Direct Rapid Antigen Test on Presumptive Non-Isolated Beta Haemolytic Streptococcus Colonies on Throat Culture Plates

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Abstract

Group A Streptococcus is frequently associated with pharyngitis, which can have potential suppurative and non-suppurative sequelae, including acute rheumatic fever. Appropriate control of Group A Streptococcus by targeted antibiotic treatment is therefore vital in prevention of diseases such as acute rheumatic fever, which remains a significant health problem in indigenous populations of industrialized countries and in the developing world. Clinical diagnosis via assessment of symptoms using Centor criteria can be helpful in establishing whether or not pharyngitis is associated with Group A Streptococcus or not, but is usually not sufficient. This necessitates laboratory diagnosis to rule Group A Streptococcus out or in. The gold standard for laboratory diagnosis is culture of throat swabs on blood agar. However, in cases where no clear conclusion can be drawn, sub-culture and additional testing by latex agglutination, for example, is necessary. This further delays diagnosis by 24 hours and can contribute to commencement of inappropriate antibiotic treatment in advance of a definitive result. In this paper, we assessed the specificity, sensitivity and accuracy of using the TestPackPlus™Strep A rapid antigen test kit directly on beta hemolytic zone samples from 100 original culture plates from pharyngitis patients where Group A Streptococcus colonies were suspected but not isolated, in comparison to use of the Streptex rapid latex agglutination test (Thermo Scientific) on sub-cultured plate. Specificity of the modified direct rapid antigen test method was 0.95, sensitivity was 0.98 and diagnostic accuracy was 97%. The method also had 100% reproducibility. Thus we propose that this direct method should be adopted in the future to expedite positive identification of GAS and ensure timely application of appropriate antibiotic treatment, and also to rule out GAS in negative cases and thus help prevent unnecessary antibiotic prescription.

Keywords: Group A Streptococcus (GAS); Beta hemolytic zone; Pharyngitis; Rapid antigen test; Sensitivity; Specificity

Abbreviations

ELISA: Enzyme-linked immunoassay; FISH: Fluorescent in situ hybridization; GAS: Group A Streptococcus; PCR: Polymerase chain reaction; RAT: Rapid antigen test

Introduction

Group A Streptococcus (GAS; Streptococcus pyogenes) is one of the most significant human pathogens, associated with acute pharyngitis and tonsillitis, as well as other diseases including impetigo and scarlet fever, with a particularly strong impact in settings where resources are limited [1]. Meta-analysis estimates suggest that 37% of sore throats in children are a result of GAS infection [2]. GAS pharyngitis is associated with high financial costs and with potential suppurative and non-suppurative sequelae, including acute rheumatic fever [1,3,4]. Thus prevention strategies for diseases such as acute rheumatic fever is dependent upon effective control of GAS pharyngitis [3,5]. Some subtle differences in symptoms can help to suggest a GAS versus a viral cause of sore throat. These differences inform the Centorscore, which has been modified since its introduction in 1981, and uses up to four clinical criteria to suggest a likely GAS cause for sore throat. These are tonsillar exudate, high fever, lack of cough and swollen, tender anterior cervical nodes [6-8]. Factors
such as the winter season, acute onset, headache, and abdominal pain also support the diagnosis of GAS pharyngitis. However, clinical examination to assess symptoms is not usually sufficient to distinguish between viral and GAS pharyngitis, and individual signs and symptoms, while they may be of moderate use, are not enough to rule out GAS pharyngitis [9]. Even when all four Centor criteria are present, the estimated risk of GAS infection being present is 51-53% [3,8]. Thus, a diagnostic test is recommended to confirm GAS presence and help inform appropriate antibiotic use [9].

In most countries, a diagnostic test based on a throat swab is recommended. However, guidelines vary widely between countries and differ both in the choice of evidence used to inform the guidelines and in how they are interpreted for clinical practice [3,8,10,11]. Bacterial culture from throat swabs on blood agar plates is considered the diagnostic gold standard [12]. However, there is some debate concerning the major disadvantage of carrying out throat swab cultures, i.e. the delay in obtaining results to inform antibiotic prescribing. This in turn can lead to commencement of sometimes unnecessary treatment in advance of results being available [3,13]. The normal bacterial microbiota can also obscure GAS presence or impede its isolation, resulting in false-negative results. Additionally, delays in reporting can be caused by attempting to re-isolate the organism.

In many laboratories, GAS is identified by rapid antigen tests (RAT) of various types, including latex agglutination tests, enzyme-linked immunoassay (ELISA), optical immunoassay, polymerase chain reaction (PCR), fluorescent in situ hybridization (FISH) or chemiluminescent DNA probes [8,12,13]. All are based on extraction of the group-specific carbohydrate antigen from the cell wall and its immunological identification [13]. While in some places, including the USA and Europe, RATs have been incorporated into GAS identification clinical practice guidelines, other countries such as Australia do not routinely include them [3,8,13]. While they are fast and easy to perform, do not require any special equipment, and give objective results, there is also variability in assay sensitivity between studies, and in the diagnostic accuracy between different RAT methodologies [8]. In a recent meta-analysis it was estimated that overall RATs have a sensitivity of 0.86 and specificity of 0.96, however there was substantial variability between methodologies. [8].

In blood culture, typical colonies of GAS appear small, white, and convex surrounded by a zone of beta-hemolysis after 24-48 hours of incubation [14]. Identity can be confirmed, for example, by using a streptococcal grouping latex agglutination RAT kit for the rapid identification of Lancefield streptococcal groups A, B, C, D, F, and G from primary culture plates [15]. However, overgrowth of normal upper respiratory tract microbiota can obscure the presence of GAS, making its identification a real challenge [16]. Thus one recommended solution is to subculture the suspected non isolated beta hemolytic colony into new blood agar plate and carry out latex agglutination RAT on the sub-culture plate colonies. However, this method requires a well-trained technologist and has the major drawback of delaying the report for least 24 hours [17]. This may increase the possibility of developing streptococcal sequelae, such as glomerulonephritis, rheumatic fever, and rheumatic heart disease and contributes to over-prescription of antibiotics, as further delay in diagnosis increases the likelihood that antibiotic treatment will have commenced in advance of the results being available [3,8]. Another recommended method is nitrous acid extraction method [18], however this method is costly, time consuming, and the isolation of the antigenic groups from the streptococcal bacteria might be disrupted by the presence of normal oral flora.

In this study, we propose that an alternative to further subculture where GAS is suspected would be direct application of RAT to the beta-hemolytic zone of initial throat swab culture plates. To this end, we compared the performance in terms of specificity, sensitivity and accuracy, of the TestPackPlus™Strep A kit directly on beta hemolytic zones samples from throat swab cultures from patients presenting with pharyngitis symptoms, to latex agglutination testing of sub-culture plates.

**Materials and Methods**

We collected beta hemolytic zones samples from throat swab cultures. Swabs were originally collected from adults and children with pharyngitis symptoms, inoculated within 30 mins on to 5% sheep blood agar plates and cultured at 37°C and 5% CO₂ for 18-24 h. 100 beta hemolytic zone samples, consisting of a mixture of suspected GAS and normal oral flora, were collected from culture plates where presence of GAS colonies was suspected but not isolated.

Beta hemolytic zone samples were subjected to direct RAT testing with the TestPackPlus™Strep A kit (Abbott Laboratories), according to the manufacturer’s instructions, as originally designed for direct identification from throat swabs. As the TestPackPlus™Strep A kit was designed to identify GAS from throat swabs that are normally carrying multiple organisms, we did not expect that there would be cross-reactivity or interference from normal oral flora from within the sample. The test allows detection of the GABHS antigen via an ELISA antigen-AC agglutination reaction.

In parallel, the beta hemolytic zone samples were sub-cultured on to fresh blood agar plates according to our current conventional method and incubated at 37°C and 5% CO₂ for 24 h, followed by serotyping by Streptex rapid latex agglutination test (Thermo Scientific, Remel Microbiology products). This test allows isolation and differentiation of Lancefield groups A, B, C, F and G, by acid extraction followed by grouping, according to the manufacturer’s instructions, and catalase and coagulase test for all creamy beta hemolytic colonies that were suspected to be Staphylococcus species.

The performance of the TestPackPlus™Strep A kit method on non-isolated presumptive GAS was compared to that of the conventional sub-culturing assay in a prospective evaluation. Sensitivity, specificity and accuracy of the direct RAT method were calculated by comparison to the conventional sub-culturing assay via a 2x2 contingency table.

To confirm the specificity of the direct test with the TestPackPlus™Strep A kit, organisms that are likely to be part of respiratory microbiota were tested at 107 organisms per ml using appropriate American Type Culture Collection (ATCC) strains. 2 tubes of thioglycolate medium were spiked by Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (27853), Neiserelalactamica (ATCC 23970), Staphylococcus aureus (25923), Streptococcus Group B (ATCC 12386), Streptococcus Group C (ATCC 12388), Streptococcus Group F (ATCC 12392), Streptococcus Group G (ATCC 12394), and Haemophilus influenza (ATCC 25238). The second thioglycolate tube was additionally spiked with the control strain for Streptococcus Group A (ATCC 19615). Five swabs were inserted consecutively in the first tube and five swabs were inserted consecutively in the second tube, and TestPackPlus™StrepA kit RAT was applied to each swab sample.

Reproducibility of TestPackPlus™Strep A kit RAT as a candidate tool for GAS identification directly from the hemolytic zone of plates was examined using five samples with non-isolated beta hemolytic species which tested negative for GAS, and five samples with non-isolated beta hemolytic species which tested positive. These ten samples were tested for three consecutive days by three different technologists to determine whether the RAT results obtained by each technologist agreed with the expected results.

**Results and Discussion**

We directly tested 100 beta-hemolytic zone samples on blood agar culture plates from throat swabs of pharyngitis patients for presence of GAS, using the TestPackPlus™Strep A RAT kit. We compared the results to those obtained by our conventional sub-culturing assay, in which sub-cultured samples were tested by latex agglutination. Of the 100 samples, 57 tested positive for both methods and 40 tested negative for both methods.

<table>
<thead>
<tr>
<th></th>
<th>RAT directly from the original plate (+)</th>
<th>RAT directly from the original plate (-)</th>
<th>Total</th>
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<tbody>
<tr>
<td>Streptex test from subcultured plate (+)*</td>
<td>57</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Streptex test from subcultured plate (-)*</td>
<td>2</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>41</td>
<td>100</td>
</tr>
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*either positive or negative for GAS

**Table 1:** 2x2 contingency table comparing GAS identification by TestPackPlus™Strep A kit on the original plate beta hemolytic zones versus Streptex latex agglutination on the subcultured plates.

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Two samples that tested negative on the latex agglutination test on the sub cultured plate tested positive by the TestPackPlus™Strep A kit on the original plate, while one sample that tested positive on the sub cultured plate tested negative on the original plate (Table 1). Thus the specificity of the TestPackPlus™Strep A kit used directly on the sub cultured plate method was 0.95 (40/42) and the sensitivity was 0.98 (57/58), while the accuracy was 97% (97/100) (Table 1). Among the 40 samples that were tested negative by both methods, 20 were found to be hemolytic S. aureus (Catalase positive, coagulase positive), seven were S. coagulase negative (Catalase positive, coagulase negative), ten were beta hemolytic Strep to coccus Group C (Streptex test kit), and three were beta hemolytic Streptococcus group G (Streptex test kit).

For confirmation of specificity of the TestPackPlus™Strep A kit, five swabs from a tube spiked with ATCC bacterial strains other than control strain for Streptococcus Group A (ATCC 19615) all tested negative for GAS. A further five swabs, which were from a tube that also contained ATCC 19615, all tested positive.

Reproducibility of results from the direct test with the TestPackPlus™Strep A kit was confirmed by testing five samples with non-isolated beta hemolytic species which tested negative for GAS, and five samples with non-isolated beta hemolytic species which tested positive, for three consecutive days by three different technologists. In every case, results obtained by each technologist agreed 100% with the expected results.

Thus we have confirmed that direct use of the TestPackPlus™Strep A RAT kit on the beta hemolytic zone of blood agar throat culture plates from pharyngitis patients can identify GAS with high specificity and sensitivity. This would reduce the time to confirmation of presence or absence of GAS by approximately 24 h. In turn, this would help inform and expedite decisions regarding antibiotic treatment, when compared with the existing method of sub culturing on-isolated beta hemolytic species and testing with Streptex latex agglutination.

The sensitivity and specificity of our direct RAT test method compares favorably to those obtained using direct blood culture. It also compares favorably to use of RATs in other studies, particularly in terms of sensitivity, which can tend to be variable, ranging from approximately 66 to 99% depending on kit and methodology used [3,8,16,17] and may vary with the severity of disease(spectrum bias) [17-19]. It has been demonstrated that such spectrum biases are relevant mainly in patients with light in ocula and it has been suggested that negative RAT is sufficient to rule out GAS in children if clinicians accept that approximately 10-14% of these patients would be positive on blood culture [8,20]. A false negative result might occur, for example, if the amount of extracted antigen is below the sensitivity of the test. Therefore in many countries, inclusion of blood culture is still recommended by expert groups laying down guidelines for diagnosis of GAS pharyngitis, particularly in case of negative direct immunoassay results [3,8,10,11].

This is especially relevant in populations where there may still be a significant incidence of complications arising from GAS pharyngitis [1]. While in the USA and Europe, the incidence of complications such as acute rheumatic fever are rare, they are more common in other populations, particularly in under-resourced settings, such as indigenous populations in industrialized countries, for example in North Queensland in Australia [1,21-23]. In developing countries, acute rheumatic fever remains a significant contributor to cardiac morbidity and mortality in many developing countries [1,23,24].

In Saudi Arabia, acute rheumatic fever continues to occur, usually preceded by recent streptococcal infection [25], although incidence is declining [26]. Thus in our laboratory we continue to use blood agar culture alongside RAT methods for GAS pharyngitis diagnosis. The modification tested in this paper, in which RAT is performed directly on beta-hemolytic zone samples from original culture plates, should reduce the time to diagnosis by 24 hours in cases of suspected non-isolated beta hemolytic colonies. Use of RAT in GAS identification has been shown to help reduce unnecessary prescription of antibiotics in pharyngitis patients. In one recent study, use of RAT in tests on children with pharyngitis reduced antibiotic prescription by over 40% and also substantially reduced antibiotic-related costs in groups with both GAS and non-GAS-associated Streptococcus pharyngitis [27]. The combination of blood agar culture and direct use of RAT on the original plates represents a compromise appropriate in our local conditions to rule GAS in or out in cases of pharyngitis would help ensure timely application of appropriate antibiotic treatment and prevention of unnecessary antibiotic prescription.
Conclusion

Our proposed method of use of direct RAT on beta hemolytic zones of original blood agar culture plates from pharyngitis patients had high sensitivity, specificity and accuracy in ruling GAS involvement in or out in cases of suspected non isolated beta hemolytic colonies. This method should be adopted in the future to expedite positive identification of GAS and ensure timely application of appropriate antibiotic treatment, and also to rule out GAS in negative cases and thus prevent unnecessary antibiotic prescription.

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Conflict Of Interest

None of the authors has a conflict of interest to declare.

Bibliography


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