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A comparison of the Quidel Solana GAS assay, the Luminex Aries Group A Strep assay and the Focus Diagnostics Simplexa Group A Strep Direct assay for detection of Group A *Streptococcus* in throat swab specimens

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ABSTRACT

We compared three commercially available group A *Streptococcus* (GAS) nucleic acid amplification tests, the Quidel Solana GAS assay, the Luminex Aries Group A Strep assay and the Focus Diagnostics Simplexa Group A Strep Direct assay, with GAS bacterial culture. A true positive result was defined as one positive by culture or positive by ≥2/3 molecular methods. Two hundred eighty-seven throat swabs (207 children, 80 adults) were collected. The sensitivity of culture was 84.8% (95% CI 77.7–90.3%) with a specificity of 100% (95% CI 97.5–100%). The Solana assay sensitivity was 94.2% (95% CI 88.9–97.5%) with a specificity of 98.7% (95% CI 95.2–99.8%). Simplexa assay sensitivity was 99.3% (95% CI 96.0–99.9%) with a specificity of 95.3% (95% CI 90.6–98.1%). Aries assay sensitivity was 96.4% (95% CI 91.8–98.8%) with a specificity of 98.0% (95% CI 94.2–99.6%). In summary, all the molecular methods evaluated showed high sensitivity and specificity and were more sensitive than culture.

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1. Introduction

Group A *Streptococcus* (GAS) is a common and important cause of pharyngitis. In more developed countries, it is estimated that approximately 15% of school-age children experience a symptomatic episode of GAS culture positive pharyngitis each year and that in less developed countries the incidence may be 5–10 times higher, leading to an estimated 616 million incident cases of GAS pharyngitis globally each year (Carapetis et al., 2005). Accurate and timely diagnosis of GAS pharyngitis is crucial to prevent complications such as acute rheumatic fever (ARF), as well as to relieve clinical symptoms and decrease risk of suppurative complications (American Academy of Pediatrics (AAP), 2018). Because of an overlap in symptoms between GAS and viral causes of pharyngitis, laboratory confirmation of the diagnosis of GAS pharyngitis is generally recommended (Zühlke et al., 2017).

Culture has been traditionally considered the gold standard for diagnosis but it is time consuming and rapid diagnosis of GAS is highly recommended in order to effectively treat patients (AAP, 2018). This has led to the use of a variety of microbiological methods for diagnosis including rapid antigen tests and nucleic acid amplification tests (NAAT) (Zühlke et al., 2017). Although rapid antigen detection tests (RADTs) are relatively inexpensive, fast, and have a high specificity, due to their lower sensitivity negative results do not reliably exclude infection (Dingle et al., 2014). This limitation has led to the development of several commercial molecular methods for GAS detection that provide more rapid results than culture but with a higher sensitivity than RADTs (Uphoff et al., 2016; Zühlke et al., 2017). This higher sensitivity appears to be clinically important, since negative RADTs sometimes are found in patients with serious complications of GAS such as peritonsillar abscesses and ARF (Dingle et al., 2014).

We therefore elected to compare three of commercial molecular methods: the Quidel Solana GAS assay, the Luminex Aries Group A

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Strep assay and the Focus Diagnostics Simplexa Group A Strep Direct assay to conventional culture detection to determine the clinical performance of the molecular tests. Of note, no previous publications have evaluated the Aries GAS assay and only limited data has been published for the other two assays.

2. Material and methods

2.1. Study site and ethics

This study was conducted at the EORLA Regional Microbiology Reference Laboratory at The Ottawa Hospital, Ottawa, and the Children's Hospital of Eastern Ontario, ON, Canada, which serve a catchment area of 1.5 million people. This study was approved by the Children's Hospital of Eastern Ontario REB (Research Ethics Board) as study number 18/45X. This study used the remainder of flocked swab specimen fluid that would have otherwise been discarded; therefore individual patient consent was not required by the REB. Patient age was recorded.

2.2. Specimen collection

Flocked swab pharyngeal specimens (BD Eswabs, BD Canada, Oakville, ON) were collected in liquid media. These swabs were set up for routine bacterial culture and the remainder of the swab fluid was then frozen at −80 °C until used for molecular methods.

2.3. Statistical analysis

We hypothesized that the molecular methods being investigated would have higher sensitivity than bacterial culture, based on previous studies conducted (Uphoff et al., 2016). Therefore a true positive result was defined as one that was positive for GAS by culture or positive for GAS by ≥2/3 molecular methods used. A true negative result was defined as one negative for GAS by culture and by ≥2/3 molecular assays. (This consensus approach to defining true positives based upon the results of the majority of assays has been used in previous comparisons of molecular tests (Binnicker et al., 2017). Test performance characteristics and 95% confidence intervals (CI) were calculated using the MedCalc Diagnostic test evaluation calculator (https://www.medcalc.org/calc/diagnostic_test.php). The McNemar test, a statistical method used to compare results for paired samples, was used to analyze differences between the molecular assays and bacterial culture methods (Liao and Lin, 2008).

2.4. GAS culture

For culture of GAS, flocked swabs were processed on the Kiestra Total Laboratory system (BD Canada) by inoculating 10 μL of the liquid transport media on blood agar plates that were then incubated in anaerobic conditions at 35 °C. Plates were imaged and processed after 24 hours of incubation. Beta-hemolytic colonies were identified using antigen detection testing (Remel™ PathoDx, ThermoFisher Scientific, Nepean, ON) and Matrix Assisted Laser Desorption Ionization Time of Flight Mass Scpectoscopy(MALDI-ToF MS) with a Bruker Biotyper instrument (Bruker Daltonics, Billerica, MA).

2.5. GAS molecular assay methods

All molecular methods were performed according to the manufacturer's instructions. Among the molecular systems being investigated, the technology of the Solana is unique in that it utilizes (isothermal) helicase dependent amplification (HDA) while both the Aries and the Simplexa assay use the polymerase chain reaction (PCR). In addition, the Solana required a brief nucleic acid (NA) extraction step prior to the amplification reaction, while the Simplexa and Aries assays were performed directly on specimens.

Briefly, for the Solana method, 50 μL of specimen collected by ESwab is vortexed, added to the Lysis buffer and then incubated at 95 °C for 5 minutes. Once the specimen is lysed, 50 μL of the Lysis Buffer is added to the Elution buffer; 50 μL of the elution buffer is then added to the reaction tubes containing the lyophilized HDA reagents including the DNase B sequence specific primers and probes. The reaction tubes are placed in the instrument and the amplification reaction takes approximately 30 minutes to run. Once the reaction is complete, the results are presented as GAS detected or not detected, without cycle threshold values, by the instrument's software. A competitive process control (PRC) is included in the lysis tube to monitor specimen processing, inhibitory substances, reagent failure and instrument failure. The total procedure time was approximately 40 minutes.

For the Simplexa, 50 μL of the specimen is placed in the specimen well and 50 μL of the thawed assay is placed in the reagent well of a Direct Amplification Disc. Within the assay, bi-functional fluorescent probe-primers are used with corresponding reverse primers to amplify the *Streptococcal pyrogenic* exotoxin B gene. The Disc is loaded onto the 3 M Integrated Cycler and the reaction is then run, taking approximately 70 minutes. Once the run is complete, the results (detected/not detected) and the corresponding cycle threshold values are presented. A DNA internal control (IC) was used to detect PCR failure and/inhibition.

For the Aries method, 200 μL of the specimen is loaded into one cassette. All the required reagents for the extraction, purification and amplification of nucleic acids are present within the cartridges, and include a Specimen Processing Control (SPC) to ensure valid results are obtained. The GAS amplification target is proprietary. Up to 6 cassettes can be loaded onto a magazine, and 12 specimens (2 magazines) can be run in one instrument. Results are presented as GAS detected or not detected by the SYNCT software after a run time of approximately 2 hours.

3. Results

3.1. Specimen overview

In total, 287 throat swab specimens were collected for the study. One gave repeatedly invalid results on the Aries assay, and was therefore excluded from the study analysis as we required valid results for all three molecular methods and culture to assign specimens as true positive. Two hundred eighty-six specimen results were therefore included in the analysis, 206 from children, and 80 from adults (>18 years of age). The median patient age was 8 years. Results of the performance of culture and of each molecular assay compared to the reference standard are shown in Tables 1–4. Of the 286 specimens, 249 gave concordant results for all 4 tests (GAS positive with all 4 tests or GAS negative for all 4 tests) and 37 gave discordant results. Results for all discordant specimens are shown in Table 5.

3.2. GAS culture results

GAS grew in culture in 117 specimens, and Group C and G streptococci in 10 specimens. Culture failed to detect GAS in 21 specimens that were defined as true positives by the definition used (Table 1). The sensitivity of culture was thus 84.8% (95% CI 77.7–90.3%) with a

Table 1
Group A *Streptococcus* culture results as compared to the true positive definition*.

		True positive		
		+	−	Totals
Culture	+	117	0	117
	−	21	148	169
	Totals	138	148	286

* a true positive result was defined as one that was positive for GAS by culture or positive for GAS by ≥2/3 molecular methods.

Table 2
Quidel Solana GAS assay results as compared to the true positive definition*.

		True positive		
		+	−	Totals
Solana	+	130	2	132
	−	8	146	154
	Totals	138	148	286

* a true positive result was defined as one that was positive for GAS by culture or positive for GAS by ≥2/3 molecular methods.

specificity of 100% (95% CI 97.5–100%). The accuracy of culture was 92.7% (95% CI 89.0–95.4%). By the McNemar test, the difference between culture detection and the true positive definition was statistically significant ($P = 0.0001$).

3.3. Solana results

No invalid results were noted with the Solana assay. As seen in Table 2, the Solana assay did not detect 8 true positive GAS specimens, and additionally two false positive specimen results were observed, one of which grew group G streptococci in culture. The sensitivity of the Solana assay was 94.2% (95% CI 88.9–97.5%) and the specificity was 98.7% (95% CI 95.2–99.8%). The accuracy of the assay was 96.5% (95% CI 93.7–98.3%). By the McNemar test, the differences between the Solana assay and the true positive results were not statistically significant ($P = 0.1138$).

3.4. Simplexa results

No invalid results were observed with the Simplexa assay. This assay failed to detect only 1 specimen that was considered a true positive (Table 3). Seven specimens were considered as false positives with this method for a sensitivity of 99.3% (95% CI 96.0–99.9%) and the specificity was 95.3% (95% CI 90.6–98.1%). The accuracy of the assay was 97.2% (95% CI 94.6–98.8%). No group C or G culture positive specimens were positive for GAS with the Simplexa assay. The difference between the Simplexa assay and the true positive results was not statistically significant ($P = 0.0771$).

3.5. Aries results

With the Aries assay, five specimens of 286 (1.75%) gave invalid results. Four of these 5 gave valid results on re-testing. One specimen gave an invalid result on re-testing and was excluded from analysis as noted above. As shown in Table 4, the Aries did not detect 5 true positives, and additionally had 3 false positive results, for a sensitivity of 96.4% (95% CI 91.8–98.8%) and specificity of 98.0% (95% CI 94.2–99.6%). The accuracy of the assay was 97.2% (95% CI 94.5–98.8%). No group C or G culture positive specimens were positive for GAS with the Aries assay. According to the McNemar test, the difference between the Aries results and the reference standard results was not statistically significant ($P = 0.7237$).

Table 3
Focus Diagnostics Simplexa Group A Strep Direct assay results as compared to the true positive definition*.

		True positive		
		+	−	Totals
Simplexa	+	137	7	144
	−	1	141	142
	Totals	138	148	286

* A true positive result was defined as one that was positive for GAS by culture or positive for GAS by ≥2/3 molecular methods.

Table 4
Luminex Aries Group A Strep assay results as compared to the true positive definition*.

		True Positive		
		+	−	Totals
Aries	+	133	3	136
	−	5	145	150
	Totals	138	148	286

* a true positive result was defined as one that was positive for GAS by culture or positive for GAS by ≥2/3 molecular methods.

4. Discussion

Culture and molecular methods have varying advantages and disadvantages over one another. Although culture can take up to 24 hours, it allows for subsequent susceptibility testing, while molecular methods do not. However, susceptibility testing is not routinely performed for GAS at our laboratory unless the patient has a beta-lactam allergy. Culture also detects other pathogens that cause symptoms of pharyngitis like group C and G *Streptococcus* (*S. dysgalactiae*).

Each molecular method evaluated varies from one another, and, depending on the molecular laboratory implementing the technology, some methods will seem more favorable than others. The Solana is the only method that relies on isothermal HDA technology to detect GAS and use of this method may in theory reduce device costs as thermocycling is not required. In addition, the Solana's run time was shorter than that of the Simplexa and the Aries. However, the Solana is not a specimen to answer method, as it requires a separate specimen nucleic acid extraction step. This step requires three solution transfers: from the specimen to the lysis buffer, to the elution buffer and finally to the reaction tube. (To our knowledge, these steps must be performed manually as we are unaware of any automation platform that can be linked to the Solana device.) Even with these manual steps, the Solana has the quickest turnaround time in this study, taking approximately 35–40 minutes for a single specimen and approximately 1 hour for a full batch of 12 specimens, including the hands-on specimen preparation steps and the 30 minute amplification step (Table 6). For a single specimen, the Simplexa takes just over an hour, and slightly longer for a full batch of 8 specimens as each specimen and the master mix for that specimen must be added to the disk. The Aries takes less than 1 minute for specimen preparation for a single specimen since the specimen is directly placed in the cartridge, and takes 2 hours in the instrument. Batch runs for a full batch of 12 specimens takes slightly longer to prepare, as shown in Table 6. An advantage of the Aries system is that Aries cartridges could be stored at room temperature, allowing for more space in freezers and fridges, whereas the Solana assays must be stored at 4 °C, and the Simplexa assays at −20 °C.

Compared to culture, all three molecular methods investigated provide a much faster turnaround time. This more rapid identification of the pathogen could aid the physician in the choice of treatment and will possibly reduce the administration of unnecessary antibiotics (Tenover, 2010).

Only a few studies have previously investigated the performance of the assays we evaluated in this study. For the Aries assay, a recent

Table 5
Discordant specimen results by group A *Streptococcus* test method.

Culture	Solana	Aries	Simplexa	Number of specimens
Positive	Positive	Negative	Positive	3
Positive	Negative	Negative	Positive	1
Negative	Positive	Positive	Positive	12
Negative	Negative	Positive	Positive	7
Negative	Negative	Negative	Positive	7
Negative	Negative	Positive	Negative	3
Negative	Positive	Negative	Negative	2
Negative	Positive	Negative	Positive	1
Negative	Positive	Positive	Negative	1

Table 6
Comparison of the characteristics of the four testing methods used.

Method	Number of specimens per run (batch size)	Approximate turnaround time for single specimen	Approximate turnaround time for full batch of specimens	Amplification Method	Relative Cost
Culture	1	24 hours	NA	NA	$
Solana	1–12	40 minutes	60 minutes	HDA	$$
Simplexa	1–8	70 minutes	80 minutes	PCR	$$
Aries	1–12	120 minutes	130 minutes	PCR	$$

Abbreviations: HDA- helicase dependent amplification, PCR-polymerase chain reaction.

multicenter evaluation reported a sensitivity of 97.5% and specificity of 97.8% (Kanwar et al., 2019). These figures were very similar to our findings of 96.4% sensitivity and specificity of 98.0% for the Aries test. For the Solana assay, one multicenter study reported a 98.2% sensitivity and 97.2% specificity (Uphoff et al., 2016), figures that were similar to our findings of 94.2% sensitivity and 98.7% specificity. The rate of detection by culture was 20.7% compared to 22.6% by the Solana method. A second study evaluated the Solana only on throat swab specimens that were negative by RADTs and reported a sensitivity of 91.4%, and specificity of 98.5% in that setting (Arbefeville et al., 2018). For the Simplexa assay, one study reported a sensitivity of 100% and specificity also of 100% in comparison to the Hologic Group A Streptococcal Direct Assay (Church et al., 2018). This compares to the sensitivity of 99.3% and specificity of 95.3% for this assay that we observed.

There are several limitations of our study that should be noted. First, for logistic reasons, we performed the study using previously frozen specimens rather than fresh liquid specimens, as would be done in clinical use. The effect of freezing and thawing on our results is not known. Freezing and thawing could potentially lead to DNA damage that might lower the sensitivity of the assays (Suomalainen et al., 2006)). Alternatively freezing and thawing has also been shown to mitigate the effect of PCR inhibitors in some studies (Toye et al., 1998). However since specimens were frozen and thawed for each molecular method, the performance of each method should have been affected to the same degree. Secondly, we did not use a GAS selective agar for culture, which may have reduced the sensitivity of culture in our study. Such media have been shown to increase the ability to detect GAS relative to the standard BA plate we used (Mirrett et al., 1987). Thirdly, the GAS culture prevalence is out study of 40.7% was higher than typically reported. Specimens were collected prospectively for this study, so this higher prevalence of GAS was unexpected. This higher prevalence may limit the generalizability of our findings to settings where lower GAS rates are seen. Forth, as with all NAAT molecular methods, the detection of target DNA could always be due to the presence of non-viable rather than live organisms. Fifth, the molecular tests used are only approved for GAS throat swab detection, and thus cannot be used to replace GAS culture from other sites such as wounds. Finally, a potential limitation of the tests studied was that they do not detect other pharyngitis pathogens such as group C and G streptococci. However, these streptococci do not lead to ARF, and are therefore less clinically important. Of note, molecular testing for group C and G, as well as group A streptococci, is offered on the Solana platform (the Solana Strep Complete Assay), so laboratories wishing to use molecular testing for GAS pharyngitis that prefer to also continue testing for group C and G streptococci may be interested in evaluating this assay.

5. Conclusions

In summary, all the molecular methods had significantly higher sensitivities than culture and much faster turnaround times for GAS detection. In terms of performance, all three assays had high sensitivity and specificity. However, depending on each laboratory's requirements, one molecular method may be more favorable to implement than the others, based on the assay workflow and throughput required.

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Data availability

Data available upon reasonable request.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Competing interests

None declared.

Ethical approval

This study was approved by the Children's Hospital of Eastern Ontario REB (Research Ethics Board), study number 18/45X.

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