

Performance Evaluation of the STANDARD F Strep A Ag FIA for the Diagnosis of Group A Streptococcal Pharyngitis

Seon A Jo¹, Sang Hyuk Ma², Seungjun Lee³, Sunjoo Kim^{3,4*}

¹Department of Laboratory Medicine, Daewoo General Hospital, Geoje, Korea

²Department of Pediatrics, Changwon Fatima Hospital, Changwon, Korea

³Department of Laboratory Medicine, Gyeongsang National University Changwon Hospital, Changwon, Korea

⁴Gyeongsang Institute of Health Sciences, Gyeongsang National University, Jinju, Korea

*Corresponding author: Sunjoo Kim, sjkim8329@hanmail.net

Copyright: © 2019 Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), permitting distribution and reproduction in any medium, provided the original work is cited.

Abstract

Background: Pharyngitis is one of the most common conditions encountered in primary health care facilities. Accurate differentiation of group A streptococcus (GAS) infection from viral infection is difficult. The STANDARD F Strep A Ag FIA (SD BIOSENSOR, Korea) is a rapid antigen detection test (RADT) that has been recently developed for diagnosing GAS pharyngitis. In this study, we evaluated the diagnostic performance of the STANDARD F Strep A Ag FIA and compared the results between the RADT and conventional throat culture. **Methods:** Throat swab samples were obtained from a total of 372 children presenting pharyngitis symptoms in five pediatric clinics in Changwon, Korea from July 2018 to October 2019. A comparative study between STANDARD F Strep A Ag FIA and Sofia Strep A FIA (Quidel, USA) was performed. Two throat swabs were taken simultaneously from each patient for RADT. The third throat swab was stored in a transport tube containing Stuart's transport medium for culture. Performance and kappa index of STANDARD F Strep A Ag FIA were evaluated. **Results:** GAS infection was detected in 29.3% (109/372) patients, using the STANDARD F Strep A Ag FIA. The sensitivity, specificity, positive predictive value, and negative predictive value were 95.0%, 95.2%, 88.1%, and 98.1%, respectively. The STANDARD F Strep A Ag FIA showed an excellent concordance rate of 96.5% and a kappa value of 0.89 compared to Sofia Strep A FIA. **Conclusion:** The STANDARD F Strep A Ag FIA demonstrated an excellent performance along with Sofia Strep A FIA for the diagnosis of GAS pharyngitis.

Keywords

Group A streptococcus
Pharyngitis
Rapid antigen test
Streptococcus pyogenes
Tonsillitis

1. Introduction

Pharyngitis is one of the most commonly encountered conditions in family medicine and pediatrics and is mainly caused by viral infections such as rhinoviruses, with approximately 10%–20% of cases being due to bacterial infections [1-3]. Group A streptococci (GAS) are the most common cause of bacterial pharyngitis, especially in children aged 5–15 years [4,5], and are associated with 20%–30% of pediatric pharyngitis and 5%–15% of adult pharyngitis [2,6,7]. Although there are many studies on the diagnosis of GAS pharyngitis in children based on symptoms and physical examination findings, five symptoms (scarlet fever rash, petechiae on the palate, purulent exudate from the pharynx, vomiting, and cervical lymph node tenderness) are associated in more than 50% of cases, these findings alone do not provide a definitive diagnosis of GAS pharyngitis [4]. As pharyngitis caused by GAS is more severe than viral pharyngitis and can lead to purulent (peritonsillar abscess, retropharyngeal abscess, lymphadenitis) and non-purulent (acute glomerulonephritis, rheumatic fever) complications [8], accurate diagnosis and antibiotic treatment are important [9]. Accurate and rapid diagnosis of GAS pharyngitis is important because it prevents unnecessary overuse of antibiotics for viral pharyngitis and ensures prompt administration of antibiotics for bacterial pharyngitis, which reduces the duration of symptoms, prevents complications and reduces contagion to others.

Traditional bacteriological culture is the standard test for identifying GAS [9], but it requires basic equipment in the microbiological laboratory and the skills of a skilled examiner, and it takes at least one to two days to identify the bacteria, so it is difficult to perform routinely in private clinics and small hospitals. In addition, the sensitivity of bacteriological culture is 90%–95% [10]. To overcome these disadvantages of bacteriological culture, rapid antigen tests have been developed with results available in less than 10 minutes, and new products are being developed to improve upon the initially low sensitivity [11-13].

To evaluate the performance of the STANDARD F Strep A Ag FIA (SDBIOSENSOR, Suwon, Korea) rapid antigen test developed by immunofluorescence in the diagnosis of GAS pharyngitis, the authors compared the results with traditional bacteriological culture, and the Sofia Strep A FIA (Quidel, San Diego, CA, USA) test, a conventional rapid antigen test, was performed simultaneously to assess the agreement of the results.

2. Materials and methods

2.1. Subject

A total of 372 patients with suspected GAS pharyngitis who visited five pediatric clinics in Changwon from July 2018 to October 2019 were included. Three specimens were collected from the tonsils of patients, and two specimens were immediately tested with the commercially available STANDARD F Strep A Ag FIA test and Sofia Strep A FIA test according to the manufacturer's instructions, and the other specimen was transported to Changwon National University Hospital for pharyngeal culture. Approval was obtained from the Institutional Review Board (IRB) of Changwon Kyungsang University Hospital before starting the study, and written informed consent was obtained from all participating subjects or their guardians (IRB No. GNUCH 2018-06-011).

2.2. Rapid antigen tests

STANDARD F Strep A Ag FIA test was performed according to the manufacturer's instructions. After adding 3 drops of extraction buffer 1 to a tube of extraction buffer 2, the surface of the tonsils was rubbed with a cotton swab, and the swab from which the sample was taken was placed in the tube and left for 1 minute, and then the swab was rubbed at least 10 times towards the floor and walls before being removed. A pipette was used to dispense 100 μ L of the sample into the test device and the results were read using the STANDARD F200 Analyzer (SDBIOSENSOR).

The Sofia Strep A FIA test was performed

according to the manufacturer's instructions. First, hold the bottle of reagent solution and break the glass bottle for the ampoule inside, shake the bottle five times to make sure the mixture is green, unscrew the lid, hold it vertically, and add about 6 drops of the mixture to the reagent tube to the marking line. The swab from which the sample was taken was placed in the reagent tube, mixed up and down, and left for 1 minute. The swab was removed and 100 μ L was dispensed by pipette into the test device and the results were read using the Sofia Fluorescence Immunoassay Device (Quidel).

2.3. Culture test

The swab from which the sample was taken was inoculated into a blood agar medium and colony counts were determined by semi-quantitative method. A bacitracin disc (0.04 U) was placed on the primary fraction and incubated for 16–18 hours in a 37°C incubator. Colonies showing complete hemolysis were observed and identified by MALDI-TOF MS (bioMérieux SA, Marcy L'Étoile, France) to determine if they were susceptible to bacitracin discs and to identify GAS.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Pearson's chi-square test was used to compare the results of the standard F Strep A Ag FIA test and oropharyngeal

culture, and a *P* value of less than 0.05 was defined as statistically significant, and sensitivity, specificity, positive predictive value, and negative predictive value were calculated accordingly. To evaluate the agreement between rapid antigen tests, the positive agreement rate, negative agreement rate, overall agreement rate, and Cohen's kappa were calculated and interpreted according to the values (0.01–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; 0.81–0.99, almost perfect agreement).

3. Results

3.1. STANDARD F Strep A Ag comparison between FIA and culture

Of the 372 specimens tested with both the STANDARD F Strep A Ag FIA and culture, 101 were positive for culture and 271 were negative; 109 were positive for the STANDARD F Strep A Ag FIA and 263 were negative (**Table 1**). For a total of 372 samples, the sensitivity of the STANDARD F Strep A Ag FIA test based on culture was 95.0% (95% CI, 88.8–98.4%), specificity was 95.2% (95% CI, 91.9–97.4%), positive predictive value was 88.1% (95% CI, 80.5–93.5%), and negative predictive value was 98.1% (95% CI, 95.6–99.4%). The age-specific positivity rate was highest in 8-year-olds at 47.8%, followed by 39.9% in 6-year-olds and 37.1% in 7-year-olds, with 57.8% of males and 42.2% of females (**Figure 1**).

Table 1. Clinical performance of STANDARD F Strep A Ag FIA and Sofia Strep A FIA compared to throat culture

Rapid antigen detection test	Results	Throat culture		Sensitivity (95% CI)	Specificity (95% CI)
		GAS positive	GAS negative		
STANDARD F Strep A Ag FIA	Positive	96	13	95% (88.8–98.4%)	95.2% (91.9–97.4%)
	Negative	5	258		
Sofia Strep A FIA	Positive	95	11	94.1% (87.5–97.8%)	95.9% (92.9–98.0%)
	Negative	6	260		

Abbreviations: GAS, group A streptococci; CI, confidence interval.

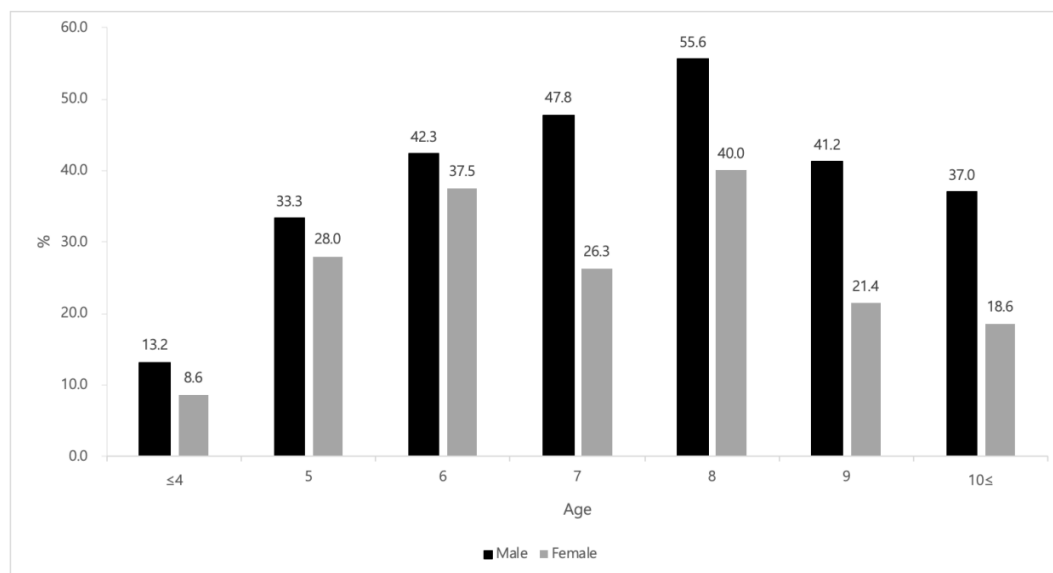


Figure 1. Distribution of age and sex of STANDARD F Strep A Ag FIA positive patients

3.2. Comparison of concordance between rapid antigen tests

The results of the STANDARD F Strep A Ag FIA test and the Sofia Strep A FIA test were combined to assess the concordance between the two devices. The positive concordance rate was 92.7% (95% CI, 86.1–96.8%), the negative concordance rate was 98.1% (95% CI, 95.6–99.4%), and the overall concordance rate was 96.5% (95% CI, 92.8–97.3%), with a Cohen's kappa value of 0.89 (95% CI, 0.84–0.94%).

3.3. Comparison of results of culture and two rapid antigen tests

When comparing the results of the culture and the two rapid tests, two specimens were positive for both the culture and the STANDARD F Strep A Ag FIA and negative for the Sofia Strep A FIA test only, and one specimen was positive for both the culture and the Sofia Strep AFIA test and negative for the STANDARD F Strep A Ag FIA test only. Four specimens were positive for the culture but negative for both rapid antigen results, 13 specimens were negative for the culture but positive for STANDARD F Strep A Ag FIA, and 11 specimens were positive for Sofia Strep A FIA.

In seven specimens, the culture was negative but both rapid antigen tests were positive.

4. Discussion

The GAS rapid antigen test is very useful in clinical practice because of its simple method and short test time (less than 10 minutes), which allows doctors to diagnose and prescribe bacterial pharyngitis without the need for multiple visits to the hospital. In addition, GAS is the most common cause of bacterial pharyngitis and is mostly sensitive to penicillin, so it can be diagnosed with a rapid antigen test without bacterial culture. However, if a patient with suspected bacterial pharyngitis has a negative GAS rapid antigen test or is refractory to penicillin and requires antimicrobial susceptibility testing, culture should be performed^[1].

Rapid antigen tests for the diagnosis of GAS pharyngitis have been developed since the 1980s and have gradually improved in performance, and the testing principles vary from immunological to molecular methods. The STANDARD F Strep A Ag FIA test evaluated in this study, a medical device that detects antigens of GAS in human throat specimens by immunochromatography and qualitatively detects them by fluorescence, showed

a high sensitivity (95%) compared with conventional throat culture, which was significantly higher than the sensitivity of rapid antigen tests (86%) reported in meta-analyses of other studies ^[3,14]. Concordance with another rapid antigen test, the Sofia Strep A FIA test, was 96.5% with a kappa of 0.89, indicating a 'near perfect agreement'. Therefore, the use of the STANDARD F Strep A Ag FIA test as a screening test in patients with suspected pharyngitis is likely to be useful in the diagnosis of bacterial pharyngitis.

The high specificity (95.2%) of the STANDARD F Strep A Ag FIA test may reduce unnecessary antimicrobial prescriptions for viral pharyngitis, which may be useful in reducing overall healthcare costs. An observational study of children with suspected pharyngitis found that empiric antimicrobials were prescribed in 80% of patients prior to the implementation of rapid antigen testing, whereas antimicrobials were prescribed in only 37% of patients when rapid antigen testing was performed and antimicrobials were prescribed based on the results, resulting in a 76% reduction in healthcare costs ^[15].

The modified Centor score (McIsaac) criteria used to guide treatment strategies for pharyngitis recommends neither rapid antigen testing nor antimicrobial therapy if the score is 0 or 1 (each worth 1 point; fever lasting 24 hours, severely inflamed tonsils, no cough or rhinorrhea, symptoms onset within 3 days, purulent tonsils), and recommends rapid antigen testing or culture to differentiate between bacterial and viral infection in patients aged 3–15 years for score 1 and for scores 2 and 3. In addition, empiric antibiotics may be considered for score 4 if the probability of GAS pharyngitis is greater than 50% ^[16,17]. The Infectious Diseases Society of America and the American Academy of Family Physicians recommend that when bacterial pharyngitis is suspected in children, a rapid antigen test should be performed first, and if the result is positive, antibacterial treatment should be initiated ^[7,18]. In Korea, GAS rapid antigen tests and modified Centor scores are not yet widely used in

practice, but as the culture of evidence-based practice takes hold and patient and societal awareness of antibiotic misuse increases, the voluntary demand for physicians and patients to perform rapid antigen tests in the management of patients with suspected pharyngitis is expected to gradually increase. Furthermore, the use of these tests in practice is part of an urgent need to improve the quality of care for accurate diagnosis and treatment, which requires improved clinician awareness of new tests and easy access to them. This may require test promotion and guideline development at the level of diagnostic laboratory societies, and active efforts to ensure that they are incorporated into departmental guidelines. On the other hand, rapid antigen tests require several caveats for clinical use. Firstly, rapid antigen tests alone cannot distinguish between carriers and infections of GAS, as can bacteriological culture. Asymptomatic carriers are positive for the rapid antigen test but do not require a prescription of antimicrobials, and the prevalence of asymptomatic group A carriage in normal school-aged children is approximately 10% ^[2]. It is also important to understand the various internal and external factors that affect the test. For example, differences in sensitivity between test devices and principles, technical proficiency of the examiner performing the test, specimen collection, transport, and storage, patient antimicrobial use, disease course, and prevalence of GAS pharyngitis ^[19-21]. In other words, discrepancies in results due to different rapid antigen tests or differences in test methods may need to be confirmed by molecular methods, and laboratories need to exclude interference from examiner factors by performing tests consistently according to guidelines. In addition, accurate specimen collection is the most important process that affects the test results, and it is necessary to rub the tonsil area relatively hard several times, and be careful not to touch the swab with other parts of the oral cavity. The sooner the specimen is transported to the laboratory, the better, and transport media should be used and the temperature maintained for prolonged transport. The specimen collector's

understanding of the test and knowledge of specimen collection and transport are often overlooked in practice but have a significant impact on the results [22]. The patient's condition is also important in interpreting test results, and culture results may be negative if the patient has been treated with antimicrobials. Although we were unable to analyze these factors in this study, it can be assumed that various factors contributed to the differences in the results of the three tests. In particular, since the swab is collected three times, the number of bacteria collected from each swab may vary.

In conclusion, the STANDARD F Strep A Ag FIA is a rapid antigen test for GAS pharyngitis with high sensitivity and specificity compared to the standard test, culture, and almost perfect agreement with the Sofia Strep A FIA rapid antigen test, which may be useful for rapid diagnosis and antimicrobial therapy decisions in pediatric pharyngitis patients with difficulty differentiating the cause based on symptoms alone. The authors encourage clinicians to use the GAS rapid antigen test when managing patients with pharyngitis.

Disclosure statement

The authors declare no conflict of interest.

Funding

This study was supported by the Investigator Clinical Trials Project (H19C0047) from the Korea Health Industry Promotion Agency, and we are grateful to the director of the Department of Paediatrics for his participation in the study.

References

- [1] Lean WL, Arnup S, Danchin M, et al., 2014, Rapid Diagnostic Tests for Group A Streptococcal Pharyngitis: A Meta-Analysis. *Pediatrics*, 134: 771–781.
- [2] Wessels MR, 2011, Clinical Practice. Streptococcal pharyngitis. *N Engl J Med*, 364: 648–655.
- [3] Cohen JF, Bertille N, Cohen R, et al., 2016, Rapid Antigen Detection Test for Group A Streptococcus in Children with Pharyngitis. *Cochrane Database Syst Rev*, 7: CD010502.
- [4] Shaikh N, Swaminathan N, Hooper EG, 2012, Accuracy and Precision of the Signs and Symptoms of Streptococcal Pharyngitis in Children: A Systematic Review. *J Pediatr*, 160: 487–493.
- [5] Vazquez MN, Sanders JE, 2017, Diagnosis and Management of Group A Streptococcal Pharyngitis and Associated Complications. *Pediatr Emerg Med Pract*, 14: 1–20.
- [6] Ebell MH, Smith MA, Barry HC, et al., 2000, The Rational Clinical Examination. Does This Patient Have Strep Throat? *JAMA*, 284: 2912–2918.
- [7] Kalra MG, Higgins KE, Perez ED, 2016, Common Questions about Streptococcal Pharyngitis. *Am Fam Physician*, 94: 24–31.
- [8] Del Mar CB, Glasziou PP, Spinks AB, 2006, Antibiotics for Sore Throat. *Cochrane Database Syst Rev*, 4: CD000023.
- [9] Bisno AL, 2001, Acute Pharyngitis. *N Engl J Med*, 344: 205–211.

- [10] Giesecke KE, Mackenzie T, Roe MH, et al., 2002, Comparison of Two Rapid Streptococcus Pyogenes Diagnostic Tests with a Rigorous Culture Standard. *Pediatr Infect Dis J*, 21: 922–927.
- [11] Gerber MA, Shulman ST, 2004, Rapid Diagnosis of Pharyngitis Caused by Group A Streptococci. *Clin Microbiol Rev*, 17: 571–580.
- [12] Fox JW, Marcon MJ, Bonsu BK, 2006, Diagnosis of Streptococcal Pharyngitis by Detection of Streptococcus Pyogenes in Posterior Pharyngeal Versus Oral Cavity Specimens. *J Clin Microbiol*, 44: 2593–2594.
- [13] Kim HN, Kim J, Jang WS, et al., 2019, Performance Evaluation of Three Rapid Antigen Tests for the Diagnosis of Group A Streptococci. *BMJ Open*, 9: e025438.
- [14] Ma SH, Choi WH, Park HW, et al., 2019, Evaluation of the SD Bioline Strep A Ultra Test in Relation with Number of Colony Forming Units and Color Intensity. *Ann Lab Med*, 39: 31–35.
- [15] Kose E, Sirin Kose S, Akca D, et al., 2016, The Effect of Rapid Antigen Detection Test on Antibiotic Prescription Decision of Clinicians and Reducing Antibiotic Costs in Children with Acute Pharyngitis. *J Trop Pediatr*, 62: 308–315.
- [16] Edmonson MB, Farwell KR, 2005, Relationship Between the Clinical Likelihood of Group A Streptococcal Pharyngitis and the Sensitivity of a Rapid Antigen-Detection Test in a Pediatric Practice. *Pediatrics*, 115: 280–285.
- [17] Randel A, 2013, IDSA Updates Guideline for Managing Group A Streptococcal Pharyngitis. *Am Fam Physician*, 88: 338–340.
- [18] Shulman ST, Bisno AL, Clegg HW, et al., 2012, Clinical Practice Guideline for the Diagnosis and Management of Group A Streptococcal Pharyngitis: 2012 Update by the Infectious Diseases Society of America. *Clin Infect Dis*, 55: 1279–1282.
- [19] Kucuk O, Bicer S, Giray T, et al., 2014, Validity of Rapid Antigen Detection Testing in Group A Beta-Hemolytic Streptococcal Tonsillopharyngitis. *Indian J Pediatr*, 81: 138–142.
- [20] Penney C, Porter R, O'Brien M, et al., 2016, Operator Influence on Blinded Diagnostic Accuracy of Point-of-Care Antigen Testing for Group A Streptococcal Pharyngitis. *Can J Infect Dis Med Microbiol*, 2016: 1710561.
- [21] Lacroix L, Cherkaoui A, Schaller D, et al., 2018, Improved Diagnostic Performance of an Immunofluorescence-Based Rapid Antigen Detection Test for Group A Streptococci in Children with Pharyngitis. *Pediatr Infect Dis J*, 37: 206–211.
- [22] Vakkila J, Koskinen JO, Brandt A, et al., 2015, Detection of Group A Streptococcus from Pharyngeal Swab Samples by Bacterial Culture is Challenged by a Novel mariPOC Point-of-Care Test. *J Clin Microbiol*, 53: 2079–2083.

Art & Technology Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.