeuMoDx Lysis Buffer 6***
400100	**NeuMoDx Wash Reagent**
400200	**NeuMoDx Release Reagent**
100100	**NeuMoDx Cartridge**
235903	**Hamilton CO-RE / CO-RE II Tips (300 µL) with Filters**
235905	**Hamilton CO-RE / CO-RE II Tips (1000 µL) with Filters**

**Note: versions of NeuMoDx System software earlier than 1.8.0.0 will recognize NeuMoDx Lysis Buffer 6 as 'Lysis Buffer 4'. See NeuMoDx Lysis Buffer 6 Instructions for Use (P/N 40600406) for detailed Warnings & Precautions.*

Instrumentation Required

NeuMoDx 288 Molecular System [REF 500100] or **NeuMoDx 96 Molecular System** [REF 500200]

WARNINGS & PRECAUTIONS

- This test is for *in vitro* diagnostic use with NeuMoDx Systems only.
- Do not use the consumables or reagents after the listed expiration date.
- Do not use any reagents if the safety seal is broken or if the packaging is damaged upon arrival.
- Do not use consumables or reagents if the protective pouch is open or broken upon arrival.
- The NeuMoDx Strep A/C/G Vantage Assay has not been validated for use with preservatives.
- Do not collect swab specimens in transport media other than liquid Amies or equivalent. The NeuMoDx Strep A/C/G Vantage Assay has not been validated for use with other transport media.
- Minimum specimen volume is dependent on the tube size/specimen tube carrier as defined in the NeuMoDx 288 and 96 Molecular System Operator's Manuals (p/n 40600108 & 40600317).
- Performing a test on throat swab specimens more than 2 days old (stored at 2 – 8 °C) may produce invalid or erroneous results when using the NeuMoDx Strep A/C/G Vantage Test Strip.
- Avoid microbial and deoxyribonuclease (DNase) contamination of reagents. The use of sterile DNase-free disposable transfer pipettes is recommended if transferring specimen to a secondary tube. Use a new pipette for each specimen.
- To avoid contamination, do not handle or break apart any NeuMoDx Cartridge post-amplification. Do not retrieve NeuMoDx Cartridges from waste containers under any circumstances. The NeuMoDx Cartridge is designed to prevent contamination.
- In cases where open-tube PCR tests are also conducted by the laboratory, care must be taken to ensure that the NeuMoDx Strep A/C/G Vantage Test Strip, the additional consumables and reagents required for testing, personal protective equipment such as gloves and lab coats, and the NeuMoDx System are not contaminated.
- Clean, powder-free, nitrile gloves should be worn when handling NeuMoDx reagents and consumables. Care should be taken not to touch the top surface of the NeuMoDx Cartridge, the foil seal surface of the NeuMoDx Strep A/C/G Vantage Test Strip or NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer 6; handling of the consumables and reagents should be done by touching side surfaces only.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[11] and in CLSI Document M29-A3.[12]
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

PRODUCT STORAGE, HANDLING & STABILITY

- Safety Data Sheets (SDS) are provided for each reagent, as applicable.
- NeuMoDx Strep A/C/G Vantage Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 15 – 23 °C.
- Do not use consumables and reagents past the stated expiration date.
- Do not use any test product if the primary or secondary packaging has been visually compromised.
- Once loaded, the NeuMoDx Strep A/C/G Vantage Test Strip may remain onboard the NeuMoDx System for 14 days. Remaining shelf life of loaded test strips is tracked by the software and reported to the user in real time. Removal of a test strip that has been in use beyond its allowable period will be prompted by the System.

SPECIMEN COLLECTION / TRANSPORT / STORAGE

- The NeuMoDx Strep A/C/G Vantage Test Strip has been tested using clinician-collected throat swab specimens. Performance with specimens other than those specified has not been evaluated.
- Collected swab specimens should be kept at the temperature recommended in the swab collection kit during transport.
- Swab specimens should be stored between 2 – 8 °C for no longer than 2 days prior to testing and a maximum of 8 hours at room temperature.

INSTRUCTIONS FOR USE

Specimen Collection / Transport

1. Clinician-collected throat swabs should be collected in liquid Amies transport medium.
2. If specimens are not tested within 8 hours, they should be stored between 2 to 8 °C for up to 2 days prior to testing.

Test Preparation

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System. The primary collection tube may be labeled and placed directly into the specimen carrier. Alternatively, an aliquot of the liquid Amies medium may be transferred to a secondary tube for processing on the NeuMoDx System.
2. Briefly vortex the swab specimen in the parent container to achieve uniform distribution.
3. If testing the swab specimen in the primary swab collection tube, place the barcode-labeled tube into a specimen tube carrier and ensure that the cap and swab are removed prior to loading on to the NeuMoDx System. DO NOT leave swab in the tube.
4. If using a secondary tube, transfer a ≥ 0.5 mL aliquot of the liquid Amies specimen to a barcoded specimen tube compatible with a NeuMoDx 32-Tube Specimen Tube Carrier.

NeuMoDx System Operation

For detailed instructions, refer to the NeuMoDx 288 and 96 Molecular System Operator's Manuals (p/n 40600108 & 40600317).

1. Populate one or more NeuMoDx test strip carrier(s) with NeuMoDx Strep A/C/G Vantage Test Strip(s) and use the touchscreen to load the test strip carrier(s) into the NeuMoDx System.
2. If prompted by the NeuMoDx system software, add the necessary required consumables to the NeuMoDx System consumable carriers and use the touchscreen to load carrier(s) into the NeuMoDx System.
3. If prompted by the NeuMoDx system software, replace NeuMoDx Wash Reagent, NeuMoDx Release Reagent, empty the Priming Waste, Biohazard Waste Container (NeuMoDx 288 only), Tip Waste Bin (NeuMoDx 96 only) or Biohazard Waste Bin (NeuMoDx 96 only) as appropriate.
4. Load the specimen tube(s) into the appropriate specimen tube carrier and ensure caps are removed from all specimen tubes.
5. Place the specimen tube carrier on the autoloader shelf and use the touchscreen to load carrier into the NeuMoDx System. This will initiate processing of the specimen(s) loaded for the tests identified.

LIMITATIONS

- The NeuMoDx Strep A/C/G Vantage Test Strip can only be used on NeuMoDx Systems.
- The performance of the NeuMoDx Strep A/C/G Vantage Test Strip has been established with clinician-collected throat swab specimens.
- Use of the NeuMoDx Strep A/C/G Vantage Test Strip with other sources has not been assessed and performance characteristics of this test are unknown for other specimen types.
- Because detection of GAS and GCS/GGS is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- Erroneous test results could occur from improper specimen collection, handling, storage, technical error, or sample mix-up. In addition, false negative results could occur because the number of organisms in the specimen is below the analytical sensitivity of the test.
- Testing is limited to use by personnel trained on the use of the NeuMoDx System.
- If the Sample Process Control does not amplify and the NeuMoDx Strep A/C/G Vantage test result is Negative, an invalid result (Indeterminate or Unresolved) will be reported and the test should be repeated.
- A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of GAS and/or GCS/GGS DNA.
- While there are no known strains/isolates of GAS lacking the region for LPXTG-motif cell wall anchor domain-containing protein or of GCS/GGS lacking the region for Nisin resistance protein, the occurrence of such a strain could lead to an erroneous result using the NeuMoDx Strep A/C/G Vantage Test Strip.
- Mutations in primer/probe binding regions may affect detection using the NeuMoDx Strep A/C/G Vantage Test Strip.
- Results from NeuMoDx Strep A/C/G Vantage Assay should be used as an adjunct to clinical observations and other information available to the physician. The test is not intended to differentiate carriers of CAS and/or GCS/GGS DNA from those with streptococcal disease.
- Test results may be affected by concurrent antibiotic therapy as GAS and GCS/GGS DNA may continue to be detected following antimicrobial therapy.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens.

RESULTS

NeuMoDx Molecular Systems

Available results may be viewed or printed from the 'Results' tab in the Results window on the NeuMoDx System touchscreen. A test result is called Positive (POS), Negative (NEG), Indeterminate (IND), or Unresolved (UNR) based on the amplification status of the target and the Sample Process Control (SPC1).

Criteria for a positive or negative call are specified in the NeuMoDx System Strep A/C/G Vantage Assay Definition File (ADF) as installed on the System(s) by NeuMoDx Molecular, Inc. Results are reported based on the ADF decision algorithm, summarized in *Table 1*, below.

Table 1. Summary of Strep A/C/G Vantage Assay Decision Algorithm

RESULT	GAS and/or GCS/GGS TARGETS	PROCESS CONTROL (SPC1)
POS	Amplified	N/A
NEG	Not Amplified	Amplified
IND	Not Amplified, System Error Detected	
UNR	Not Amplified, No System Error Detected	

Invalid Results

If a NeuMoDx Strep A/C/G Vantage Assay performed on the NeuMoDx System fails to produce a valid result, it will be reported as either Indeterminate or Unresolved based on the type of error that occurred, and the test should be repeated to obtain a valid result.

An Indeterminate result will be reported if a NeuMoDx System error is detected during sample processing.

An Unresolved result will be reported if no target is detected and there is no amplification of the Sample Process Control, which indicates possible reagent failure or the presence of inhibitors.

Quality Control

Local regulations typically specify that the laboratory is responsible for control procedures that monitor accuracy and precision of the complete analytical process, and must establish the number, type, and frequency of testing control materials using verified performance specifications for an unmodified, approved test system.

1. External (User-Defined) control materials will not be provided by NeuMoDx Molecular, Inc. Appropriate controls must be chosen and validated by the laboratory. The controls must meet the same minimum volume specifications as clinical samples specified. The user may define specific barcodes per Positive and Negative Control or assign barcode(s) at random.
2. Recommended: 1 *Streptococcus pyogenes* LYFO DISK™ (Microbiologics® 0508L) and 1 *Streptococcus dysgalactiae* subsp. *equisimilis* LYFO DISK (Microbiologics® 0602L) reconstituted according to manufacturer's instructions, diluted in 50 mL liquid Amies, stored and used in 0.5 mL aliquots. If processing controls, place the labeled controls in a specimen tube carrier and use the touchscreen to load the carrier into NeuMoDx System from the autoloader shelf. The NeuMoDx System will recognize the barcodes (if predefined by the user) and start processing controls unless adequate regents or consumables required for testing are not available.
3. The primers and probe specific for Sample Process Control 1 (SPC1) are included in each NeuMoDx Strep A/C/G Vantage Test Strip. This Sample Process Control allows the NeuMoDx System to monitor the efficacy of the DNA extraction and PCR amplification processes.
4. A positive test result reported for a negative control sample indicates a specimen contamination problem. Please refer to NeuMoDx *288 or 96 Molecular System Operator's Manual* for Troubleshooting tips.
5. A negative result reported for a positive control sample may indicate there is a reagent or NeuMoDx System related problem. Please refer to *NeuMoDx 288 or 96 Molecular System Operator's Manual* for Troubleshooting tips.

PERFORMANCE CHARACTERISTICS

Clinical Performance

Clinical performance characteristics of the NeuMoDx Strep Vantage A/C/G Assay were determined using an internal retrospective method comparison study using residual throat swab specimens sourced from two geographically diverse clinical laboratory locations.

Residual throat swab specimens from symptomatic patients were de-identified and given a unique ID number by clinical laboratories, establishing a confidential list linking the patient ID to the de-identified specimens tested for study purposes. A total of 230 residual specimens provided from two clinical laboratories were tested. Among the 230 samples, 68 samples were identified as GAS positive and 47 samples were identified as GCS/GGS positive by the clinical laboratories. One specimen tested positive for both GAS and GCS/GGS, indicating a dual or co-infection. The test status of these samples was withheld from the operator to implement a "single blind study". Results reported from the specific FDA- and CE-approved, legally marketed molecular devices utilized by the laboratories for standard of care testing were used to perform the method comparison analysis.

Results of the NeuMoDx Strep A/C/G Vantage test provided a Clinical Sensitivity of 100% for the GAS target and 95.9% for the GCS/GGS target, both reported at 95% confidence interval (CI). The Clinical Specificity from the study was determined to be 100% for both GAS and GCS/GGS, again using 95% CI. The lower and upper limits of the 95% CI presented in *Tables 2A* and *2B* below were calculated using the Wilson procedure with continuity correction.

Table 2A. Clinical Performance Summary – NeuMoDx Strep A/C/G Vantage Test Strip Detection of *S. pyogenes*

GAS		FDA / CE Approved Reference Test Result		
		POS	NEG	Total
NeuMoDx Strep A/C/G	POS	68	0	68
	NEG	0	162	162
	Total	68	162	230
Clinical Sensitivity (GAS) = 100% (93.3 - 100)				
Clinical Specificity (GAS) = 100% (97.1 - 100)				

Table 2B. Clinical Performance Summary – NeuMoDx Strep A/C/G Vantage Test Strip Detection of *S. dysgalactiae*

GCS/GGS		FDA / CE Approved Reference Test Result		
		POS	NEG	Total
NeuMoDx Strep A/C/G	POS	47	0	47
	NEG	2	181	183
	Total	49	181	230
Clinical Sensitivity (GCS/GGS) = 95.9% (84.9 – 99.3)				
Clinical Specificity (GCS/GGS) = 100% (97.4 - 100)				

Analytical Sensitivity

The Limit of Detection (LoD) of the NeuMoDx Strep A/C/G Vantage Assay was determined in negative clinical throat swabs spiked with GAS, GCS and GGS targets: *Streptococcus pyogenes* (ATCC 700294), *Streptococcus dysgalatiae* subsp.*equisimilis* (ATCC 35666), and *Streptococcus dysgalactiae* subsp. *equisimilis* (ATCC 12384), respectively. All samples for the study were prepared in pooled and screened Streptococcus-negative clinical throat swab specimens and spiked separately with targets at concentrations of 50 CFU/mL GAS, 2500 CFU/mL GCS, or 10,000 CFU/mL GGS. Each target was tested in 40 replicates and hit-rate analysis was used to confirm that a ≥ 95% detection rate was achieved, allowing these concentrations to be accepted as the LoD of the given targets. Findings of the Limit of Detection study are summarized in *Table 3*, below.

Table 3. Hit-rate determination of NeuMoDx Strep A/C/G Vantage Assay Limit of Detection

Target	Concentration (CFU/mL)	n	# Positive	% Positive	LoD (Hit-Rate)
GAS	50	40	40	100	50 CFU/mL
GCS	2,500	40	40	100	2,500 CFU/mL
GGS	10,000	40	40	100	10,000 CFU/mL

The Limit of Detection of the NeuMoDx Strep A/C/G Vantage Assay is claimed to be 50 CFU/mL for GAS, 2,500 CFU/mL for GCS, and 10,000 CFU/mL GGS.

Detection of Variants

The analytical sensitivity of NeuMoDx Strep A/C/G Vantage Assay was further confirmed with 11 different GAS strains, 7 GCS strains, and 9 GGS strains. Testing was performed using the GAS, GCS and GGS strains listed below in *Table 4*. Targets at the specified levels were spiked into negative clinical swab specimens prior to testing at 2X the relevant LoD as listed above to confirm ≥ 95% detection. Variant strains that did not meet this requirement were retested at higher concentrations until ≥ 95% detection was achieved. The level at which this was achieved for each strain is reported in *Table 4* as the LoD for that variant.

Table 4. Variant GAS, GCS, and GGS Strains Tested

	Strain	n	Concentration CFU/mL	Positive	Negative	Detection Rate (%)
S. pyogenes (Group A)	M3	5	100	5	0	100
	M82	5	100	5	0	100
	M4	5	100	5	0	100
	M18	20	100	19	1	95
	M28	20	300	19	1	95
	M73	20	500	20	0	100
	M78	20	500	20	0	100
	M77	19	500	19	0	100
	M12	20	500	20	0	100
	M75	20	1500	20	0	100
	M49	20	2500	19	1	95
S. dysgalactiae subsp. *equisimilis* (Group C)	C74	5	5000	5	0	100
	13-166	5	5000	5	0	100
	1180	5	5000	5	0	100
	C46	5	5000	5	0	100
	UCM 74/02P	5	5000	5	0	100
	SVA XVI 172	5	5000	5	0	100
	Lancefield H64	5	5000	5	0	100
	CCUG 28238	5	5000	5	0	100
S. dysgalactiae subsp. *equisimilis* (Group G)	NIH 1129	5	10000	5	0	100
	G16	5	10000	5	0	100
	CCUG 15679	5	10000	5	0	100
	G47	5	10000	5	0	100
	CCUG 27483	5	10000	5	0	100
	CCUG 33802	5	10000	5	0	100
	CCUG 502	5	10000	5	0	100
	CCUG 15680	5	20000	5	0	100
	CCUG 24070	5	20000	5	0	100

Analytical Specificity

A total of 45 culture isolates or DNA from organisms potentially cohabiting or phylogenetically similar to GAS or GCS/GGS were evaluated for possible cross-reactivity when testing with the NeuMoDx Strep A/C/G Vantage Assay. Organisms were prepared in pools of 3 to 6 organisms each and tested at a high concentration. Bacterial organisms were spiked into GAS/GCS/GGS negative liquid Amies at 6 – 9X10^6 CFU/mL and viral agents at 1x10^6 copies DNA/mL, except where otherwise noted. No cross reactivity was observed with any of the pathogens tested in this study. The list of organisms tested is shown in *Table 5*.

Table 5. List of Pathogens Used to Demonstrate Analytical Specificity

Bacteria	Bacteria	Bacteria
Acinetobacter lwoffii	*Neisseria gonorrhoeae*	*Streptococcus mitis*
Arcanobacterium haemolyticum	*Neisseria subflava*	*Streptococcus mutans*
Bacillus cereus	*Peptostreptococcus micros* (*Parvimonas micra*)	*Streptococcus oralis*
Bordetella pertussis†	*Pseudomonas aeruginosa*	*Streptococcus pneumoniae*
Burkholderia cepacia	*Serratia marcescens*	*Streptococcus salivarius*
Candida albicans	*Staphylococcus aureus* (MRSA)	*Streptococcus sanguinis*
Corynebacterium diphtheria	*Staphylococcus epidermidis* (MSRE)	*Streptococcus suis*
Enterococcus faecalis	*Stenotrophomonas maltophilia*	**Viruses**
Escherichia coli	*Streptococcus agalactiae*	
Fusobacterium necrophorum	*Streptococcus anginosus*	Adenovirus Type I*
Klebsiella pneumonia	*Streptococcus bovis*	Haemophilus influenzae Type A
Lactobacillus acidophilus	*Streptococcus canis*	Influenza A
Lactococcus lactis	*Streptococcus canis* (STR T1)	Influenza B
Legionella micdadei	*Streptococcus equi* subsp. *zooepidemicus* (group C)	Parainfluenza Type 4b†
Legionella pneumophila	*Streptococcus gordonii*	Rhinovirus Type 1A
Moraxella cartarrhalis	*Streptococcus intermedius*	

* Adenovirus Type I was spiked at $1x10^6$ TCID50/mL

† *Bordetella pertussis* and Parainfluenza Type 4b were spiked at 10 ng/mL

Interfering Substances - Commensal Organisms

The NeuMoDx Strep A/C/G Vantage Assay was tested for interference in the presence of non-target organisms (co-habiting posterior pharynx) by evaluating the performance of the NeuMoDx Strep A/C/G Vantage Assay at low levels of GAS and GCS/GGS on the NeuMoDx Molecular System. The same panel of 45 organisms *[Table 5]* used for assessing cross-reactivity was used for this study. The organisms were pooled into groups of 3 to 6 in GAS/GCS/GGS negative liquid Amies and spiked with 150 CFU/mL GAS, 7500 CFU/mL GCS, and 30000 CFU/mL GGS targets. No interference was observed with any of the commensal organisms.

Interfering Substances - Endogenous and Exogenous Substances Encountered in Clinical Throat Swab Specimens

Performance of the NeuMoDx Strep A/C/G Vantage Assay was assessed in the presence of potentially interfering substances that may be associated with collection of a throat swab from a patient *[Table 6]*. All agents were tested for potential interference in the absence and presence of GAS, GCS, and GGS. Liquid Amies samples spiked at 3X LoD were dosed with endogenous and exogenous moieties dissolved or diluted in molecular grade water at the specified concentrations using a saturated swab. None of the substances tested had an adverse effect on the detection of GAS or GCS/GGS.

Table 6. Exogenous and Endogenous Interfering Agents Tested Liquid Amies Swab Specimens

	Interfering Substance	Stock Concentration
Exogenous	Altoids™ (Spearmint)	10% (w/v)
	Aspirin™	10% (w/v)
	CEPACOL® Extra Strength Sore Throat & Cough Lozenges	5% (w/v)
	Children's Dimetapp® Cold & Cough	15% (v/v)
	Chloraseptic® Max Sore Throat Lozenges	10% (w/v)
	Chloraseptic Sore Throat Spray	10% (v/v)
	Cold-EEZE® Zinc Lozenges	15% (w/v)
	Crest® Pro-Health Advanced Gum Protection	4% (w/v)
	Halls™ Cough Drops (Cherry)	15% (w/v)
	Halls Cough Drops (Menthol-Lyptus)	15% (w/v)
	ICE BREAKERS® Mints (Cool Mint)	10% (w/v)
	LISTERINE® Total Care Mouthwash	15% (v/v)
	LISTERINE Ultra-clean Antiseptic Mouthwash	15% (v/v)
	*Ricola® Original Swiss Sugar Free Herb Cough Suppressant Throat Drops	15% (w/v)
	Robitussin® Max Strength Nighttime Cough DM	10% (v/v)
	Sucrets® Sore Throat & Cough Lozenges (Vapor Cherry)	5% (w/v)
	Tic Tac® Freshmints	10% (w/v)
	Wal-Tussin DM Max Cough Syrup	10% (v/v)
Endogenous	Saliva	100%
	Whole Blood	10% (v/v)

** Initially 1 of the 3 GAS samples tested at 3X LoD did not amplify in the presence of Ricola Throat Drops but performed as expected upon re-test.*

Lot-to-Lot Reproducibility

Lot-to-lot reproducibility of the NeuMoDx Strep A/C/G Vantage Assay was verified by retrospective analysis of quality test data for three separate lots of NeuMoDx Strep A/C/G Vantage Test Strip and NeuMoDx Lysis Buffer 6. These data were generated through functional testing of the reagents on liquid Amies transport media spiked with representative strains of GAS and GCS at the LoD for those targets. A total of 64 positive and 16 negative replicates were processed per lot of NeuMoDx Strep A/C/G Vantage Test Strip; evaluation of NeuMoDx Lysis Buffer 6 involved 16 positive and 8 negative replicates. The variation across production lots was analyzed by determining average C_t value, standard deviation and coefficient of variation percentage (%CV) shown in *Table 7*. Standard deviation values ≤ 1.1 and coefficient of variation values ≤ 3.0 % for both GAS and GCS targets demonstrated excellent reproducibility across lots of NeuMoDx Strep A/C/G Vantage Assay key reagents.

Table 7. %CV Analysis by Targets Across NeuMoDx Strep A/C/G Vantage Assay Key Component Lots

	GAS			GCS			All Results		
(Across 3 Lots)	$\bar{C}_t$	C_t SD	%CV	$\bar{C}_t$	C_t SD	%CV	$\bar{C}_t$	C_t SD	%CV
Strep A/C/G Test Strip	35.83	1.06	3.0%	34.93	0.76	2.2%	34.06	0.60	1.8%
Lysis Buffer 6	35.71	1.01	2.80%	34.86	0.63	1.80%	34.15	0.67	2.0%

Fresh versus Frozen Specimen Equivalence

Testing was performed to demonstrate specimen matrix equivalency between fresh and frozen throat swab specimens. Negative clinical specimens were spiked with GAS, GCS, and GGS targets at 3X LoD of the NeuMoDx Strep A/C/G Vantage Assay and processed using the NeuMoDx Strep A/C/G Vantage Assay. Each sample was then kept at -80 °C until frozen, thawed, and reprocessed. Results from fresh versus frozen swab specimens were compared for equivalency by regression analysis. The data demonstrated excellent equivalency between fresh and frozen swab specimens.

Effectiveness of Control

The efficacy of the Sample Process Control included in the NeuMoDx Strep A/C/G Vantage Test Strip to report any process step failures or inhibition affecting NeuMoDx A/C/G Vantage Assay performance was assessed on the NeuMoDx Molecular System. The conditions tested are representative of critical process step failures that could potentially occur during sample processing and *may not be detected* by the onboard sensors that are monitoring the performance of the NeuMoDx System. Effectiveness of control was evaluated by simulating failure of various sample process flow steps to mimic a potential system error and by spiking specimen with a known inhibitor to observe the effect of inefficient inhibitor mitigation on detection of the Sample Process Control (see *Table 8*). In instances where the processing errors did not adversely impact the performance of the Sample Process Control (NO WASH/NO WASH BLOWOUT), the test was repeated with specimens containing low levels of GAS and GCS/GGS (near LoD) to confirm the process error also had no adverse effect on the detection of GAS or GCS/GGS target as well. *Table 8* summarizes the results of the efficacy of control verification test.

Table 8. Effectiveness of Control Data Summary

Condition	Expected Result	Observed Result
Normal Processing	Negative	Negative
Normal Processing + Inhibitor	Unresolved	Unresolved
No Wash Reagent	Unresolved or Negative	Negative*
No Wash Blowout	Unresolved or Negative	Negative
No Release Reagent	Indeterminate	Indeterminate
No PCR Master Mix Reagents	Indeterminate	Indeterminate

* In rare instances, low positive GAS samples were found to produce a False Negative result when coupled with a system failure in the delivery of Wash Reagent. This was observed at GAS levels under 500 CFU/mL, well below the average concentration of a positive clinical swab specimen, and in most cases can be expected to be resolved by the likely occurrence of repeat testing following one-time false negative results.

On-board Sample Stability of Swab Specimens

Streptococcus negative clinical swab specimens were spiked with GAS, GCS, and GGS targets at 10-15X LoD, stored at 4 °C for 48 hours, and then processed using the NeuMoDx Strep A/C/G Vantage Assay along with an equal number of negative specimens. At the end of processing, all the positive and negative specimen tubes were left on the system worktable at room temperature for 8 hours and then reprocessed. The expected result at all the 0- and 8-hour time points was POSITIVE (for the appropriate target) for all the swab specimens spiked with GAS, GCS, or GGS target and NEGATIVE (for both targets) in the swab specimens that were not spiked with target. 100% concordance with expected result was observed at both time points, indicting that an on-board stability of 8 hours was demonstrated for testing with the NeuMoDx Strep A/C/G Vantage Test Strip. Results are summarized in *Table 9*, below.

Table 9. On-board Sample Stability Data Summary

On-Board Specimen Stability	% Positive, T_0			% Positive, 8 hr		
	GAS	GCS/GGS	SPC1	GAS	GGS/GCS	SPC1
GAS [ATCC 700294]	100	0	100	100	0	100
GCS [ATCC 35666]	0	100	100	0	100	100
GGS [ATCC 12384]	0	100	100	0	100	100
Negative	0	0	100	0	100	100

REFERENCES

1. Bisno AL, Gerber MA, Gwaltney JM Jr, Kaplan EL, Schwartz RH, for the Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of group A streptococcal pharyngitis. *Clin Infect Dis*. 2002;35:(2):113–125.

2. Trupti B Naik, Shobha D Nadagir,[1] and Asmabegaum Biradar Prevalence of Beta-Hemolytic Streptococci Groups A, C, and G in Patients with Acute Pharyngitis. J Lab Physicians. 2016 Jan-Jun; 8(1): 45–49.

3. David B. Haslam, Joseph W. St. GemeIII, in Principles and Practice of Pediatric Infectious Diseases (Fifth Edition), 2018.

4. Mobin Shah, MD, Robert M. Centor, MD, and May Jennings, MD. Severe Acute Pharyngitis Caused by Group C Streptococcus. J Gen Intern Med. 2007 Feb; 22(2): 272–274.

5. Shimomura Y, Okumura K, Murayama SY, Yagi J, Ubukata K, Kirikae T, Miyoshi-Akiyama T. Complete genome sequencing and analysis of a Lancefield group G Streptococcus dysgalactiae subsp. equisimilis strain causing streptococcal toxic shock syndrome (STSS). BMC Genomics. 2011;12:17. Epub 2011 Jan 11.

6. Gerber MA, Shulman ST. Rapid diagnosis of pharyngitis caused by group A streptococci. Clin Microbiol Rev. 2004;17(3):571–580. doi: 10.1128/CMR.17.3.571-580.2004.

7. Rimoin AW, Walker CL, Hamza HS, Elminawi N, Ghafar HA, Vince A, Da Cunha AL, Qazi S, Gardovska D, Steinhoff MC. The utility of rapid antigen detection testing for the diagnosis of streptococcal pharyngitis in low-resource settings. Int J Infect Dis. 2010;14(12):e1048–e1053. doi: 10.1016/j.ijid.2010.02.2269.

8. Slinger R, Goldfarb D, Rajakumar D, Moldovan I, Barrowman N, Tam R, Chan F. Rapid PCR detection of group A streptococcus from flocked throat swabs: a retrospective clinical study. Ann Clin Microbiol Antimicrob. 2011;10(1):33. doi: 10.1186/1476-0711-10-33.

9. Uhl JR, Adamson SC, Vetter EA, Schleck CD, Harmsen WS, Iverson LK, Santrach PJ, Henry NK, Cockerill FR. Comparison of LightCycler PCR, rapid antigen immunoassay, and culture for detection of group A streptococci from throat swabs. J Clin Microbiol. 2003;41(1):242–249. doi: 10.1128/JCM.41.1.242-249.2003.

10. Wei Ling Lean, Sarah Arnup, Margie Danchin, Andrew C. Steer. Rapid Diagnostic Tests for Group A Streptococcal Pharyngitis: A Meta-analysis. Pediatrics, October 2014, VOLUME 134 / ISSUE 4.

11. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th edition. HHS Publication No. (CDC) 21-1112, Revised December 2009.

12. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Fourth Edition. CLSI document M29-A4; May 2014

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LOT	**Batch code**
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	**Contains sufficient for <*n*> tests**
	**Consult instructions for use**
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