QUIDEL

AmpliVue® GAS ASSAY

For the qualitative detection of Group A β-hemolytic Streptococcus (*Streptococcus pyogenes*) nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis, such as sore throat

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

Contents

INTENDED USE

The AmpliVue GAS Assay is an *in vitro* diagnostic test for the qualitative detection of Group A β-hemolytic Streptococcus (*Streptococcus pyogenes*) nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis, such as sore throat.

The AmpliVue GAS Assay is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.

SUMMARY AND EXPLANATION

Group A streptococcus (GAS; *Streptococcus pyogenes*) is the most common bacterial cause of acute pharyngitis and GAS pharyngitis or "Strep throat" is most common in school-age children, affecting approximately 1 in 10 children per year,[1] GAS pharyngitis is a costly disease to society due to medical care and absence from school. In the United States, it is estimated that GAS pharyngitis costs the community up to 500 million USD per year.[2]

Acute pharyngitis is one of the most frequent illnesses for which pediatricians, internists, and other primary care physicians are consulted. However, only a small percentage of patients with this condition are infected by GAS.[3] In addition to pain and discomfort, GAS pharyngitis can lead to suppurative complications such as otitis media and peri-tonsillar abscess, and non-suppurative sequelae such as rheumatic fever.[4] Since GAS pharyngitis is the only commonly occurring form of acute pharyngitis that needs antibiotic therapy, for a patient with acute pharyngitis, the clinical decision that usually needs to be made is whether the pharyngitis is attributable to GAS.[3]

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, rapid and accurate detection is important.[5-8] In addition, accurate diagnosis can reduce the unnecessary use of antibiotics and potential development of antibiotic resistance, as most pharyngitis is viral in origin.[9,10] However, accurate diagnosis of GAS pharyngitis is difficult for a number of reasons. First, diagnosis of GAS pharyngitis using clinical signs alone is unreliable; physicians miss up to 50% of GAS pharyngitis cases and identify 20%-40% of non-GAS sore throat cases as requiring antibiotics.[11] Second, the standard procedure for laboratory detection of GAS, culture on blood agar, typically requires 24–48 hours. Physicians must therefore treat patients presumptively while awaiting culture results or withhold antibiotic therapy until the presence of Streptococcus pyogenes is confirmed with culture. Third, many children are asymptomatic carriers of GAS, with the prevalence of GAS throat carriage estimated at 12%.[12] Since the 1980s, commercial rapid antigen detection tests (RADTs) have been available as a means of GAS detection. The advantage of rapid diagnostic tests is that they can be quickly performed in the physician's office.

AmpliVue GAS Assay allows for the accurate detection of GAS without the need for culture confirmation. The assay detects GAS DNA by isothermal Helicase Dependent Amplification (HDA) reaction which amplifies a GAS specific sequence in the presence of a process control sequence.[13,14] The amplicons are subsequently detected by a DNA test strip embedded in the cross-contamination-resistant cassette.[15–20]

PRINCIPLE OF THE PROCEDURE

The AmpliVue GAS Assay detects GAS DNA isolated from throat swab specimens obtained from symptomatic patients. The assay consists of three major steps: (1) specimen preparation, (2) isothermal Helicase-Dependent Amplification (HDA) of target amplicons specific to GAS, and (3) detection of the amplified DNA by target-specific

hybridization probes via a colorimetric reaction on a lateral-flow strip which is embedded in a self-contained disposable cassette to prevent amplicon contamination.[17–20]

Patient specimen on a throat swab is transferred to a Lysis Tube and subjected to heat-treatment at 95°C for 10 minutes. The heat-treated sample is diluted 10-fold in a Dilution Tube, and then transferred to a Reaction Tube.

A HDA reaction is carried out in the Reaction Tube which contains lyophilized HDA reagents, dNTPs, primers and probes. Incubation at 64°C for 35 minutes results in isothermal amplification of the target sequence by GAS specific primers. The amplified DNA is detected by a set of specific detection probes included in the Reaction Tube: GAS target hybridizes to two specific probes labeled with Biotin (BioTEG) and 6-carboxyfluorescein (6-FAM). A competitive process control (PRC) is included in the Lysis Tube to monitor sample processing, inhibitory substances in clinical samples, reagent failure or device failure. The PRC target is amplified by GAS specific primers and hybridizes to the IC specific probes labeled with Biotin (BioTEG) and 2,4-dinitrophenyl (DNP-TEG).

Detection of the amplified DNA with specific probes is achieved by AmpliVue cassettes. The cross-contamination-resistant AmpliVue cassettes carry lateral-flow DNA detection strips coated with anti-DNP antibodies (C-line) and anti-FAM antibodies (T2-line). GAS amplicon with BioTEG and FAM-labeled probes is captured by anti-FAM antibodies at the T2-Line, while the PRC amplicon with BioTEG and DNP-labeled probes is captured by anti-DNP antibodies at the C-Line. The Biotin in the amplicon-probe complexes captures the streptavidin-conjugated color particles for visualization and the test result is shown as colored lines that are visually read.

A positive result for GAS (detection of GAS DNA) is reported when the T2-line is visible through the detection window of the cassette. A negative result (no detection of GAS DNA) is reported when only the C-line is displayed. The assay result is regarded as invalid when the T2-line and C-line are not present and the assay should be repeated.

MATERIALS PROVIDED

Cat. #M212
16 Tests per Kit

Component	Quantity	Storage
Detection Cassettes	16/kit	2°C to 30°C
Dilution Buffer	16 tubes/kit 0.5 mL	2°C to 8°C
Lysis Buffer	16 tubes/kit 0.5 mL	2°C to 8°C
Reaction Tubes	16 tubes/kit	2°C to 8°C
Amplicon Cartridge	16/kit	2°C to 30°C

MATERIALS REQUIRED BUT NOT PROVIDED

- External controls for Group A Streptococcus (e.g. Quidel Molecular Strep A+G Control Set, Cat. #M111, which contains positive and negative controls, serves as an external processing and extraction control)
- Sterile DNAse-free filter-blocked or positive displacement micropipettor tips
- Micropipettor
- Stopwatch or timer
- Scissors or a blade
- Micro tube tray
- Heat block capable of 95°C ± 2°C temperature
- Heat block with heated lid capable of 64°C ± 2°C temperature
- Thermometer

WARNINGS AND PRECAUTIONS

1. All reagents are for *in vitro* diagnostic use only.
2. Treat all specimen/samples as potentially infectious.[21] Follow universal precautions when handling samples, this kit and its contents.[22]
3. All tubes should be capped tightly prior to vortexing.
4. Proper sample collection, storage and transport are essential for correct results.
5. Store assay reagents as indicated on their individual labels.
6. Reagents are not interchangeable between lots.
7. Never pool reagents from different tubes even if they are from the same lot.
8. Do not use the reagents after their expiration date.
9. Do not interchange caps among reagents as contamination may occur and compromise test results.
10. Only open the tubes when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
11. To avoid contamination of the environment with GAS amplicons, do not open the reaction tubes post-amplification.
12. Avoid microbial and deoxyribonuclease (DNAse) contamination of reagents when removing aliquots from tubes. The use of sterile DNAse-free disposable filter-blocked or positive displacement pipettor tips is recommended.
13. Use a new pipettor tip for each specimen or reagents.
14. Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.
15. To avoid exposure to excessive heat, care should be taken when inserting and removing tubes from the heat blocks, and when handling the heated tubes.
16. Additional controls may be tested according to guidelines or requirements of Local, State, Provincial and/or Federal regulations or accrediting organizations.
17. In cases where open-tube PCR tests are conducted in the same general area by the laboratory, separated or segregated working areas should be used for specimen preparation and amplification/detection activities. Supplies and equipment should be dedicated to each area and should not be moved from one area to another. Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating the reagents.
18. Wash hands thoroughly after performing the test.
19. Do not pipette by mouth.
20. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
21. Dispose of unused reagents and waste in accordance with County, Federal, Provincial, State and Local regulations.
22. Wear suitable protective clothing, gloves, eye and face protection when using this kit.
23. For accurate results, pipette carefully using only calibrated equipment.
24. Thoroughly clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
25. Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
26. MSDS is available upon request or can be accessed on the product website.

STORAGE AND HANDLING OF KIT REAGENTS

Store the assay kit at 2°C to 8°C until the expiration date listed on the outer kit box.

SPECIMEN COLLECTION, STORAGE AND HANDLING

During clinical studies, the AmpliVue GAS Assay was evaluated with Liquid Amies Single Plastic Applicator, Liquid Stuart Single Plastic Applicator, Puritan Liquid Amies Transport System, and Sterile Rayon and Polyester Throat Swabs.

Analytical studies performed with contrived specimens containing group A streptococci, near LOD (2x LOD) demonstrated that samples can be stored at 25°C ± 2°C for 2 days and then at 2°C to 8°C for up to 8 more days before testing or at ≤–15°C or ≤–70°C for up to 32 days before testing with the AmpliVue GAS Assay. Specific

requirements for shipping specimens should follow recommendations found in section 42 and 49 of the Code of Federal Regulation, CFR.

ASSAY PROCEDURE

Heat Lysis

1. 25 minutes prior to the heat lysis step, warm a heating block to 95°C.
2. Place the required number of Lysis Tubes in a rack. Mark the Lysis Tubes on the cap and/or side of the tube.
   Note: One (1) Lysis Tube is required for each specimen to be tested.
3. Place a throat swab in a patient-identified Lysis Tube and vigorously twirl the swab for 10 seconds to elute specimen material. When ESwab was used for specimen collection, vortex the ESwab collection device for 5 seconds and transfer 50 µL of the ESwab transport medium to a patient-identified Lysis Tube.
   Note: the specimens in Lysis Tubes may be stored at room temperature (20°C to 25°C) or at 2°C to 8°C for up to 24-hours.
4. Heat the Lysis Tubes at 95°C for 10 minutes and then vortex the Lysis Tubes for 5 seconds.
   Note: Begin 10 minute lysis procedure after placing tubes in block and waiting until block returns to 95°C
   Note: The specimens in Lysis Tubes may be stored at room temperature (20°C to 25°C) or at 2°C to 8°C for up to 24-hours.
5. Place the required number of Dilution Tubes in a rack. Mark the Dilution Tubes on the cap and/or side of the tube.
   Note: One (1) Dilution Tube is required for each specimen to be tested.
6. Transfer 50 µL of each Lysis Tube to a patient-identified Dilution Tube (blue cap). Close the cap and mix the solution well by vortexing the tubes for 5 seconds.
   Note: Use a new pipette tip for each specimen.
   Note: The diluted specimen can be stored at room temperature (20°C to 25°C) or at 2°C to 8°C for up to 24-hours.

Amplification

1. 15 minutes prior to the amplification step, warm a heating block with a heated lid to 64°C ± 2°C.
2. Transfer 50 µL of the diluted specimen to a labeled Reaction Tube, mix the solution by pipetting up and down a minimum of 5 times and close the cap. The solution should be clear, free of solid material.
   Note: Remove the required number of Reaction Tubes from the protective pouch, remove the excess air and reseal the bag. Unused Reaction Tubes must be stored at 2°C to 8°C.
   Note: Use a new pipette tip for each diluted sample.
   Note: Proceed immediately to the next step. Do not allow reconstituted reaction mix to sit for longer than 15 minutes.
3. Incubate the Reaction Tubes at 64°C for 35 minutes in a heating block with a heated lid.
   Note: Be sure that all tubes are in tight contact with heat block.
   Note: To avoid laboratory contamination, once the tube has been closed and the amplification reaction started, **DO NOT** open the Reaction Tube.

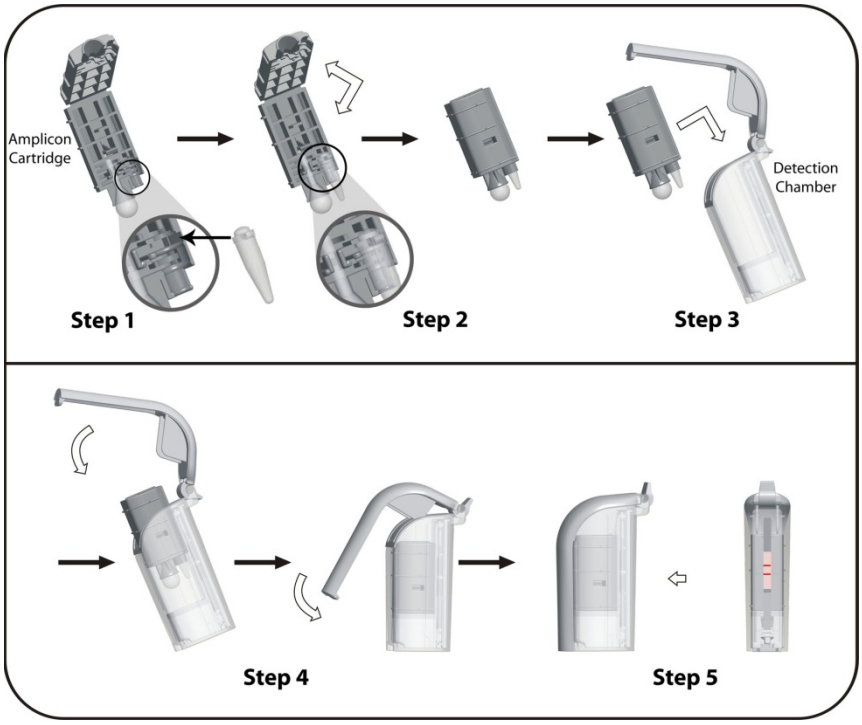
Detection

1. Tear open a new Detection Cassette package. Label the Cassette appropriately.
   Make sure a buffer bulb is attached in the Amplicon Cartridge.
2. Place the Reaction Tube into the Amplicon Cartridge (Figure 1, step 1). Be sure to place the HINGE of the Reaction Tube cap into the largest slot adjacent to the buffer bulb.
3. Close the Amplicon Cartridge (Figure 1, step 2) ensuring that it snaps shut.
   If the Cartridge does not snap shut, reposition the tube within the cartridge.
4. Insert the closed Amplicon Cartridge into the Detection Cassette until resistance is felt on the cartridge (Figure 1, step 3).

Make sure the arrow faces the detection strip (Reaction Tube should face the razor blade and the plastic bulb containing the running buffer should face the pin). Identify the Cassette on the top and/or side of the outer casing.

5. Keep the device upright and press the handle of the outer casing to close the device (Figure 1, step 4). The handle will lock into place when closed completely (Figure 1, step 5).

Figure 1



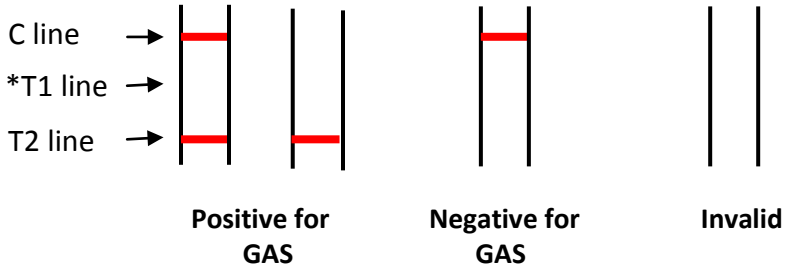
6. Results are read at 10 minutes. **Note: the results are stable for up to 60 minutes.**
7. Discard the used Detection Chambers in sealed bags and/or as appropriate for your laboratory.

Warning

1. **DO NOT** open the AmpliVue Detection Cassette after use. Opening the Cassette after use may result in amplicon contamination of the test area.
2. Remove the required number of Reaction Tubes from the protective pouch, remove the excess air and reseal the bag.

INTERPRETATION OF RESULTS

- Any pink to red colored visible line should be recorded as positive (+) and no line should be recorded as (–); for example, "T2+" = Visible T2 line and "T2–" = No T2 line (See diagram below).
- The T2 line detects GAS DNA.
- The C line detects the process control DNA in the absence of the target GAS DNA. In the presence of the target GAS DNA, the C line detects amplified products from both the GAS and the process control DNA. The control line intensity may vary with each test. Any pink to red colored visible line in the control signifies a valid test.



The interpretation of the assay results is done according to the following criteria:

Test line (T) Reading	Control line (C) Reading	Interpretation of result
T2+	C+	GAS DNA detected (Positive)
T2+	C–	GAS DNA detected (Positive)
T2–	C+	No GAS DNA detected (Negative)
T2–	C–	Invalid: failure due to inhibitory specimen, reagent failure, or device failure. Repeat test, starting at the **Heat Lysis** step 4.
***Note 1:** The T1 line is not used on this assay. The presence of a T1 line should be considered invalid for this assay. Repeat test, starting at the **Heat Lysis** step 4.		

QUALITY CONTROL

The AmpliVue GAS Assay incorporates several controls to monitor assay performance.

1. The process control is used to monitor sample processing, to detect HDA inhibitory specimens and to confirm the integrity of assay reagents and cassette detection. The process control is included in the Lysis Buffer tube.
2. The external positive control may be treated as a patient specimen. The control should be sampled and tested as if it were a swab specimen and processed as described above in the Assay Procedure. The external positive control is intended to monitor substantial reagent and cassette failure.
3. The external negative control may be treated as a patient specimen. The control should be sampled and tested as if it were a swab specimen and processed as described above in the Assay Procedure. The external negative control is used to detect reagent or environmental contamination (or carry-over) by GAS DNA or amplicon.

It is recommended that the reactivity of each new lot and each new shipment of the AmpliVue GAS Assay be verified on receipt and before use.[23] External control tests should be performed thereafter in accordance with appropriate federal, state and local guidelines. The AmpliVue GAS Assay should not be used in patient testing if the external controls do not produce the correct results.

LIMITATIONS

- Additional follow-up testing using the culture method is required if the result is negative and clinical symptoms persist, or in the event of an acute rheumatic fever (ARF) outbreak.
- The main laboratory technique required is pipetting. Good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all reagents, especially in cases where multiple aliquots are taken from a tube.
- The AmpliVue GAS Assay does not distinguish between viable and non-viable organisms.

- As with other assays of this type, there is a risk of false negative results due to the presence of sequence variants in the amplification targets.

EXPECTED VALUES

Performance characteristics of the AmpliVue GAS Assay were established during a prospective study conducted January to March 2014. One thousand one hundred ninety-two (1192) fresh throat swab specimens from female and male patients were collected at five distinct geographical sites across the United States. A single specimen was collected per patient. Samples were collected on Polyester or Rayon Swab with liquid Amies or Polyester Swab or Rayon with liquid Stuart's.

The expected value of Group A β-hemolytic Streptococcus (*Streptococcus pyogenes*) detected with the AmpliVue GAS Assay has been calculated for the combined sites based on the age of the patient and overall.

The gender and age demographics for each category are listed below.

Combined Study – Age and Gender Distribution (N=1192)		
Gender	**Female**	**Male**
Total	683	509
Age		
≤2 years	29	29
3 to 12 years	234	233
13 to 21 years	185	103
≥22 years	235	144

The prevalence of Group A β-hemolytic Streptococcus (*Streptococcus pyogenes*) detected with the AmpliVue GAS Assay has been calculated for the combined sites based on the age of the patient. One (1) specimen (0.08%) was invalid (in both the initial and repeat test neither the T2 or control lines were detected) and has been removed from the Expected Values table. The table below presents the data for the remaining one thousand one hundred ninety-one (1191) specimens.

Combined Study – Expected Values (N=1191*)			
	Group A β-hemolytic Streptococcus (by Amplivue®)		
Age	**Total #**	**Total Positive**	**Prevalence**
≤2 years	58	4	6.9%
3 to 12 years	466*	139	29.8%
13 to 21 years	288	36	12.5%
≥22 years	379	60	15.8%

*One (1) invalid specimen was excluded.

CLINICAL PERFORMANCE

Performance characteristics of the AmpliVue GAS Assay were established during a prospective study conducted January to March 2014. One thousand one hundred ninety-two (1192) fresh throat swab specimens from female and male patients were collected at five distinct geographical sites across the United States. A single specimen was collected per patient. Samples were collected on Polyester or Rayon Swab with liquid Amie's or Polyester Swab or Rayon with liquid Stuart's. The swabs were inoculated by conventional streak-stab culture technique onto a trypticase soy agar plate containing 5% horse red blood cells. Testing with the AmpliVue device was performed at the five external laboratories using the same swab that was plated for the culture. All residual specimen transport media from the samples was shipped daily (with cold packs) to a central location. The transport media was cultured using the same testing protocol as that employed by the clinical sites.

One thousand one hundred ninety-two (1192) fresh throat specimens were cultured for Group A β-hemolytic Streptococcus and tested with the AmpliVue GAS Assay. The specimens were cultured at the testing sites and the

transport media was cultured at a central location. The specimen was considered positive if either the swab or the transport media was positive for β-hemolytic Streptococcus (Composite Culture) and typed as Lancefield group A by latex agglutination. The table below details the overall performance using composite culture results as a reference.

Performance Results of AmpliVue GAS Assay for Group A β-hemolytic Streptococcus			
Overall Performance (All Sites)			
	Composite Culture		
AmpliVue GAS Assay	**Positive**	**Negative**	**Total**
Positive	189	50*	239
Negative	3**	949	952
Total	192	999	1191
			95% CI
Sensitivity	189/192	98.4%	95.5% to 99.5%
Specificity	949/999	95.0%	93.5 % to 96.2%
*Of the fifty (50) discordant specimens, thirty-one (31) of these specimens were positive for GAS when tested with an additional FDA-cleared molecular device, eighteen (18) were negative. One (1) specimen was unavailable for discordant testing. **Of the three (3) discordant specimen, two (2) were negative when tested with an additional FDA-cleared molecular device.			
Site 1 Performance			
	Composite Culture		
AmpliVue GAS Assay	**Positive**	**Negative**	**Total**
Positive	82	15	97
Negative	1	402	403
Total	83	417	500
			95% CI
Sensitivity	82/83	98.8%	93.5% to 99.8%
Specificity	402/417	96.4%	94.2 % to 97.8%
Site 2 Performance			
	Composite Culture		
AmpliVue GAS Assay	**Positive**	**Negative**	**Total**
Positive	45	17	62
Negative	0	132	132
Total	45	149	194
			95% CI
Sensitivity	45/45	100%	92.1% to 100%
Specificity	132/149	88.6%	82.5 % to 92.8%
Site 3 Performance			
	Composite Culture		
AmpliVue GAS Assay	**Positive**	**Negative**	**Total**
Positive	16	9	25
Negative	0	174	174
Total	16	183	199
			95% CI
Sensitivity	16/16	100%	80.6% to 100%
Specificity	174/183	95.1%	90.9 % to 97.4%

Site 4 Performance			
	Composite Culture		
AmpliVue GAS Assay	**Positive**	**Negative**	**Total**
Positive	8	3	11
Negative	0	89	89
Total	8	92	100
			95% CI
Sensitivity	8/8	100%	67.6% to 100%
Specificity	89/92	96.7%	90.8 % to 98.9%
Site 5 Performance			
	Composite Culture		
AmpliVue GAS Assay	**Positive**	**Negative**	**Total**
Positive	38	6	44
Negative	2	152	154
Total	40	158	198
			95% CI
Sensitivity	38/40	95.0%	83.5% to 98.6%
Specificity	152/158	96.2%	92.0 % to 98.2%

ANALYTICAL PERFORMANCE

Limit of Detection

The analytical sensitivity (limit of detection or LOD) of the AmpliVue GAS Assay was determined using quantified (CFU/mL) cultures of two (2) *Streptococcus pyogenes* strains serially diluted in a contrived negative matrix. Analytical sensitivity (LOD) is defined as the lowest concentration at which 95% of all replicates tested positive.

The LOD for the 2 *Streptococcus pyogenes* strains tested were $1.90x10^4$ CFU/mL (ATCC #19615) and $2.74x10^4$ (ATCC #12344). Based on this data the reported LOD for the AmpliVue GAS Assay is $2.74x10^4$ CFU/mL.

Analytical Reactivity (Inclusivity)

The reactivity of the AmpliVue GAS Assay was evaluated against an additional seven (7) strains of *Streptococcus pyogenes*. The testing was performed near the level of detection for the assay (1x LOD). The seven (7) strains were detected by the AmpliVue GAS Assay in this study at a LOD of $2.74x10^4$ CFU/mL.

Bacterial Strain	Concentration CFU/mL	Strain Detected (Yes/No)
ATCC 12384	$2.74x10^4$	Yes
NCIMB 13285	$2.74x10^4$	Yes
CCUG 33061	$2.74x10^4$	Yes
CCUG 33409	$2.74x10^4$	Yes
CCUG 39158	$2.74x10^4$	Yes
ATCC 49399	$2.74x10^4$	Yes
CCUG 53553	$2.74x10^4$	Yes

Repeatability Study

The Precision/Within Laboratory Repeatability was determined via a study, where a four-member panel (3x, 1x, 0.3x LOD and a negative sample) was tested by two (2) operators, twice a day (2x) for twelve (12) days.

The AmpliVue GAS Assay produces results that are highly reproducible. This observation is based on the following findings:

- All negative samples generated negative results for GAS.
- The percentage of positive High Negative (0.3x LOD) samples is 53%, this is within the target range of 20% to 80%.
- The percentage of positive of the Low Positive (1x LOD) samples was 100%.
- The percentage of positive of the Moderate Positive (3x LOD) samples was 100%.

Reproducibility Study

In order to confirm the reproducibility of the AmpliVue GAS Assay a blinded and randomized study panel containing *Streptococcus pyogenes* negative and positive samples (3x, 1x, 0.3x LOD) were tested at three (3) test sites (one in-house laboratory and two (2) clinical sites). Each site tested a reproducibility panel and Assay Controls for five (5) days in triplicate. Testing was done by two operators at each site. Each operator ran the panel once a day using one lot of AmpliVue GAS Assay. A total of five hundred forty (540) specimens were tested (including controls). The AmpliVue GAS Assay generated reproducible results in this study.

Category	SITE						Overall Percent Agreement		95% Confidence Interval
	Site #1		Site #2		Site #3				
	#expected results/# tested	*% Agreement*	*#expected results/# tested*	*% Agreement*	*#expected results/# tested*	*% Agreement*			
GAS High Negative	18/30	60%	19/30	63%	13/30	43%	50/90	56%	45% to 65%
GAS Low Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
GAS Moderate Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
GAS Negative	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
GAS Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
GAS Negative Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%

Analytical Specificity – Cross-reactivity and Microbial Interference

An *in silico* BLAST analysis of primers used in the AmpliVue GAS Assay against sixty-one (61) potential interfering organisms (see below) did not show evidence of cross-reactivity.

Arcanobacterium sp.	Human adenovirus F	*Lactobacillus* sp.[1]
Bacillus sp.	Human adenovirus G	*Legionella pneumophila*
Bacteroides sp.[2]	Human coronavirus 229E	Measles virus
Bordetella sp.	Human coronavirus HKU1	Human Metapneumovirus
Branhamella sp.	Human coronavirus NL63	*Moraxella* sp.
Burkholderia sp.	Human enterovirus A	Mumps virus
Campylobacter sp.[3]	Human enterovirus B	*Mycoplasma pneumoniae*
Candida sp.	Human enterovirus C	*Neisseria* sp.
Corynebacterium sp.	Human enterovirus D	*Peptostreptococcus* sp.
Cytomegalovirus	Human herpesvirus 1	*Proteus* sp.
Enterobacterio phage MS2	Human herpesvirus 2	*Pseudomonas* sp.
Enterococcus sp.	Human herpesvirus 4	Respiratory syncytial virus Type B

[1] Includes *L. acidophilus*
[2] Includes *B. ovatus*
[3] Includes *C. rectus*

Escherichia coli	Human parainfluenza virus 1	*Saccharomyces cerevisiae*
Fusobacterium sp.	Human parainfluenza virus 2	*Serratia* sp.
Haemophilus sp.	Human parainfluenza virus 3	*Staphylococcus* sp.
Human adenovirus A	Human parainfluenza virus 4a and 4b	*Treponema* sp.
Human adenovirus B	Influenza virus A	*Veillonella* sp.
Human adenovirus C	Influenza virus B	*Yersinia* sp.
Human adenovirus D	Influenza virus C	*Prevotella oralis*[4]
Human adenovirus E	*Klebsiella* sp.	*Parvimonas micra*[5]
Veillonella parvula		

A study was performed to evaluate the performance of the AmpliVue GAS Assay in the presence of forty-seven (47) other microorganisms commonly found in throat specimens. Each potentially interfering microorganism was tested in the presence of 2x LOD Group A *Streptococcus* (2 strains) in the presence of clinically relevant levels of viruses (10^5pfu/ml) and bacteria (10^6cfu/mL) or higher. All strain combinations were spiked into contrived negative matrix. The strains included in the cross-reactivity study are shown in the table below.

Arcanobacterium haemolyticum	*Lactobacillus acidophilus*
Burkholderia cepacia	*Moraxella cartarrhalis*
Corynebacterium diphtheria	*Neisseria gonorrhoeae*
Influenza A	*Staphylococcus aureus MRSA*
Influenza B	*Staphylococcus epidermidis MRSE*
Parainfluenza Type 4B (VR-1377)	*Streptococcus agalactiae*
Fusobacterium necrophorum	*Streptococcus salivarius*
Peptostreptococcus micros (aka Parvimonas micra)	*Acinetobacter lwoffii*
Candida albicans	Adenovirus Type 1
Enterococcus faecalis	Adenovirus Type 11 (Slobitski)
Escherichia coli	*Bacillus cereus*
Klebsiella pneumonia	*Lactococcus lactis*
Legionella pneumophila	*Legionella jordanis*
Pseudomonas aeruginosa	*Legionella micdadei*
Stenotrophomonas maltophilia	*Neisseria subflava*
Streptococcus bovis	Parainfluenza virus 4a
Streptococcus dysgalactiae subsp equisimilis	Rhinovirus Type 15 (1734)
Streptococcus canis	*Serratia marcescens*
Streptococcus intermedius	*Streptococcus anginosus*
Streptococcus mutans	*Streptococcus gordonii (Virdans type)*
Streptococcus oralis	*Streptococcus mitis*
Streptococcus suis	*Streptococcus pneumoniae*
Bordetella pertussis	*Streptococcus sanguinis*
Haemophilis influenzae type A	

None of the organisms or viruses tested above cross-reacts with the performance of the AmpliVue GAS Assay.

[4] In NCBI, *Bacteroides oralis* is *Prevotella oralis*.
[5] In NCBI, *Peptostreptococcus micros* is *Parvimonas micra*.

Analytical Specificity – Interfering Substances

A study was conducted using two strains of *Streptococcus pyogenes* (ATCC 19615 and 12344) tested near LOD to evaluate the AmpliVue GAS Assay for potential interference using a panel consisting of twenty-eight (28) common biological and chemical substances found in throat samples. Substances were introduced into the assay dilution tubes at concentrations which were medically relevant. Each of the strains was tested for each substance. None of the substances tested were found to interfere with the AmpliVue GAS Assay.

Substance Name	Test Concentration	Interference? (Y/N)
Children's Dimetapp DM Cold & Cough Elixir	25% v/v	No
Chloraseptic Max: Sore Throat Relief	10% v/v	No
BreathSavers 3 Hour Mint-Spearmint	10% w/v	No
Cepacol Sore Throat: Cherry Flavor	5% w/v	No
Robitussin Cough & Cold-CF Max	10% v/v	No
Ricola Mountain Herb Throat Drops-Sugar Free	15% w/v	No
Human Saliva	10% v/v	No
Robitussin Nighttime Cold, & Flu	10% v/v	No
Crest Pro-Health Night Mint	25% v/v	No
CVS Tussin CF	15% v/v	No
Chloraseptic Throat Cherry lozenge	10% w/v	No
Halls Cherry Mentholyptus	15% w/v	No
Tic Tac Freshmints	10% w/v	No
Zicam® Oral Mist	0.625% v/v	No
Sucrets Complete-Vapor Cherry	5% w/v	No
Acetaminophen	19.5 mg/mL	No
Aspirin	12.3 mg/mL	No
Ibuprofen	15.6 mg/mL	No
Benadryl	2.7 mg/mL	No
Crest® Complete Toothpaste	5% w/v	No
Contac® Cold + Flu Caplets Night	10% w/v	No
Children's Wal-Tap Elixir Cold & Allergy (Dimetap Children's Cold and Allergy)	25% v/v	No
Children's Wal-Tap DM Elixir Cold & Cough	25% v/v	No
Robitussin Nighttime Cough, Cold, & Flu (peak cold)	10% v/v	No
Halls Mentholyptus (not cherry flavor)	15% w/v	No
Listerine Cool Mint Antiseptic	15% v/v	No
Whole Blood	5% v/v	No
Mucin (Bovine Submaxillary Gland, type I-S)	5.0 mg/mL	No

Carryover – Cross Contamination

Three (3) runs of twenty-two (22) samples consisting of eleven (11) negative and eleven (11) high *S. pyogenes* positive (4.33×10^7 CFU/mL) samples were tested in an alternating pattern. All positive samples were reported as positive (33 total) and all negative samples were reported as negative (33 total). No carry over contamination was seen when performing the AmpliVue GAS Assay according to the Package Insert.

CUSTOMER AND TECHNICAL ASSISTANCE

To place an order or for Technical Support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact custserv@quidel.com or technicalsupport@quidel.com. For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website **quidel.com**.

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 Intended use	 Catalog number
 Contents / Contains	 Contains sufficient for XX determinations
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