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Detection of Group A *Streptococcus* in Pharyngeal Swab Specimens by Use of the AmpliVue GAS Isothermal Helicase-Dependent Amplification Assay

Matthew L. Faron,[a] Nathan A. Ledeboer,[a] Paul Granato,[b] Judy A. Daly,[c,d] Kristina Pierce,[c] Preeti Pancholi,[e] Timothy S. Uphoff,[f] Blake W. Buchan[a]

Medical College of Wisconsin, Milwaukee, Wisconsin, USA[a]; Laboratory Alliance of Central New York, Syracuse, New York, USA[b]; University of Utah[c] and Primary Children's Medical Center,[d] Salt Lake City, Utah, USA; The Ohio State University Medical Center, Columbus, Ohio, USA[e]; Marshfield Labs, Marshfield Clinic, Marshfield, Wisconsin, USA[f]

We evaluated the clinical performance (sensitivity and specificity) of the AmpliVue group A *Streptococcus* (GAS) isothermal helicase-dependent amplification assay using 1,192 pharyngeal swab specimens. AmpliVue GAS assay results were compared to the results of routine throat cultures on selective streptococcal blood agar plates. The sensitivity and specificity of the AmpliVue GAS assay were 98.3% (95% confidence interval [CI], 95 to 100%) and 93.2% (95% CI, 91 to 95%), respectively.

Streptococcus pyogenes, or group A *Streptococcus* (GAS), is a leading cause of bacterial pharyngitis (1, 2). Antibiotic treatment, especially for pediatric patients, is important to reduce the risk of life-threatening nonsuppurative sequelae, including acute rheumatic fever (ARF) and acute glomerulonephritis (AGN) (3, 4). GAS remains universally susceptible to penicillin, and identification of the organism is sufficient to guide empirical therapy. Therefore, assays capable of rapid accurate identification of GAS can be beneficial in determining the need for antibiotic therapy for patients with clinical symptoms of pharyngitis (5).

We conducted a multicenter evaluation of the FDA-cleared AmpliVue GAS assay (Quidel, San Diego, CA). The goal of this study was to establish the performance of the AmpliVue GAS assay in comparison with the established reference culture method for identification of GAS in pharyngeal specimens. A total of 1,192 pharyngeal swab specimens were prospectively collected and tested in February and March 2014 at five clinical centers, in accordance with site-specific institutional review board-approved protocols. Pharyngeal specimens were collected using ESwabs ($n = 481$) or wound fiber swabs ($n = 711$) with liquid Stuart or gel Amies nonnutritive medium. All testing was performed using residual material within 72 h after collection.

Reference cultures were performed by direct inoculation of wound fiber swabs or 10 μl of ESwab liquid medium to selective streptococcal agar (SXT blood agar; Remel, Lenexa, KS). Inoculated plates were stabbed several times and were incubated at 35°C to 37°C in 5% CO_2 for up to 48 h. Latex typing was performed for all beta-hemolytic, Gram-positive, catalase-negative cocci, to definitively identify GAS. Following solid medium inoculation, specimens were tested using the AmpliVue GAS assay in accordance with the package insert. Briefly, a swab specimen or 50 μl of ESwab medium was added to a lysis buffer tube and heat treated at 95°C for 10 min. A 50-μl aliquot of the lysed specimen was added to a dilution buffer tube and mixed, and 50 μl of the diluted specimen was then added to a reaction tube containing lyophilized reagents required for isothermal amplification and labeling of the GAS target sequence (*sdaB* region). Reaction tubes were incubated at 64°C for 35 min for isothermal helicase-dependent amplification (HDA) of the target. Following HDA, the reaction tube was loaded into the AmpliVue test cartridge and then placed

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Address correspondence to Blake W. Buchan, bbuchan@mcw.edu.

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TABLE 1 Performance of AmpliVue GAS assay compared to reference culture

Clinical test site	No. of specimens tested	Result (no.)[a]				Performance (% [95% CI])[b]			
		TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
1	500	78	401	20	1	98.7 (92-99.9)	95.2 (93-97)	79.6 (70–87)	99.8 (98–100)
2	194	38	132	24	0	100 (89-100)	84.6 (78-90)	61.3 (48–73)	100 (96–100)
3	200[c]	13	174	12	0	100 (72-100)	93.5 (89-96)	52.0 (32–72)	100 (97–100)
4	100	5	89	6	0	100 (46-100)	93.7 (85-97)	45.4 (18–75)	100 (95–100)
5	198	37	152	7	2	94.9 (81-99)	95.6 (91-98)	84.1 (69–93)	98.7 (95–100)
Total	1,192	171	948	69	3	98.3 (95-100)	93.2 (91-95)	71.2 (65–77)	99.7 (99–100)

[a] TP, true positive; TN, true negative; FP, false positive; FN, false negative.
[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[c] One specimen was invalid after a repeat test.

TABLE 2 Discrepant analysis using Lyra Direct Strep assay

No. of samples	AmpliVue GAS assay result	Throat culture result	PCR assay result	Average C_T[a]
46	Positive	Negative	Positive	28.3
23	Positive	Negative	Negative	NA
1	Negative	Positive	Positive	29.4
2	Negative	Positive	Negative	NA

[a] C_T, cycle threshold of positive results using the Lyra assay; NA, not applicable.

in the AmpliVue detection device, which released the amplification product to a lateral-flow membrane strip. Test results were read after 10 min. Each reaction had an internal process control to monitor for HDA reaction failure. All invalid specimens were retested once from the remaining elution lysis buffer. If the repeat test yielded a second invalid test result, then no further testing was performed and the result for the specimen was reported as invalid.

Specimens demonstrating discrepant results between the AmpliVue GAS assay and bacterial culture were analyzed at a central laboratory using an FDA-cleared real-time PCR assay (Lyra Direct Strep assay; Quidel, San Diego, CA) that amplifies a region of *comx1.1*. Fifty microliters of ESwab medium or excess fluid extracted from the residual sponge in the transport device was used as the assay input.

The performance of the AmpliVue GAS assay is summarized in Table 1. During the study, two specimens were initially reported as invalid, resulting in a first-run success rate of 99.8% (1,190/1,192 specimens). One specimen remained invalid after repeat testing, reducing the total number of specimens used to calculate assay performance values to 1,191. GAS was detected by the AmpliVue assay and culture in 171/1,191 specimens (14.4%), and 948/1,191 specimens (79.6%) were negative for GAS by both the AmpliVue assay and culture. Seventy-two specimens (6.0%) had discrepant results, consisting of 69 false-positive (FP) and 3 false-negative (FN) results. These data establish sensitivity and specificity of 98.3% and 93.2%, respectively, for the AmpliVue GAS assay in comparison with culture. AmpliVue GAS assay performance results were similar across all 5 clinical test sites, demonstrating sensitivities of 98.3% (78/79 specimens), 100% (38/38 specimens), 100% (13/13 specimens), 100% (5/5 specimens), and 94.9% (37/39 specimens) (Table 1). The specificity of the AmpliVue GAS assay was also consistent across 4 of the 5 clinical sites, being 95.2% (401/421 specimens), 93.5% (174/186 specimens), 93.7% (89/95 specimens), and 95.6% (152/159 specimens). Clinical test site 2 demonstrated specificity results (84.6% [132/156 specimens]) that were outside the 95% confidence interval (CI) calculated for the entire study set.

Discrepant analysis results are summarized in Table 2. The Lyra assay was positive for GAS in 46/69 specimens (66.7%) initially categorized as FP. The average Lyra cycle threshold (C_T) for these specimens was 28.3, compared to an average C_T of 24.1 for culture-positive specimens. This suggests that the amounts of GAS in these specimens were small and might have been below the culture limit of detection. The Lyra assay confirmed the presence of GAS in 1/3 specimens (33.3%) initially categorized as FN, with a C_T of 29.4. The Lyra assay failed to detect GAS in the remaining 2 FN samples, suggesting misidentification by reference culture or the presence of a streptococcal species other than *S. pyogenes* with group A antigen (6). Following the analysis of discrepant results, the final sensitivity and specificity of the AmpliVue GAS assay were 99.5% (95% CI, 97 to 100%) and 97.6% (95% CI, 96 to 98%), respectively, resulting in a 90.4% positive predictive value and a 99.9% negative predictive value. Additionally, no statistical difference in performance among sites was noted after discrepant analysis.

Recently, the addition of the ESwab system has been of interest for comparisons of collection devices (7, 8). In this study, 483 specimens (40.5%) were collected using ESwabs, compared to 709 specimens (59.5%) collected using wound fiber swabs (liquid Stuart or gel Amies medium). Using the AmpliVue GAS assay, ESwabs and wound fiber swabs were found to be equivalent (Table 3). The specificity for specimens collected using ESwabs fell outside the 95% CI for all specimens tested; however, this difference was not significant after the discrepant analysis.

The study was not free of limitations. Specimens collected using wound swabs typically included only a single swab. Therefore, prior to AmpliVue assay and reference culture testing, all specimens were inoculated to culture plates as the routine standard of care. This could result in contamination of the swab or reduced bacterial burden prior to study enrollment. Another limitation is that all analyses of discrepant results for wound swab specimens required extraction of material from the empty collection device, which could reduce the sensitivity of the molecular test used as the determining method.

Current Infectious Diseases Society of America (IDSA) guidelines for the detection of GAS in pharyngeal specimens recommend performing direct antigen detection, with all cases with negative results being referred to throat culture or a molecular test for patients <18 years of age (9). Rapid antigen testing allows point-of-care GAS detection, but the sensitivity of this method is low, ranging from 58% to 96% (10). Throat culture is the current gold standard for detecting GAS and is more sensitive than rapid antigen assays (11). However, culture is a time-dependent, labor-intensive process that requires 24 to 48 h to confirm the presence of GAS in a throat specimen. The results of this study demonstrate that the AmpliVue GAS assay is both sensitive and specific for detection of GAS in pharyngeal specimens. The assay takes less than 1 h to complete and requires less than 2 min of hands-on time per sample. In addition, HDA technology allows the test to be completed using a stationary heat block. Combined with the hand-held, lateral-flow design of the AmpliVue detection device, the assay can be performed on demand, without other specialized

TABLE 3 Performance of AmpliVue GAS assay based on collection device

Collection device	No. of specimens tested	Result (no.)[a]				Performance (% [95% CI])[b]			
		TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
ESwab	481	76	385	19	1	98.7 (92-100)	95.2 (93-97)	80.0 (70–87)	99.7 (98–100)
Wound fiber swab	711[c]	95	563	50	2	97.9 (92-100)	91.8 (89-94)	65.5 (57–73)	100 (98–100)

[a] TP, true positive; TN, true negative; FP, false positive; FN, false negative.
[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[c] One specimen was invalid after a repeat test.

laboratory equipment. This may be beneficial for use in near-point-of-care laboratories certified to perform tests of moderate complexity.

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