REFERENCEs


Extragaryngeal group A Streptococcus infection: diagnostic accuracy and utility of rapid antigen testing

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Background. Antigen tests have been well-studied and are widely used in pediatric practice for rapid detection of group A Streptococcus (GAS) infections in the throat, but they have not been examined sufficiently for the detection of infection of skin sites, such as the perineal region or impetiginous lesions.

Methods. During the 3-year period 1999 to 2002, we evaluated 239 patients with suspected GAS skin infection, in 5 pediatric practices, using 3 Dacron swabs for each site. The first swab was tested in the pediatric office laboratory with an antigen detection kit. For the first 91 patients,
the Abbott Test Pack Plus antigen detection test (ADT) was used. The Abbott Signify Strep A ADT was used to test subsequent patients. The second swab was tested with BD Directigen 1-2-3 ADT in the hospital laboratory. The third swab was placed in modified Stuart’s transport medium for comparison of recovery of GAS from culture in broth or on agar. A positive culture served as the reference standard. Test performance and test accuracy were determined for each ADT.

**Results.** Of the 247 ADTs and cultures performed on 239 patients, 91 with suspected skin infection were tested with the Test Pack Plus test, 149 with the Signify Strep A test and 247 with the Directigen test. Eighty-six (35%) cultures were positive, 73 from perineal sites (54 rectal, 13 vaginal, 6 penile) and 13 from impetiginous lesions. There was 100% concordance for the 86 cultures positive for GAS in a comparison between dry Dacron swabs and swabs that had been placed in modified Stuart’s transport medium. Test Pack Plus and Signify Strep A ADTs had similar performance characteristics for skin infections: sensitivity, 92 and 88%; specificity, 99 and 97%; positive predictive value, 96 and 94%; and negative predictive value, 97 and 93%. Directigen ADT had sensitivity 78%, specificity 100%, positive predictive value 100% and negative predictive value 89%. Accuracy for the tests varied from 92 to 97%.

**Conclusion.** Tests designed to detect GAS carbohydrate antigen in patients with pharyngitis can be used rapidly and accurately to detect GAS antigen in patients with cutaneous lesions suspected of GAS infection.

**INTRODUCTION**

Rapid antigen testing has been well-studied and applied in clinical practice for group A *Streptococcus* (GAS) infection in the throat,

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but limited attention has been given to antigen detection testing for other sites.

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The skin and soft tissues are the primary nonthroat sites of GAS infection in children.

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Perineal GAS infection is a recognized, perhaps underdiagnosed, clinical entity in children and includes perianal infection characterized by perianal erythema and/or itching, anal fissure, painful defecation, blood-streaked stools and mucopurulent discharge;

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vulvovaginitis in prepubertal girls;

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and balanitis in prepubertal, especially uncircumcised, boys.

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Group A streptococci are also a common cause of impetigo in children.

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Culture results for skin samples may take 24 to 72 h, and most office laboratories do not have the capability of performing the service. An antigen detection test (ADT) may aid in rapid differentiation of cutaneous GAS infection from entities such as candidiasis, non-specific diaper dermatitis, pinworm infestation, apparent fissures with local trauma or even suspected sexual abuse. We undertook this study to determine whether ADTs could be accurately and rapidly used to detect GAS at the common sites of GAS infection other than the pharynx, particularly perineal and other superficial skin sites. Secondary aims were to evaluate the concordance of ADTs in use in our office and hospital laboratories and to assess the need for transport media for recovery of GAS from skin sites.

**MATERIALS AND METHODS**

**Patients.** Patients eligible for inclusion were children 0 to 18 years of age with the clinical suspicion of GAS infection in the perineal region (anus/vagina/penis) and other superficial skin sites seen in our 5 pediatric office practices (22 providers). Clinical criteria for suspected perineal GAS infection included erythema and/or maceration in the perineal area or these findings with or without discharge from the vagina in girls or penis in boys and also included perianal itching, anal fissure, painful defecation, blood-streaked stools and discharge from or about the anus, vagina or penis. Skin lesions consistent with streptococcal impetigo were usually nonbullous, had erythematous bases and had honey-crusted exudates or crusts. We attempted to enroll consecutive patients meeting these criteria.

Children treated with an antibiotic within the previous week or who had another diagnosis explaining the findings were excluded. The protocol was approved by the Institutional Review Board of Presbyterian Hospital, Charlotte, NC. Informed, written consent was obtained at study entry.

**Specimen collection and culture methods.** Specimens were obtained by sequentially rubbing the affected site with three, sterile Dacron-tipped swabs (Fig. 1). The first two swabs were replaced in their original paper packets, and the third swab was placed in modified Stuart’s transport medium (Starswab; Starplex Scientific, Etobicoke, Canada), as is the standard protocol for specimens for culture taken from the perineum or skin. These swabs were either hand-carried or transported by courier the same day (usually within a few hours) to the Eastover Pediatrics Clinic office on-site laboratory. This was staffed by a certified medical technologist and is Clinical Laboratory Improvement Amendments (CLIA)-approved for high complexity testing. Swabs 2 (dry) and 3 (in Stuart’s transport medium) were then transported the same day by courier to the Presbyterian Hospital microbiology laboratory where all tests for these swabs were done by one of the authors (SD).

The first swab was inoculated onto a sheep blood agar (SBA) plate (Remel, Lenexa, KS) using standard
Six to eight stabs were made into the agar, and a bacitracin disk (Taxo A disk, 0.04 unit; BD Biosciences, Franklin Lakes, NJ) was applied at the junction of the primary and secondary streaks. Plates were incubated overnight aerobically at 35–37°C and interpreted by one of the authors (HWC or OFR). Plates negative for beta hemolysis the first day were reincubated and reinterpreted the next day. All plates were also evaluated in blinded manner by a microbiologist (SD). The second and third swabs were inoculated onto SBA. The third swab was then placed in a tube of Todd-Hewitt enrichment broth (THB) which was incubated at 35–37°C and room air, then subcultured onto SBA, and incubated and interpreted as described above.

If isolated colonies suggestive of beta hemolysis were present on the original plates or after subculturing, a confirmatory GAS latex agglutination test was performed following the manufacturer’s instructions (PathoDx Strep Grouping kit, Diagnostic Products Corp., Los Angeles, CA). If the organism was positive for GAS antigen, no further testing was performed. If the test was negative, the organism was Gram-stained and tested for group B, C, F and G antigens with the same grouping kit.

**Test evaluation.** After it was used to inoculate SBA at the Eastover Pediatrics Clinic office laboratory, the first swab was used by the office laboratory technologist to perform the rapid antigen test. The Abbott Test Pack Plus test (Abbott Laboratories, Abbott Park, IL), a CLIA-moderately complex enzyme immunoassay, was selected because it was the test kit in use for GAS antigen detection testing for throat specimens in our office laboratory when the study began. In 2000 this test kit and several others were removed from the market by an Food and Drug Administration consent decree because of general quality control issues (and not specifically related to kit problems) at Abbott Laboratories. Ninety-one specimens had been evaluated with this test kit. Abbott substituted the kit with Signify Strep A, a CLIA-waived, color immunochromatographic test. This was used for the remainder of the study (as well as for ADT for throat specimens in the office). When this change was made, the first swab obtained from the patient (see Fig. 1) was placed in modified Stuart’s transport medium (Starswab), because a prior verification exercise in our office laboratory with the Signify Strep A kit for throat specimens had revealed improved sensitivity compared with a dry swab (unpublished data).

In the Presbyterian Hospital laboratory, the second dry swab (after replacement in its paper packet), was inoculated onto SBA and then tested for antigen using BD Directigen 1-2-3 (BD Biosciences), a CLIA-moderately complex liposome immunoassay. It was selected because it was the test kit in use for the hospital laboratory for throat specimens.

For each ADT the manufacturer’s guidelines were followed in the performance and interpretation of the tests. Those reading the ADTs were blinded to the results of each other, and the readers of the culture plates were blinded to the ADT results.

**Statistical analyses.** The reference standard for the definitive presence of GAS was considered to be the isolation of a serologically confirmed group A *Streptococcus* by any one of the four cultures. Test performance characteristics (sensitivity, specificity, positive and negative predictive values) were calculated with standard definitions from 2 × 2 contingency tables along with 95% confidence intervals to estimate uncertainty. Clinical accuracy, or test efficiency, was determined by dividing the total true positives and negatives by the total number of specimens tested.

**RESULTS**

During the 3-year study period, 247 ADTs and cultures were performed on 239 patients with suspected extrapharyngeal GAS infection. There were 8 episodes of recurrent infection (in 6 patients), each >3 weeks apart, and these were included. Ninety-one were tested with the Test Pack Plus ADT, 156 with the Signify Strep A ADT and 247 with the Directigen ADT (Table 1). For the Signify Strep A ADT, 7 were excluded (for a total of 149) because the test was performed by someone other than the office medical technologist. The study group had a median age of 4.9 years (range, 0.9 to 18.8 years) for those with positive cultures and 3.9 years (range, 0.1 to 17.5 years) for those with negative cultures.

Of the 247 specimens cultured, 86 were positive (35%) with 73 from perineal sites (54 rectal, 13 vaginal and 6 penile) and 13 from nonperineal skin sites (all were impetiginous lesions). All 4 cultures (3 SBA plates and THB) were positive in 80 of 86 (93%). In no instance was the THB the only positive culture, and the THB culture was negative 3 times when at least one other culture was positive.

In the culture comparison for GAS between the dry
swab and the swab placed in modified Stuart’s transport medium, there was 100% concordance for the 86 positive cultures for GAS. Of the 247 specimens transported in liquid transport medium, overgrowth of Gram-positive organisms occasionally occurred, the most common organism being *Staphylococcus aureus*. Only 2 cultures were overgrown with Gram-negative organisms, one with *Proteus* sp. and the other with *Pseudomonas* sp. Meticulous subculturing usually resolved any issues. However, cultures from dry swabs rarely grew organisms other than GAS to any degree.

Table 1 shows the performance characteristics for the three ADTs evaluated. In comparison with the reference standard (any positive culture), the Test Pack Plus ADT gave sensitivity 92%, specificity 99%, positive predictive value 96% and negative predictive value 97%. The Signify Strep A ADT had sensitivity 88%, specificity 97%, positive predictive value 94% and negative predictive value 93%. The sensitivity of the Directigen ADT tended to be lower in comparison with that of the Signify Strep A ADT, but this was not significant (78% vs. 88%, *P* = 0.125, chi square test). The Directigen ADT gave 100% specificity. The overall accuracy for the tests varied from 92 to 97%.

**DISCUSSION**

In previous studies of ADTs applied to extrapharyngeal sites, only two have included a substantial number of patients. Kokx et al. identified perianal GAS infection in 31 children, 27 of whom had an ADT (Culturette Group A Rapid Strep ID, Marion Laboratories, Kansas City, MO) performed. Of these children 89% had a positive ADT, whereas none in a group of 119 well children was positive by ADT. Kaplan et al. evaluated an ADT (Culturette Group A Rapid Strep ID) in 129 children with impetigo (84 culture-positive) and found a sensitivity of 94% and a specificity of 96%. Coffey found positive ADTs in 6 children with impetigo, and Bourgeois and Bourgeois used an ADT to diagnose GAS skin and soft tissue infection in 8 children. In case reports others have reported use of an ADT to make a rapid diagnosis of extrapharyngeal GAS infection.6–8

In our evaluation the Test Pack Plus ADT performed slightly better than its successor, Signify Strep A, for extrapharyngeal specimens, but the Test Pack Plus test is no longer commercially available. The waived ADT, Signify Strep A, had better sensitivity than the moderately complex Directigen test (88% vs. 78%), but the Directigen test was slightly more specific (100 vs. 97%). It is possible that the delay in performance of the Directigen test (because of transportation to the hospital laboratory) resulted in its poorer sensitivity, but we did not evaluate this possibility by direct parallel comparison in the office or hospital laboratory. We sought to compare the tests under the conditions in which they would usually be done (the waived test in an office and the moderately complex test in a referral or hospital laboratory).

Our findings are consistent with those in published studies of Test Pack Plus, Signify Strep A and Directigen ADTs for throat specimens. Sensitivities in these studies ranged from 82 to 91% for Test Pack Plus, 80 to 89% for Signify Strep A and 39 to 95% for Directigen. In these same studies specificities are reported as between 85 and 100%, 19–30 As with throat swabs for the recovery of GAS, it appears that a moistened swab from perineal and other skin sites is not necessary for the culture recovery of GAS as the dry swab and swab placed in modified Stuart’s transport medium showed 100% concordance for the 86 positive cultures. Use of a dry swab from these sites may also make interpretation easier as there was little overgrowth of other organisms with the dry swab compared with results from the swab placed in transport medium.

This study has several limitations. We enrolled only 42 patients with specimens from nonperineal cutaneous infections, and only 13 of these patients had positive cultures from impetiginous lesions. However, our findings are in accordance with those of the one other study that addressed ADT use for impetigo. We also encountered no patients with invasive soft tissue or toxin-associated streptococcal infections. Nonetheless the spectrum of patients who were included represents the usual variety of suspected cutaneous GAS infections seen in pediatric outpatients.

We conclude that the three ADTs evaluated have acceptable performance characteristics for the detection of GAS infections in extrapharyngeal sites. Their use can aid in the rapid differentiation of cutaneous...
lesions, especially in the perianal area, and may limit inappropriate antibiotic therapy for non-GAS lesions. The Signify Strep A test is particularly attractive for office laboratory use given its excellent performance characteristics and its waived status. For hospitalized patients with suspected necrotizing fasciitis, a GAS ADT might be very useful in making a presumptive, rapid etiologic diagnosis.

Although the current generation of ADTs for GAS may eventually be supplanted by newer technology, ADTs for GAS can be used for extrapharyngeal specimens as with throat specimens. The controlled conditions in this study may not reflect those in an office or other setting where an occasional rapid test for GAS may be done and, as with throat specimens, a negative result of a rapid test for an extrapharyngeal site should be confirmed with a conventional culture. ADTs for GAS should also be validated in the individual laboratory setting in which they will be used, especially when used for sites of infection for which there is no formal Food and Drug Administration approval.

APPENDIX


REFERENCES

Culture-negative osteomyelitis

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Objective. To examine the natural history, clinical manifestations, laboratory changes and outcomes of children with culture-negative osteomyelitis and to compare findings with cases of culture-positive infection treated during the same time period.

Design. Retrospective review of consecutive admissions for osteomyelitis during the 4-year period 1998 through 2001, with a minimum follow-up of 1 year.

Setting. A single urban children’s hospital with a large orthopedic referral service.

Clinical and laboratory measures. Age, gender, predisposing factors, clinical manifestations, maximum temperature, duration of pain, bone involved, laboratory changes, results of cultures from infected bone and blood and outcome after treatment.

Results. A total of 85 patients fulfilled study criteria, of whom 40 were culture-negative. Compared with culture-positive cases, culture-negative osteomyelitis patients were less likely to have antecedent trauma \( (P = 0.0357) \) and overlying skin changes \( (P = 0.0001) \), duration of pain and other symptoms was longer \( (P = 0.0396) \) and skeletal residua were rare. They were also older, with this difference approaching statistical significance \( (P = 0.0586) \).

Conclusions. Children with culture-negative osteomyelitis present initially differently from culture-positive cases and can be managed as presumed staphylococcal disease with excellent long term results.

INTRODUCTION

The incidence of osteomyelitis in children has changed very little from the 1970s, primarily because *Staphylococcus aureus* accounts for the majority of cases and little progress has been made in management and prevention of this pathogen. Diagnosis and management have also changed very little. Although magnetic resonance imaging (MRI) is often the diagnostic procedure of choice rather than the technetium bone scan as used during the previous three decades, initial empiric therapy has remained relatively constant.

A review of current text chapters indicates that most references for pathophysiology, natural history, clinical presentations and outcome were published before 1980. The only new observations have been the emergence of methicillin resistant *S. aureus*, *Kingella kingae* as a significant pathogen in childhood septic arthritis occasionally involving bone and the occasional case report of unusual pathogens such as *Bartonella henselae* and *Borrelia burgdorferi* causing bone infection.

One aspect of osteomyelitis that has received little attention in the literature is the natural history and outcome of those cases in which no bacterial pathogen...