

# *Performance Evaluation of Strep A Rapid Test Cassette for Detecting Strep A Antigens in Throat Swab Specimens*

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**Abstract:** Group A *Streptococcus* (GAS) is a bacterium that is commonly present in the throat and on the skin surfaces of humans, typically as a gram-positive organism. It is a commonly found pathogen that can cause a variety of infections and diseases. Timely diagnosis and appropriate treatment with antibiotics are crucial to prevent complications and further transmission of the bacteria. The study aimed to evaluate the diagnostic capacity of the Strep A Rapid Test in qualitatively identifying Strep A antigens through specific antibodies that target whole-cell Lancefield Group A *Streptococcus*. The test detects the existence of Strep A antigens in specimens obtained from throat swabs to aid in the diagnosis of Group A Streptococcal infection. 526 throat swabs from pharyngitis patients across three medical centers were gathered to gauge the sensitivity, specificity, and accuracy of the Strep A rapid test. The study findings revealed that the Strep A rapid test accurately identified 95.1% of the true positive cases and correctly detected 97.8% of the negative cases, yielding an overall accuracy of 97.1%. Among the 526 specimens examined, 404 tested negative, whereas 122 tested positive through culture-based testing, with one instance of a false positive result. Furthermore, cross-reactivity testing involving three distinct Strep F strains produced negative results, further confirming the specificity of the Strep A rapid test. This test developed by Hangzhou AllTest Biotech Co., Ltd has demonstrated excellent performance in detecting Strep A antigens in specimens collected from throat swabs, rendering it suitable for clinical application as an auxiliary diagnostic tool, particularly beneficial in resource-limited environments due to its high accuracy, reliability, and prompt results.

## 1. Introduction

Group A *Streptococcus* (GAS), also known as *Streptococcus pyogenes* or Group A beta-hemolytic *Streptococcus*, is a gram-positive bacterium responsible for causing a variety of diseases and associated complications.<sup>[1]</sup> It can lead to pharyngitis (infection of the throat), infections of the skin, as well as more severe complications like glomerulonephritis, sepsis, rheumatic heart disease, necrotizing fasciitis, and toxic shock syndrome.<sup>[2]</sup> According to statistical

data, GAS is responsible for approximately 20% to 40% of pharyngitis cases in children and 5% to 15% in adults.<sup>[3]</sup> In children aged 3 months to 5 years, approximately 15% of pharyngitis cases are attributed to Group A beta-hemolytic *Streptococcus*.

Pharyngitis can arise from a diverse array of viruses and bacteria, encompassing viral pathogens like the coronavirus, influenza virus, and Chlamydia, as well as bacterial pathogens such as Group A *Streptococcus*, Groups C and G *Streptococcus*, and *Corynebacterium diphtheriae*. Accordingly, the key objective in diagnosing and managing pharyngitis is to effectively differentiate between viral pharyngitis in children and Group A streptococcal pharyngitis. While antibiotics are efficacious against bacterial pharyngitis, they prove ineffective against viral infections. Given the rising concern of antimicrobial resistance, the unwarranted use of antibiotics can yield adverse consequences, underscoring the significance of accurate diagnosis in guiding appropriate treatment choices. The ability to make this distinction is crucial for reducing unnecessary antibiotic administration in pediatric patients. By ensuring a precise differentiation between viral and bacterial pharyngitis, healthcare practitioners can implement suitable treatment approaches while mitigating the risks associated with excessive antibiotic utilization in children.<sup>[4]</sup>

Throat culture-based diagnostic tests are generally considered the gold standard for detecting GAS.<sup>[5]</sup> However, these tests entail a time lag between sample collection and result acquisition, which may extend up to 48 hours.<sup>[6]</sup> In certain situations, it may be impractical for patients to revisit the clinic for treatment based on test results, or there is a risk of their symptoms worsening while awaiting the results. PCR tests, while susceptible to contamination and necessitating laboratory processing, also add to the overall turnaround time.

In order to tackle these challenges, the utilization of Point-of-Care (POC) rapid antigen detection tests (RADTs) has emerged as a potential solution for detecting Group A *Streptococcus* (GAS) in specimens from throat swabs collected at the clinic. RADTs offer the advantage of a shorter turnaround time, enabling more prompt diagnosis of GAS pharyngitis. The objective of this research was to evaluate the diagnostic efficacy of the immunochromatographic-based rapid test developed by Hangzhou AllTest Biotech Co., Ltd. in identifying Group A *Streptococcus*. In a diagnostic laboratory, throat swab samples obtained from individuals with specific pharyngitis were assessed using the rapid test. Subsequently, the obtained results were compared with culture findings to determine the assay's efficacy.

## 2. Materials and Methods

### 2.1 Sample Collection

526 throat swab specimens were collected from individuals presenting symptoms of pharyngitis, and the assessment was carried out across three medical facilities. Healthcare professionals should use the sterile swab provided in the kit for collecting the throat swab specimen. Alternatively, one can also use swabs containing modified Stuart's or Amies medium. During swabbing, healthcare professionals should focus on posterior pharynx, tonsils, and any inflamed areas, while refraining from contacting the tongue, cheeks, and teeth. It is advisable to conduct the testing promptly after specimen collection. After collection, swab specimens can be stored for up to 8 hours at room temperature or 72 hours at 2-8 °C. When a culture is required, it is recommended to lightly roll the swab tip on a Group A selective blood agar plate before utilizing it in the Strep A Rapid Test. This step helps facilitate the culture process for subsequent analysis.

### 2.2 Screen test

Before starting the test, it is crucial to ensure that the test cassette, reagents, specimen, and

controls have reached a temperature range of approximately 15-30 °C. To initiate the testing procedure, healthcare professionals should remove the test from its sealed foil pouch and ensure its use within one hour of opening. For optimal results, it is advisable to conduct the test promptly after unsealing the foil pouch. The test comprises particles coated with Strep A antibodies and Strep A antibodies that are affixed to the membrane. Subsequently, the healthcare professional should hold the Extraction Reagent 1 bottle in a vertical position and dispense 4 complete drops (approximately 240 µL) of the red-colored Extraction Reagent 1 into an extraction tube. Similarly, they should vertically hold the Extraction Reagent 2 bottle and add 4 complete drops (about 160 µL) of the colorless Extraction Reagent 2 into the same tube. Then, gently swirling the contents of the extraction tube will achieve homogeneity, leading to a color transition from red to yellow.

The swab should be promptly added into the extraction tube by the user without any delay, followed by vigorous agitation 15 times. It is important to allow the swab to remain in the extraction test tube for a duration of 1 minute. Afterward, the swab should be pressed against the side of the tube, and while removing the swab, the user should squeeze the bottom of the tube to ensure that most of the liquid remains within it. The used swab should be disposed of appropriately. Next, the user should attach the dropper tip to the extraction tube's top. The test should then be placed on a clean and flat surface. The user should carefully add three drops (approximately 100 µL) of the solution into the sample well and start the timer.

The Strep A Rapid Test is a lateral flow immunoassay used to qualitatively identify the Strep A carbohydrate antigen present in a throat swab specimen. Specific antibodies to the Strep A carbohydrate antigen are applied to the test line region of the test. As the test is conducted, the extracted throat swab specimen comes into contact with particles coated with Strep A antibodies. The mixture moves across the membrane, interacts with the antibodies specific to Strep A on the membrane, and generates a line of color in the designated test region. The color line's presence signifies a positive outcome, whereas its absence signifies a negative outcome. To function as a control, a line of color will consistently manifest in the control line area, validating the addition of the appropriate specimen volume and the occurrence of membrane wicking. The Strep A Rapid Test can be set up and completed within approximately 5 minutes. The results can be visually interpreted. However, it is important to note that the test results should not be interpreted after 10 minutes, as prolonged exposure to the test components may lead to inaccurate results.

This study employed GAS culture as the comparative method. 526 throat swabs were obtained from individuals presenting with pharyngitis symptoms. The swabs were subsequently streaked onto plates for isolation and incubated at a temperature of 37°C with 5-10% CO<sub>2</sub>. To aid in identification, a Bacitracin disk was added and the plates were incubated for a duration of 18-24 hours. The negative culture plates underwent an additional incubation period of 18-24 hours. Any potential colonies of GAS were subcultured and their identification was confirmed using a commercially available latex agglutination grouping kit.

### 2.3 Quality Control

The test includes internal procedural controls to ensure reliable results. Within the test, there is a control region (C) where a colored line appears. This line serves as an internal procedural control, indicating that the test has been conducted correctly. It confirms that there was an adequate volume of the specimen, the membrane absorbed the sample properly, and the procedural technique was performed correctly.

To ensure the accuracy and reliability of the test results, it is advisable to include both positive and negative external controls when using the kit. These controls are provided within the kit itself. Furthermore, if deemed essential as per the laboratory's internal protocols, external controls comprising different reference strains of Group A and non-Group A *Streptococcus* can be utilized.

### 3. Results and Discussion

#### 3.1 Results

A comprehensive assessment was conducted across three medical centers, gathering a total of 526 throat swabs from individuals presenting with pharyngitis symptoms. Each swab was carefully rolled onto a plate containing sheep blood agar, followed by testing with the Strep A Rapid Test Cassette (Throat Swab). The culture results revealed that out of the samples, 122 tested positive, while 404 tested negative.

As shown in Table 1, a total of 526 throat swab specimens were subjected to Strep A testing to compare the performance of rapid testing and culture. Among the 122 culture-positive samples, the rapid test identified 116 as positive, resulting in a relative sensitivity of 95.1%. Among the 404 culture-negative samples, the rapid test correctly classified 395 as negative, yielding a relative specificity of 97.8%. The overall accuracy of the rapid test was 97.1%. During the course of this study, one Strep F specimen exhibited positive results with the test. However, upon re-culturing and re-testing, it yielded a negative result. In addition, three distinct Strep F strains were cultured and tested for potential cross-reactivity, all of which produced negative results.

Table 1: Performance Characteristics of Strep A Rapid Test

Method		Culture		Total Results
Results		Positive	Negative	
Strep A Rapid Test Cassette	Positive	116	9	125
	Negative	6	395	401
Total Results		122	404	526

Relative Sensitivity: 95.1% (95% CI\*: 89.6%-98.2%);

Relative Specificity: 97.8% (95% CI\*: 95.8%-99%);

Accuracy: 97.1% (95% CI\*: 95.3%-98.4%);

\*: Confidence Interval.

From Table 2, it can be observed that a total of 122 samples tested positive for Strep A were categorized based on their growth level during culture, which ranged from "rare growth" to "4+ growth."<sup>[7]</sup> This classification was done to analyze the sensitivity of the Strep A Rapid Test. Among the Strep A positive samples categorized as "Rare growth," the test demonstrated a sensitivity of 80.0%. For samples categorized as "1+ growth," the sensitivity of the test was 90.0%. In cases where samples showed "2+ growth," the test exhibited a sensitivity of 95.0%. When samples reached "3+ growth" during culture, the test's sensitivity increased to 97.1%.

Table 2: Sensitivity Analysis of Strep A Rapid Test Based on Growth Level.

Positive Culture Classification	Strep A Rapid Test/Culture	% Agreement
Rare	8/10	80.0%
1+	18/20	90.0%
2+	19/20	95.0%
3+	33/34	97.1%
4+	38/38	100.0%

#### 3.2 Discussion

Distinguishing between viral pharyngitis and Group A Streptococcal (GAS) pharyngitis based solely on clinical symptoms presents a challenge. Thus, it becomes essential to perform a rapid antigen detection test (RADT) or a throat culture. The throat culture method is considered the gold standard, but it necessitates specialized laboratories, skilled personnel, and approximately 1-2 days for obtaining results. As acute pharyngitis is predominantly caused by viral infections, the

prescription of antibiotics without proper testing can result in unnecessary healthcare expenses and the misuse of antibiotics. It has been estimated that bacterial antimicrobial resistance (AMR) was directly accountable for 1.27 million deaths worldwide in 2019 and contributed to an additional 4.95 million deaths.<sup>[8]</sup> The utilization of RADT can contribute to the reduction of antibiotic resistance and facilitate appropriate patient management. Utilizing uncomplicated equipment or analyzers, RADT can yield easily interpretable outcomes in approximately 10-15 minutes, rendering it suitable for implementation in small hospitals or private clinics. Based on the aforementioned data, the performance of the Strep A Rapid Test demonstrates exceptional accuracy, specificity, and sensitivity. The AllTest Strep A Rapid Test does not necessitate a specific analyzer, and the results can be interpreted following the instructions provided by the manufacturer. By furnishing dependable and precise outcomes, this testing kit can aid healthcare professionals in making well-informed decisions, enabling timely intervention, and thereby contributing to the advancement of public health and safety.

It should be acknowledged that this test is specifically designed for the detection of Strep A antigen in throat swab specimens and may not be suitable for other sample types. Additionally, this test cannot distinguish between current infection and past exposure to Strep A. To address these limitations, further research and development may be necessary to enhance the test's specificity and its ability to differentiate between active infection and previous exposure. Moreover, efforts could be made to develop a quantitative test that provides information about Strep A antigen concentration, allowing for more precise clinical interpretations.

#### 4. Conclusion

The Strep A Rapid Test, manufactured by Hangzhou AllTest Biotech Co., Ltd, exhibits excellent sensitivity and specificity, delivering results within a span of 10 minutes. As a result, it proves to be an ideal primary diagnostic tool, particularly in scenarios where patients necessitate testing and treatment during a single consultation. Additionally, it can serve as a screening method in regions characterized by elevated infection rates or in settings where resources are limited. Nonetheless, additional investigations are warranted to ascertain the practicality and cost-effectiveness of the Strep A Rapid Test in these particular applications.

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