Novel, Rapid Optical Immunoassay Technique for Detection of Group A Streptococci from Pharyngeal Specimens: Comparison with Standard Culture Methods

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A novel immunoassay system based on the changes in the reflection of light, termed an optical immunoassay (OIA), was utilized to directly detect group A streptococcal (GAS) carbohydrate antigen from clinical specimens. In two studies, a total of 1,275 throat swabs were tested for the presence of this antigen with the Strep A OIA rapid detection system and the results were compared with those of standard culture methods. In both studies, the Strep A OIA yielded more positive results than plating of the throat swab onto a selective agar, Trypticase soy agar containing sheep blood, or an enriched broth. In one study, the sensitivity and specificity of Strep A OIA compared with those of the broth-enriched culture were 97.4 and 95.6%, respectively. In a second study a sensitivity of 98.9% and a specificity of 98.6% were achieved. It was also shown that the carbohydrate antigen could be detected in the absence of viable GAS organisms. The Strep A OIA is an easily interpretable method and was shown to be more sensitive than routine culture methods for detecting GAS infections directly from throat swabs.

Group A streptococci (GAS) are some of the most significant pathogens responsible for tonsillitis and pharyngitis in both children and young adults. Various methods, including clinical diagnosis and culture methods, have been used to diagnose GAS pharyngitis. It is difficult to clinically distinguish between streptococcal and nonstreptococcal pharyngitis, and most physicians tend to overestimate the likelihood of positive GAS cultures (19). Early treatment of infection results in rapid resolution of the symptoms and helps prevent further complications such as acute rheumatic fever, acute glomerulonephritis, and streptococcal bacteraemias (2). Recently, there has been an increase in GAS bacteraemia (23, 24) and in documented cases of acute rheumatic fever in a number of geographically distinct locations (3, 21). It has been noted that accurate, early diagnosis and treatment of GAS pharyngitis not only can prevent further complications (6, 20) but also can shorten the duration of clinical illness and the transmission of disease and, in negative cases, eliminate unnecessary treatment with antibiotics. Thus, there exists a need for a rapid, reliable test for GAS to assist in early diagnosis of pharyngitis.

Over the past several years a large number of commercial products for the direct rapid detection of GAS from clinical specimens have been introduced. These procedures depend on the detection of a carbohydrate antigen specific for GAS. In general, many of the procedures employed by these commercial products are immunologic, using either a monoclonal or polyclonal anti-GAS carbohydrate antibody in a latex agglutination assay or enzyme immunoassay. Most of the procedures are easy to perform with a well-defined end point but suffer from poor sensitivity relative to that of conventional culture. Because of this, many facilities either choose not to perform these rapid techniques or elect to perform a traditional throat culture when the results are negative. Throat culture results are usually obtained by use of either blood agar or a selective medium. The sensitivity and specificity of these culture methods are dependent on a number of variables, including sample collection, time and conditions of transport to the laboratory, and choice of media and culture conditions. Sensitivities from 75 to 95% are reported with a single blood agar or selective culture plate when the results of these methods are compared with the total number of positive isolates encountered with several methods, including broth-enriched methods and dual-plate systems (5, 11, 15, 25, 26).

The direct visualization of an antibody bound to an immobilized antigen on a reflecting surface was originally described in 1938 by Shaffer and Dingle (22). Others have taken this technique and have designed simple and sensitive immunologic surface tests yielding sensitivities as low as 1 ng of antibody per ml (1, 10, 18). The concept behind these surface immunoassays is that the direct visualization of a second antigen layer applied to an existing monolayer results in changes in the reflective properties. In this study, we compared standard culture techniques for the detection of GAS with a novel, rapid, surface immunoassay termed optical immunoassay (OIA) to detect GAS carbohydrate antigen directly from clinical isolates.

MATERIALS AND METHODS

Two studies were performed to compare the Strep A OIA (BioStar, Inc., Boulder, Colo.) rapid detection system with culture on either streptococcus-selective agar or Trypticase soy agar containing 5% sheep blood (TSA). Both studies included the use of an enriched-broth culture. The study design for each method is shown in Fig. 1 and was as follows.

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Study design. (i) Study 1: selective agar and Strep A OIA. Swabs were collected from patients presenting symptoms of pharyngitis by using a single, synthetic-fiber swab (S/P Culturette System; Baxter Diagnostics, Inc., Deerfield, Ill.; or Transtub; Medical Wire Equipment Co., Sparta, N.J.) and standard throat culture collection techniques (8). Swabs were collected from several clinical sites, including a large metropolitan children’s hospital, a university teaching hospital, and National Jewish Center, a large referral institution for respiratory diseases. Immediately after the specimen was collected, the swab was returned to the transport tube, which contained modified Stuart’s transport medium. Swabs were transported to the microbiology laboratory at National Jewish Center on a daily basis at ambient temperature and inoculated onto a GAS-selective agar (Group A Selective Strep Agar; BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) (SSA), a selective medium used for isolation and presumptive identification of GAS from throat cultures. After the plate was streaked for colony isolation, the agar was stabbed in several areas to aid in the detection of hemolysis. Swabs were cultured generally within 24 h of collection and in no case after 48 h. Plates were incubated at 35°C in 5% CO₂. If plates were negative for GAS after 24 h, they were held for an additional 24 h. Plates with positive cultures were processed as soon as those cultures were detected. All cultures positive for beta-hemolytic streptococci were serotyped by a latex agglutination procedure (Wellcome Streptex; Burroughs Wellcome Co., Research Triangle Park, N.C.). Following the plating procedure, the Strep A OIA was
performed by using the swab and following the manufacturer's package insert as described below.

A broth-enriched culture method was designed to confirm the presence of GAS. The pledges (plugs separating the media and swabs in the transport tubes) were aseptically removed from the tubes, placed in Todd-Hewitt broth (THB) (BBL), and incubated for 24 h at 35°C. All specimens were subcultured by placing an aliquot of THB onto SSA. These plates were incubated for 24 to 48 h at 35°C in CO₂. If growth patterns consistent with GAS were observed, colonies were selected and reisolated if necessary and their identification was confirmed by the latex agglutination assay (Welchome Streptex).

(ii) Study 2: TSA culture and Strep A OIA. Study 2 followed the same protocol as study 1 except for the following: (i) swabs in transport medium were obtained from a large private physician's practice and a university student health center; (ii) all swabs were cultured at the site of origin prior to OIA; (iii) all sites used TSA (BBL) instead of SSA, and the physician's laboratory practice incubated the TSA cultures anaerobically; (iv) PathoDx Strept Typing Sera (Diagnostic Products Corp., Los Angeles, Calif.) was used as the serotyping kit for confirmation of positive cultures; and (v) only OIA-positive and culture/OIA-discrepant (OIA-positive, agar-negative or OIA-negative, agar-positive) results, rather than all of the THB cultures, were subcultured to TSA. Subcultures from THB were incubated anaerobically (BBL GasPak).

Description of the OIA. OIA allows the direct visual detection of the physical change in thickness of thin films resulting from the binding reactions between antigens and antibodies. A color change is generated by the change in the reflection of light through the films formed on the optical substrate (Fig. 2). The test surface has an affinity-purified anti-GAS polyclonal rabbit antibody attached to it. Light reflected through this series of thin films results in a gold color. The color change will not occur unless the thickness of the film is changed. When a liquid sample containing antigen is placed on the surface, binding occurs between the antigen and the immobilized antibody. When the sample is followed by a substrate, there is an increase in the thickness of the film. Once this reaction takes place, the optical path through the film is changed, causing the surface to appear purple (Fig. 2). If the antigen is not present in the sample, no binding takes place and the original thickness remains unchanged so that the test surface retains its original gold color. This optical detection system provides a clear end point.

In the 8-min Strep A OIA, carbohydrate antigen is extracted from the swab with 0.3 M acetic acid (9). Three drops of this solution are added to the extraction tubes provided in the kit. After a 2-min incubation, the solution is neutralized with 3 drops of 1.5 M MOPS [3-(N-morpholino)-2-hydroxypropanesulfonic acid] buffer containing 0.2% Tween 20, pH 7.3. A horseradish peroxidase-labeled rabbit anti-GAS antibody is added to the neutralized extraction solution to allow the immune complex to form. This sample is then deposited onto the OIA device and incubated for 2 min to allow the immune complex to attach to the OIA surface. To remove unbound sample from the surface, the OIA surface is washed with approximately 2 ml of a stream of water. Next the substrate (tetramethylbenzidine containing H₂O₂) is applied for 4 min to react with the bound enzyme-labeled antibody and precipitates on the surface. The test surface is washed again with a stream of water, and the OIA device is read by eye. If the streptococcal antigen is present in the sample, the reaction area will appear as a purple spot. If the streptococcal antigen is not present in the sample, the surface retains its initial gold color. For the validation of each assay a procedure control is incorporated onto each test surface.

Specificity studies. Specificity studies were conducted using American Type Culture Collection (Rockville, Md.) strains of most of the common upper respiratory bacterial pathogens. The strains tested were *Staphylococcus aureus* (ATCC 25923), *Streptococcus group C* (ATCC 12388), *Corynebacterium diphtheriae* (ATCC 11913), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus group D* (ATCC 9809), *Moraxella catarrhalis* (ATCC 25240), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus group B* (ATCC 12386), *Streptococcus pneumoniae* (ATCC 27336), *Candida albicans* (ATCC 14053), *Haemophilus influenzae* B (ATCC 10211), *Streptococcus group F* (ATCC 12392), *Streptococcus group G* (ATCC 12394), *Escherichia coli* (ATCC 25922), *Neisseria gonorrhoeae* (ATCC 43070), and *Neisseria meningitidis* group B (ATCC 13090).

The *Neisseria* species, *H. influenzae*, and *M. catarrhals* were inoculated onto chocolate agar (BBL) and incubated in CO₂ pouches (BBL). The *Corynebacterium diphtheriae* was inoculated onto Loeffler's slants (BBL), and all remaining organisms were inoculated onto TSA. All organisms were incubated at 35°C for 24 to 48 h. After incubation, the organisms were inoculated into sterile normal saline to achieve a density equivalent to a McFarland no. 9 standard (approximately 2.7 × 10⁹ cells per ml). Next, 100 µl of each organism suspension was placed onto a Culturette and processed by the Strep A OIA according to the package insert in the same manner as a pharyngeal specimen.

Culture viability and antigenicity studies. To assess whether organism viability could impact the OIA results, a
TABLE 1. Results of Strep A OIA and SSA versus THB cultures for detecting the presence of GAS

<table>
<thead>
<tr>
<th>THB culture result</th>
<th>OIA*</th>
<th>SSA culture*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>111</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>16</td>
<td>345</td>
</tr>
</tbody>
</table>

* In comparison with THB culture, OIA had 97.4% sensitivity and 95.6% specificity.

* In comparison with THB culture, SSA had 75.4% sensitivity and 99.2% specificity.

Study was conducted using the following conditions to determine the effects of time, temperature, and moisture on the recovery of GAS from a Culturette swab. Sixty Culturette swabs were inoculated with 100 µl of a bacterial suspension containing approximately 2.7 × 10^9 or 2.7 × 10^6 GAS per swab. Thirty of the swabs were stored with the medium ampoule crushed to provide a moist environment for the organisms. The remaining 30 swabs were stored with the medium ampoule intact. Twelve swabs each were stored at temperatures of −20, 4, 25, 35, and 45°C for 24, 48, and 72 h. After 24, 48, or 72 h, four swabs stored at each of the temperatures were inoculated onto TSA and incubated aerobically at 35°C. The plates were examined for the presence or absence of beta-hemolytic streptococci after 24 h. This procedure was repeated with four swabs from each temperature at 48 and 72 h. Enriched-broth culture was performed by aseptically removing the pledgets from a Culturette swab and placing the pledgets in THB for 24 h. All negative THB cultures were subcultured after 24 h to TSA and examined 24 h later for beta-hemolytic streptococci.

In order to assess whether the GAS carbohydrate antigen remains detectable when stored on a Culturette swab, a simultaneous study was conducted utilizing conditions identical to those employed in the culture viability study. Twenty swabs were inoculated with 100 µl of a bacterial suspension containing approximately 2.7 × 10^9 to 2.7 × 10^6 cells per swab and stored for 24, 48, or 72 h under the same conditions as the culture viability study. By the Strep A OIA, four swabs were tested for detectable antigen at 0, 24, 48, or 72 h for each temperature.

Data analysis. Sensitivity was calculated by dividing the true-positive results by the sum of the true-positive and false-negative results. Specificity was calculated by dividing the true-negative results by the sum of the true-negative and false-positive results. The percent agreement was calculated by dividing the sum of the true-positive and true-negative results by the total number of samples.

RESULTS

Study 1: selective agar and Strep A OIA. In the study comparing SSA culture with Strep A OIA, a total of 475 samples were evaluated. A summary of the results for study 1 is shown in Table 1. THB-enriched culture determined 114 samples to be positive, and SSA culture determined 89 samples to be positive. Compared with the broth-enriched method, Strep A OIA demonstrated a sensitivity of 97.4%, a specificity of 95.6%, and 96% agreement. Compared with the broth-enriched method, the SSA culture method had a sensitivity of 75.4% and a specificity of 99.2%. The agreement between Strep A OIA and SSA culture was 89.5%. When Strep A OIA was compared with SSA culture, 28 of the 44 apparent false positives by OIA were actually true positives as determined by the broth-enriched method. Strep A OIA-negative and SSA culture-positive samples (n = 6) had low colony counts (fewer than 20 colonies per plate) on original culture, and three of the six low-colony-count samples were THB positive. An additional seven cultures with colony counts of fewer than 20 colonies per plate were detected by OIA.

Study 2: TSA culture and Strep A OIA. In the study comparing TSA culture with Strep A OIA, a total of 800 samples were examined. The results of the TSA culture and OIA comparison are summarized in Table 2. The THB-enriched method determined 93 samples to be positive, Strep A OIA determined 103 samples to be positive, and TSA culture determined 71 samples to be positive. Compared with the broth-enriched method, Strep A OIA had a sensitivity of 98.9%, a specificity of 98.4%, and 98.5% agreement. The sensitivity of TSA culture compared with that of broth-enriched methods was 76.3%, and the specificity was 99.5%. The agreement between TSA culture and Strep A OIA was 94.8%. Four of the five TSA culture positives that were Strep A OIA negative had very low colony counts on original culture (fewer than 20 colonies per plate), and all but one were broth-enriched culture negative. An additional five cultures that had fewer than 20 colonies per plate were detected by OIA. When Strep A OIA is compared with TSA culture methods, 26 of the 37 apparent false positives were actually true positives as determined by the broth-enriched method.

Specificity studies. Of the 17 common respiratory pathogens tested (see Materials and Methods for organism list), all organisms yielded negative results with the Strep A OIA. The lack of cross-reactivity with other streptococcal species was also demonstrated by both studies 1 and 2. A total of 109 non-GAS isolates, including 20 group B, 23 group C, 10 group F, and 7 group G isolates and 49 isolates identified as non-group A without additional serotyping, were detected in these two studies. None of these organisms yielded a positive result in the Strep A OIA procedure.

Culture viability and antigenicity studies. This study was designed to evaluate the recovery of GAS inoculated on Culturette swabs when subjected to a variety of transport conditions for up to 72 h. Additionally, the ability of routine and THB culture methods to recover viable microorganisms after the swabs have been stored under routine and compromised conditions was examined and compared.

All Culturette swabs stored at temperatures of −20 and 4°C demonstrated 100% viability by both culture methods. Inoculated swabs stored at 25°C showed decreasing viability through 72 h by routine culture methods. Only 25% of the
swabs inoculated were still viable at 72 h. By contrast, 75% of swabs inoculated and cultured by the THB culture method were still positive at 72 h. The swabs stored at 35 and 45°C were all negative at 24 h when tested by routine culture methods. Only 25% of the swabs stored at 35°C were still positive at 72 h when tested by the THB culture method. At 45°C, only 25% of all swabs were positive when tested by the THB culture method at 24 h and 100% were negative when tested at 48 and 72 h.

No differences in the recovery of viability of GAS from Culturette swabs stored under moist versus dry environmental conditions were observed; therefore, the data are not discussed in this paper.

Strep A OIA detected GAS antigen on all inoculated swabs prior to exposure of the swabs to the environmental transport conditions. At 24, 48, and 72 h, GAS antigen was detected by Strep A OIA from 100% of the swabs for all study conditions.

**DISCUSSION**

All in vitro immunologic diagnostic tests to detect GAS, including Strep A OIA, require the binding of GAS antigen to a specific antibody. The Strep A OIA utilizes a direct visual detection of the immunological binding event by relying on the interaction of light with thin films on a silicon surface. Membrane-based assays have a relatively higher nonspecific binding that results in a reduction in sensitivity. Strep A OIA’s combination of optical thin-film detection on a silicon crystal surface allows for a rapid method with an enhanced sensitivity without sacrificing specificity.

The reference culture method used to determine the sensitivity and specificity of any rapid test may affect the study results. The lack of a definitive method to use as a standard reference method for the detection of GAS from throat swabs adds to the problem. In both the SSA (study 1) and TSA (study 2) studies, the data collected support the results of other studies showing that conventional culturing methods are less sensitive than broth-enriched methods when culturing for GAS (5, 11). Broth-enriched methods, however, are not widely used because of the added cost and turnaround time. Routine culturing using SSA and TSA compared with the broth-enriched cultures demonstrated sensitivities of 75.4 and 76.3%, respectively. Despite these findings, the throat culture is still used as the “gold standard” for GAS testing and considered to be more sensitive than most rapid methods (12, 16, 17, 21, 26).

The Strep A OIA demonstrated sensitivities of 97.4 and 98.9% in studies 1 and 2, respectively, compared with broth-enriched culture. Our findings indicate Strep A OIA to be significantly more sensitive than standard culture and equivalent in sensitivity to the broth-enriched methods for GAS. This excellent sensitivity eliminates the need for subsequent culture of negative Strep A OIA results, as has been indicated as necessary by other investigators for other rapid antigen tests (4, 7, 12, 13). It is important to note that in both studies 1 and 2, the performance results represent all data collected, including all low colony counts, even those of fewer than 20 colonies per plate. Since a single swab was used first to inoculate the agar and second to perform the Strep A OIA, the majority of the sample from the low-colony-count positives may have been transferred to the plate, thereby leaving very little of the sample to test with Strep A OIA. Seven cultures with low colony counts on SSA culture and five cultures on TSA were detected by Strep A OIA using the single-swab system.

The absence of positive reactivity to upper respiratory pathogens in the specificity studies shows that the Strep A OIA is a highly specific test, as has been observed with other rapid detection methods (6, 7, 13, 25). Compared with the reference broth-enriched method, specificities of 95.6% in study 1 and 98.4% in study 2 were achieved. In both clinical studies, all beta-hemolytic non-GAS organisms encountered had negative Strep A OIA results.

The transport of throat swabs is an inefficient process (14) and may compromise the viability and subsequent recovery of the pathogen by culture techniques. Frequently, clinical laboratories receive from sources outside of the hospital environment swabs requiring transport to the laboratory and have little control of this transport process. Lengthy transport delays may occur, and the swabs in transit may be exposed to a variety of environmental conditions that may impact streptococcal viability. Devices with holding media are often utilized to increase the survival rate of microorganisms during transport. Most transport devices only provide a moist and not a nutritional support type of environment; therefore, delays in plating for culture decrease the survival rate of streptococcal organisms and increase the probability of false-negative results by culture.

The relative insensitivity of routine culture with aerobic incubation has been well documented (25); however, enriched-broth culture methods have been shown by other investigators to perform significantly better (5, 11). Data generated in these studies have demonstrated that the THB method was superior to routine culture and that the ability of any culture method to recover and subsequently identify GAS is entirely dependent on whether the organisms are still viable for growth and recovery. The data generated in the antigenicity and viability studies clearly demonstrate that routine culture viability loss can easily occur during sample handling and transport. As such, we cannot eliminate the possibility that the Strep A OIA false positives are in fact samples in which the organism viability has been lost while the antigenicity was retained.

GAS antigen, when subjected to the same environmental conditions as those employed in the viability study, remained detectable after 72 h by Strep A OIA. Results demonstrated antigen present on 100% of the Culturette swabs inoculated with GAS on day zero and again when tested at 24, 48, and 72 h. These results show that for the situation in which transport conditions could impact the diagnostic result, the antigen detection method is preferable, if the sensitivity of such an antigen detection method is acceptable.

There are over 30 million throat swabs processed for GAS annually in the United States (17). However, the use of culture as a detection method for GAS has little impact on patient management (7). Physicians tend to overestimate the likelihood of positive results and subsequently overtreat with antibiotics. Poses et al. report that of 104 patients treated with antibiotics prior to available culture results, only 8 actually had cultures positive for GAS (17). The most likely cause for the low impact of throat cultures on patient management is the 48-h turnaround time involved (3, 7). Facklam cites several investigators who claim that 87 to 97% of physicians may begin therapy prior to obtaining culture results and 40 to 42% of these physicians will continue to treat regardless of the culture results (7). As these numbers indicate, throat cultures are having very little impact on patient management in a clinical setting.

Direct rapid antigen tests for the detection of GAS have begun to eliminate the lengthy turnaround times associated
with cultures. Most of the rapid methods can be performed in less than 10 min, eliminating the need to begin and then discontinue treatment or to withhold treatment altogether until culture results are available. However, no rapid GAS antigen test has been available to the medical community which could approach the sensitivity of conventional culture across all samples. This fact has resulted in the development of a clinical treatment in which a swab may be obtained once from the patient for a rapid antigen test and, if the test result is positive, the patient is treated. If the result is negative, however, a second swab may be obtained and a culture is performed. This protocol has been developed as a result of the excellent specificity but relatively poor sensitivity of previously available rapid antigen tests.

It has been postulated that the recurrence of rheumatic fever may be linked to the misuse of current rapid assays. This may have occurred because the physicians neglected to have a culture performed on negative samples after obtaining results on a less sensitive rapid assay. The Strep A OIA is the first GAS rapid antigen assay available with a demonstrated performance combining excellent specificity with a sensitivity equivalent to that of an enriched broth culture and better than those of the conventional culture methods used in this study (SSA and TSA). This development eliminates the need to perform confirmatory cultures of negative samples and can offer a significant enhancement in patient care while reducing the cost of patient management.

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