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Evaluation of Two Rapid Antigen Assays, BioStar Strep A OIA and Pacific Biotech CARDS O.S., and Culture for Detection of Group A Streptococci in Throat Swabs

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Two rapid methods, BioStar Strep A OIA (OIA; BioStar, Inc., Boulder, Colo.), an optical immunoassay, and CARDS O.S. (O.S.; Pacific Biotech, Inc., San Diego, Calif.), a color immunochromographic assay, and two culture methods, one with 5% sheep blood agar (SBA) and one with Todd-Hewitt broth (TH; Remel, Lenexa, Kans.), were evaluated for use in the detection of *Streptococcus pyogenes* from pharnygeal swabs. Seven hundred forty-six double swabs (Culturette II) were processed, with OIA and SBA culture performed on one swab and O.S. and SBA culture performed on the other swab. The pledget from the Culturette II was incubated overnight in TH and was subcultured onto SBA for an additional 48 h in ambient air. All beta-hemolytic streptococci from culture were tested by a direct fluorescent-antibody test (Difco Laboratories, Detroit, Mich.). Specimens with discordant fluorescent-antibody test and rapid test results were also tested by using the Streptex latex agglutination reagent (Murex Diagnostics Limited, Dartford, England). The results obtained by all testing methods were compared with a combined test result ("gold standard"), which was defined as any positive culture detected by the SBA or TH culture methods and confirmed by Streptex latex agglutination or, in the case of negative results by both culture methods, a concomitant positive result by OIA and O.S. antigen testing. Sensitivity and specificity results for each of the methods were as follows, respectively: OIA, 81.0 and 97.5%; O.S., 74.4 and 99.0%; SBA culture, 92.3 and 98.3%; and TH culture, 86.4 and 100%. Both OIA and O.S. are suitable screening methods for detecting *S. pyogenes* directly from throat swabs but are of insufficient sensitivity to eliminate the need for backup cultures for specimens with negative OIA or O.S. results.

A number of commercial test kits are available for the rapid diagnosis of group A streptococcal pharyngitis. Because of limitations in the sensitivities of these assays and the need to prevent complications after streptococcal infection, confirmatory backup cultures are generally indicated for negative rapid test results.

The Strep A OIA (OIA; BioStar, Inc., Boulder, Colo.) is a new rapid method for detecting *Streptococcus pyogenes* directly from throat swabs. In this optical immunoassay, antibody bound to an immobilized group A streptococcal carbohydrate antigen on a reflecting surface is directly visualized (3). In the CARDS O.S. (O.S.; Pacific Biotech, Inc., San Diego, Calif.) color immunochromographic assay, group A streptococcal carbohydrate antigen is bound to a membrane coated with antistreptococcal antibody conjugated with blue latex beads.

At the Mayo Medical Center in Rochester, Minn., we evaluated the performance characteristics of two rapid antigens tests, OIA and O.S., and two cultures methods, a 48-h 5% sheep blood agar (SBA; Becton Dickinson Microbiology Systems, Cockeysville, Md.) plate culture method and a 72-h Todd-Hewitt broth (TH; Remel, Lenexa, Kans.) culture method, for the detection of *S. pyogenes* from throat swabs.

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MATERIALS AND METHODS

A total of 746 throat swabs were obtained from patients presenting with pharyngitis at Mayo Medical Center in Rochester, Minn., during a 6-week period from 26 October to 7 December 1993. A double swab (Culturette II; Becton Dickinson Microbiology Systems) was used for specimen collection. Immediately after collection the dual throat swabs were delivered at ambient temperature via a mechanical transport system to the outpatient phlebotomy area. Upon receipt, the specimens were first plated onto Trypticase soy agar with 5% sheep blood (SBA; Becton Dickinson Microbiology Systems), and then the two rapid antigen testing methods were performed in alternating order by different technicians. For each dual swab, SBA culture and OIA testing were performed on one swab and SBA culture and O.S. testing were performed on the other swab.

The SBA culture and the Culturette tube were transported to the Clinical Microbiology Laboratory for the remainder of the processing and evaluation. The SBA cultures were incubated at 35°C in ambient air for 48 h. Within 8 h of specimen collection, the pledget (the plug separating the swab from the transport medium) from the Culturette was aseptically removed, placed in a 3 ml of TH, and incubated overnight at 35°C in ambient air. The TH was subcultured onto SBA, which was incubated at 35°C in ambient air for 48 h. All beta-hemolytic streptococci from primary SBA cultures were tested by our routine direct fluorescent-antibody test for *S. pyogenes* (FA; Difco Laboratories, Detroit, Mich.). Growth of beta-hemolytic streptococci on SBA was quantitated from 1+ to 4+

TABLE 1. Comparison of combined test results[a]

Test method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
BioStar Strep A OIA	81.0 (74.3, 86.2)[b]	97.4 (95.8, 98.4)	90.1 (84.7, 94)	94.6 (92.5, 96.1)
Pacific Biotech CARDS O.S.	74.4 (67.1, 80.2)	99.0 (97.9, 99.5)	95.4 (90.8, 97.7)	93.0 (90.7, 94.8)
SBA culture[c]	92.3 (87.5, 95.2)	98.3 (96.9, 99.1)	94.1 (89.7, 97.0)	97.7 (96.2, 98.8)
SBA culture[d]	94.1 (88.7, 96.4)	98.3 (96.9, 99.1)	94.1 (89.8, 97.0)	98.3 (96.7, 99.0)
TH	86.4 (80.4, 91.1)	100 (99.5, 100)	100 (97.9, 100)	96.1 (94.3, 97.5)

[a] For the combined tests, a true-positive result is defined as a positive result for any of the three cultures confirmed to be group A streptococci by Streptex latex agglutination or if both of the rapid antigen tests are positive. Data for six patients were excluded because of either indeterminate OIA or O.S. results or the inability to isolate the organism for determination of the species of the organism.

[b] Values in parentheses are 95% confidence intervals.

[c] Performed on the same swab used to perform the OIA test.

[d] Performed on the same swab used to perform the O.S. test.

(see footnote *a* of Table 2 for definitions of quadrant growth). The beta-hemolytic streptococci isolated from TH subcultures were serogrouped by using the Streptex latex agglutination reagent (Murex Diagnostics Limited, Dartford, England). Specimens with discordant fluorescent-antibody and rapid antigen test results were also tested by using the Streptex reagent by subculturing the colonies from the original SBA culture and testing them with the Streptex reagent.

OIA and O.S. were performed according to the manufacturer's instructions. To perform the OIA procedure, three drops of extraction reagent containing 0.3 M acetic acid were added to the extraction tube provided. The throat swab was mixed with the reagent, and they were allowed to incubate for 2 min. The solution was neutralized with 3 drops of a mixture of 1.5 M MOPSO, 20 mM ethylene glycol-bis(β-aminoethyl ether)-*N*, *N*, *N'*, *N'*-tetraacetic acid, and 0.2% Tween 20. Fluid was expressed from the swab, and 1 drop of horseradish peroxidase-labeled rabbit anti-group A streptococcus antibody was added to the extract. By using a transfer pipette, 1 drop (0.05 ml) of the solution was placed onto the surface of the test device and was allowed to incubate for 2 to 5 min. The surface was rinsed vigorously for 3 to 4 s by using the provided wash solution. One drop of the tetramethylbenzidine and hydrogen peroxide substrate was added to the test surface and was allowed to incubate for 4 to 10 min. The surface of the test device was rinsed again in the same fashion. The test device was examined for the presence of a blue to purple circle on the test surface, the indicator of a positive test result.

In the O.S. procedure, the throat swab was placed in the provided extraction cup, mixed thoroughly with 6 drops of each of the two extraction reagents, and incubated at room temperature for at least 1 min. By using the pipette provided, 0.22 ml of extract was transferred to the reaction unit containing a membrane strip which provides the solid support for the assay. As the extract moves along the membrane, rabbit anti-*Streptococcus* group A antibody conjugated with blue latex beads is mobilized. The extract continues to move to the antibody-blue latex conjugate across the membrane to the immobilized anti-*Streptococcus* group A zone. If *Streptococcus* group A is present in the specimen, a "sandwich" of solid phase-*Streptococcus* group A antigen-blue latex is formed, resulting in a visible blue positive sign in the result window of the reaction unit.

For all patients whose specimens had discrepant results between the rapid tests and Streptex-confirmed culture results, a review of the patient's medical record was conducted to identify those patients with a history of antibiotic usage within 1 month of specimen collection.

For statistical analysis of the data, confidence intervals for sensitivity, specificity, and positive and negative predictive values were based on exact binomial probabilities. The difference between sensitivities was evaluated by a McNemar's test.

RESULTS

Results of the study are summarized in Tables 1 and 2. Data from six patients were excluded because of either indeterminate rapid antigen detection results (two patients) or the inability to isolate the organism for Streptex latex agglutination confirmation (four patients). For the analysis shown in Table 1, the "gold standard" by which all test methods were compared was defined as any positive culture detected by the SBA or TH culture methods and confirmed by Streptex latex agglutination or, in the case of negative results for both culture methods, a concomitant positive result by OIA and O.S. antigen testing. Results of two analyses are displayed in Table 1 for SBA culture because the source of specimen for SBA culture varied as to the swab processed. By using the two-sided McNemar's test, the sensitivity of the OIA was significantly different from that of O.S. ($P = 0.002$). Table 2 shows the OIA and O.S. results in relation to the quantity of *S. pyogenes* on SBA culture.

A medical record review was conducted for each patient whose OIA or O.S. result did not agree with the Streptex-confirmed culture result ($n = 70$) to determine whether the patient had received antimicrobial agents within 30 days of specimen collection. Seven of 70 patients whose specimens gave discrepant results had received antimicrobial agents within 30 days of specimen collection. Specimens from two of these seven patients with positive rapid antigen test results

TABLE 2. Comparison of OIA and O.S. by quantity of *S. pyogenes* on SBA

Quantity of *S. pyogenes* on SBA[a]	No. of specimens			
	OIA		O.S.	
	Positive	Negative	Positive	Negative
4+	91	1	85	4
3+	27	6	27	11
2+	9	14	6	19
1+	3	5	1	5

[a] Streptex confirmed. 1+, <10 colonies in first quadrant streak area; 2+, ≥10 colonies in first quadrant streak area and <5 colonies in second quadrant streak area; 3+, >10 colonies in first quadrant streak area, ≥5 colonies in second quadrant streak area, and <5 colonies in third quadrant streak area; 4+, >10 colonies in first quadrant streak area, >5 colonies in second quadrant streak area, and ≥5 colonies in third quadrant streak area.

were negative on culture: one had positive results by both OIA and O.S. and the second had a positive result by O.S. only. The specimen from one patient was negative in the cultures and indeterminant by both OIA and O.S. Specimens from four of seven patients were positive on cultures but had variable rapid antigen results: one had negative results by both OIA and O.S., two had negative results by OIA only, and one had a negative result by O.S. only.

DISCUSSION

The rapid diagnosis of *S. pyogenes* from throat swabs is preferred by many physicians and patients and has recently become possible by virtue of immunoassays which identify group A streptococcal carbohydrate antigen (1, 3, 5, 9) or DNA probe assays which detect unique nucleic acid sequences (4, 7, 8). A problem with conventional rapid immunoassays has been a lack of sensitivity compared with that of culture. Many of these assays, unlike the optical immunoassay evaluated in the present study, use either a monoclonal or a polyclonal anti-group A streptococcal carbohydrate antibody in a latex agglutination or enzyme immunoassay format. Anhalt and colleagues (1) noted a sensitivity of 68% for one conventional rapid antigen assay, the Abbott TestPack Strep A assay, when compared with that of any positive culture obtained by one of three methods consisting of SBA incubated aerobically, selective streptococcal agar incubated in 5 to 10% CO_2, and a subculture in TH. Recently, Wegner and colleagues (9) reported a sensitivity ranging from 31 to 50% for five conventional rapid group A streptococcal antigen assays when they were compared with a two-plate culture method consisting of a 5% SBA plate containing sullfamethoxazole incubated anaerobically and 5% sheep blood Trypticase soy broth incubated aerobically. These results corroborated prior findings for similar antigen detection methods by Hoffman (5) and Gerber (2). Wegner et al. (9) concluded that for these five rapid methods, backup culture of specimens with negative results was required.

The specificities of rapid antigen assays have generally been less of a problem. Anhalt and colleagues (1) noted a specificity of 99% for the Abbott TestPack Strep A assay. Wegner and colleagues (9) observed specificities ranging from 95.1 to 100% for four of the five antigen assays that they evaluated; however, one of the assays had a 28.3% rate of false positivity.

The rapid diagnosis of *S. pyogenes* by using DNA probe technology has recently been reported by others and us. The sensitivity of this method varied from 86 to 93.5%, and specificities ranged from 95 to 99.7% (4, 7, 8).

A highly sensitive and specific rapid test for group A streptococci which does not require a routine backup culture is of obvious utility. In our experience with the O.S. assay, approximately 80% of all throat swabs are negative for group A streptococci. Backup cultures therefore represent a significant portion of our workload. In our laboratory, the cost of performing an O.S. rapid assay and the cost of performing a culture, when adjusted for the average distribution of positive and negative specimens, is similar. A rapid GAS method for the detection of group A streptococci, even if it is more expensive than current kits on the market, could be cost-effective if the expenses related to the backup culture could be eliminated. Other potential laboratory benefits would include a reduction in personnel training and proficiency testing requirements. These changes could have a positive impact on smaller laboratories, including physician office laboratories. Additional benefits could be realized by physicians, including improved efficiency of the practice, fewer follow-up telephone calls, and improved patient satisfaction.

In the current study, we evaluated the new optical immunoassay antigen detection method, BioStar Strep A OIA. Two recent evaluations of this method by Harbeck and colleagues (3) showed high degrees of sensitivity (97.4 to 98.9%) and specificity (95.6 to 98.6%) when compared with those of TH-enriched culture method. In both studies, swabs were cultured in TH and on solid agar consisting of selective streptococcal agar inoculated in 5% CO_2 in one study and Trypticase soy agar containing 5% sheep blood incubated in 5% CO_2 in the other study. In both evaluations, culture on TH resulted in the recovery of more group A streptococci than culture on agar plates. Harbeck and colleagues (3) concluded that the OIA method is more sensitive than the standard culture methods that they evaluated for detecting group A streptococci, and because the OIA assay was highly specific it should eliminate the need to perform confirmatory cultures of negative samples.

In the current study we did not observe the same sensitivities and specificities for OIA reported by Harbeck and colleagues (3). Both studies used TH as a culture method. Harbeck et al. (3) used, in addition to TH, either a selective streptococcal agar culture incubated at 35°C in 5% CO_2 for 48 h with confirmation of streptococci by Streptex latex agglutination or a Trypticase soy blood agar culture incubated at 35°C anaerobically for 48 h confirmed by using PathoDx Strep Typing Sera (Diagnostic Products Corp. Los Angeles, Calif.). In contrast, we used, in addition to TH, 5% SBA incubated at 35°C in ambient air for 48 h, with confirmation of group A streptococci by Streptex latex agglutination. Of interest, the performance of our standard SBA protocol, which uses 5% SBA incubated in 35°C ambient air for 48 h with fluorescent-antibody instead of Streptex latex agglutination for confirmation of group A streptococci, exceeded those of the culture methods used by Harbeck et al. (3) when all culture methods were compared with the results of culture in TH. The sensitivity of our standard SBA protocol was 97.2% compared with 86% for the culture method of Harbeck et al. (3).

In our study we chose to compare all testing methods against a combination of test results which we felt best represented a true-positive or gold standard result. We also evaluated a rapid antigen assay, the Pacific Biotech CARDS O.S. In our evaluation, compared with the combined test result, the sensitivity of OIA (81.0%) was greater than that of the O.S. rapid testing method (74.4%), but was less than that of our standard SBA culture method (92.3 and 94.1% compared with OIA and O.S., respectively).

The comparison of OIA and O.S with the quantity of *S. pyogenes* on SBA showed, for both antigen detection methods, a better correlation when the growth was 4+ and 3+ (94.4% agreement for OIA and 88.2% agreement for O.S.) than when the growth was 2+ or 1+ (38.7% and 23.3% agreement, respectively). The clinical significance of the presence of a low number of colonies on SBA in uncertain and may represent colonization. In addition, since our study involved the inoculation of SBA plates prior to rapid antigen testing, fewer organisms may have been available on the swabs for antigen detection. However, Libertin and colleagues (6) showed that with sequential inoculation of six culture plates with the same swab there was no difference between the number of *S. pyogenes* organisms isolated on the first plate and the number isolated on the last plate.

We conducted a review of the medical histories of the 70 patients whose specimens showed discrepancies between the results obtained by the antigen detection methods and those

requiring organism growth. In 7 of 70 (10%) patients whose specimens had discrepant results, where was a history of antibiotic use within 1 month of specimen collection which could possibly account for some of the discrepant results if the treatment resulted in growth inhibition or the presence of nonviable organisms in the pharynx. In such cases, cultures might be expected to be negative while the rapid antigen detection methods were positive. However, in our study, four of seven (57%) of patients with discrepant results and a history of recent antimicrobial agent use provided specimens that were culture positive and negative by a rapid antigen detection method. For these patients sampling discrepancies may have occurred.

The results of the current study indicate that the BioStar OIA is a suitable screening method for detecting *S. pyogenes* directly from throat swabs. Compared with our standard culture method, OIA is of insufficient sensitivity to eliminate the need for backup cultures for specimens with negative OIA results.

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