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Specificity Study of Kits for Detection of Group A Streptococci Directly from Throat Swabs

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A total of 78 *Streptococcus* strains, 15 *Staphylococcus* strains, and 2 *Stomatococcus* strains were used to test the specificity of 18 different antigen detection systems for group A streptococci and five products that detect a specific enzyme associated with group A streptococci. All streptococcal strains possessing the group A antigen were correctly identified with 31 different lots of reagents in the 18 antigen detection systems. The specificities of the 31 different lots of reagents ranged between 88.5 and 100%. A limited number of nonspecific reactions were observed with *Enterococcus gallinarium*, group C *Streptococcus* strain C23, and *Staphylococcus aureus* F49 and Cowan 1. The antigen detection kits that used enzymes as the extraction reagent gave slightly more specific results than did the kits that used chemical extraction reagents. The reagents in the five kits designed to detect the enzyme pyroglutamic acid arylamidase in *Streptococcus pyogenes* reacted positively with *S. pyogenes* (group A streptococcus); however, the reagents also reacted positively with all group D enterococcal streptococci and with about half of the staphylococcal strains treated. The nonspecificity of tests based on pyroglutamic acid arylamidase detection would seem to limit the usefulness of these kits with mixed cultures.

There are approximately 27 million throat swabs processed annually in the United States. Two recent studies have reported that, despite broad use of throat cultures for the detection of group A streptococci (GAS), throat swabs processed by conventional means have very little impact on the management of patients (5, 12). Holmberg and Faich (12), in their survey of physicians practicing in Rhode Island, reported that 87% of primary-care physicians prescribed antimicrobial therapy before culture results were known and that 40% continued antimicrobial therapy for 10 days regardless of culture results. These authors concluded that current throat-culturing practices probably have very little influence on the treatment of streptococcal pharyngitis in the state. Cochi et al. (5), in a nationwide survey of practicing physicians, reported that 25% of physicians always take specimens for culture, 52% selectively do so, and 23% never do so for patients with acute sore throats. Pediatricians were more likely to always take culture specimens (54%) and less likely (5%) to never take culture specimens. When primary-care physicians were asked whether they begin treatment before culture results are known, 42% said yes, always; 55% said yes, selectively; and 3% said no. When physicians who took throat cultures were asked whether they discontinued antimicrobial therapy if the culture was negative, 58% said yes and 42% said no. From these responses, we can conclude that throat cultures as they are now used in the United States have very little impact on the management of patients.

For any microbiologic test to be useful, it should have some influence on the management of patients. The most often stated reason for not using the results of conventional throat cultures to influence the decision to prescribe or not to prescribe antimicrobial therapy was the delay in getting the culture results (5).

The development of new antigen detection systems which claim to identify GAS directly from throat swabs seems to have the potential for improving the management of patients with acute sore throats, because the identification of GAS is made within 10 to 70 min of taking the swab from the patient. Some of these kits have been evaluated for specificity and sensitivity in various clinical settings (2–4, 8–11, 13, 27, 28).

This study was undertaken to attempt to measure the probable specificity of as many kits as could be obtained from commercial suppliers. Most of the kits studied have not had extensive specificity evaluations, and reports of extensive clinical evaluations have been limited to only two of these products.

MATERIALS AND METHODS

Strains. All strains were stock strains from our culture collection and were identified by conventional means (7). The following strains were used in this study: 18 strains with group A antigen (11 typical beta-hemolytic *Streptococcus pyogenes*, each with a different M type; 2 nonhemolytic *S. pyogenes*; 2 beta-hemolytic *S. anginosus*; 3 alpha-hemolytic *S. intermedius*); 9 typical group B strains representing 6 different serotypes; 8 group C *Streptococcus* (7 *S. equisimilis* and 1 *S. zooepidemicus*); 10 group D *Streptococcus* representing 7 different species; 5 group F *Streptococcus*; 7 group G *Streptococcus*; and 4 strains representing Ottens types I through IV. In addition, I used 14 viridans group *Streptococcus* strains, all representing different antigenic characteristics (6, 29); 15 *Staphylococcus* strains representing 7 species; 3 *S. pneumoniae* strains; and 2 *Stomatococcus mucilaginosus* strains (1). I believe that these strains are representative of bacteria that are commonly found in the posterior pharynx of humans in high enough concentrations to cause potential reactions with the GAS antigen detection kits.

Antigen detection systems. Culturette Brand 10-Minute Group A Strep ID kits were obtained from Marion Laboratories, Kansas City, Mo. DAI Strep A Latex and Direct Antigen ID Strep A Tests were obtained from Difco Laboratories, Detroit, Mich. Detect-A-Strep kits were obtained from Antibodies, Inc., Davis, Calif. Directigen Group A Strep and Directigen Rapid Group A Strep Tests were obtained from Hynson, Wescott and Dunning, Baltimore,

TABLE 1. Specificity of direct antigen test kits for group A *Streptococcus* identification

Method and kit	Lot tested	Specificity (%)
A. Chemical extraction, slide agglutination detection		
Phadirect Strep A 50	A8326	97.8
	1089	98.7
	8798	98.5
	27200	100
Culturette Brand 10-minute Group A Strep ID	401603	100
	40667	97.7
Detect-A-Strep	7AH31W	100
	5A0003	100
PathoDx Strep A	009	100
	017	98.3
Directigen Rapid Group A Strep Test	300	98.3
	500	97.3
	502	97.4
Q Test Stat Strep	303	98.6
DAI Strep A Latex	R00256	100
Respiralex	LK14	88.5
	LK16	96.0
SKD RapidTest-Strep	2015	97.4
Streptogen 100	080968	97.4
B. Chemical extraction, ELISA detection		
Murex SUDS Group A Strep Test	No lot no.	100
Quidal Strep Group A	0309D23901	100
	0305E0402	100
Testpack Strep A	89484EG	97.4
Ventrescreen Strep A	01944	100
	02754	100
C. Enzyme extraction, slide agglutination detection		
Direct Antigen ID Strep A Test	1964	98.4
	733315	100
Directigen Group A Strep Test	202	100
Q Test Strep	614	100
Streptex Direct A	K878860	100
	K967110	100

Md. Murex SUDS Group A Strep Tests were obtained from Murex Corp., Norcross, Ga. PathoDx Strep A kits were obtained from Diagnostic Products Corp., Los Angeles, Calif. Phadirect Strep A 50 kits were obtained from Pharmacia, Inc., Piscataway, N.J. Quidal Strep A kits were obtained from Quidal, La Jolla, Calif. Q Test Stat Strep and Q Test Strep kits were obtained from Clay Adams, Parsippany, N.J. Respiralex kits were obtained from Medical Technology Corp., Somerset, N.J. SKD RapidTest-Strep kits were obtained from SmithKline Diagnostics, Sunnyvale, Calif. Streptex Direct A kits were obtained from Wellcome Diagnostics, Research Triangle Park, N.C. The Streptogen 100 test kit was obtained from New Horizon Diagnostics, Columbia, Md. Testpack Strep A kits were obtained from Abbott Laboratories, North Chicago, Ill. Ventrescreen Strep A kits were obtained from Ventrex Laboratories, Inc., Portland, Maine.

Non-antigen detection systems. Several commercial systems that measure the presence or absence of pyrrolidonylarylamidase (PYRase) were also evaluated. Identicult-AE, Minitek PYR Disc, PYR-broth, Strep-A-Fluor, and Strep-A-Chek test kits were obtained from Scott Laboratories, Fiskeville, R.I.; BBL Microbiology Systems, Cockeysville, Md.; BioSpec, Inc., Dublin, Calif.; Carr-Scarborough Microbiologicals, Inc., Stone Mountain Ga.; and EY Laboratories, Inc., San Mateo, Calif., respectively.

Evaluation procedure. Strains were streaked to coded tryptic soy-blood-agar plates containing 5% sheep blood (BBL). Plates were incubated overnight in a candle extinction jar at 35°C. Dacron-tipped swabs with plastic shafts were used, unless swabs of other material were recommended or provided by the supplier. Swabs were streaked over approximately a 30-mm portion of pure growth of the test strains. All extractions, slide agglutination tests, modified enzyme-linked immunosorbent assays (ELISAs), and PYRase tests were performed according to the instructions provided by the manufacturers in the package inserts. All GAS giving negative identification test results and all non-group A strains giving positive identification test results were retested with the product being evaluated. Repeated misidentifications were reidentified by conventional tests.

The specificities of the products were calculated by dividing the number of true-negative reactions by the true-negative plus false-negative reactions.

RESULTS

The specificities and lot numbers of 31 different lots of 18 products are listed in Table 1. The products listed in part A of Table 1 are based on chemical extraction of the group A antigen and slide agglutination testing for the antigen in the extract. The time required to process the swabs from patient to final identification (turnaround time) was about 5 to 12 min. The products listed in part B of Table 1 are based on chemical extraction of the group A antigen and modified ELISA detection of the antigen in the extract. The turnaround times for these procedures were 10 to 20 min. The products listed in part C of Table 1 were based on enzyme extraction of the gorup A antigen and slide agglutination testing for the antigen in the extract. The turnaround times for these products were 60 to 70 min.

All of the products listed in Table 1 gave positive reactions with all strains possessing the group A antigen.

Only one lot of one product had a specificity of less than 96%. The first lot of Respiralex (LK14) cross-reacted with nearly all the *Staphylococcus* strains used in this study. These cross-reactions were not present in the second lot (LK16) of reagents.

Staphylococcus aureus strains were more likely to cross-react than other strains used in this study. *S. aureus* F49 and Cowan 1 reacted with five and four products, respectively, whereas strains 42BP and 64BP reacted with two products each. Of the streptococci, group C strain C23 and *Enterococcus gallinarium* each reacted with five products. No other strain reacted with more than two lots of reagents.

In general, the specificities for the chemical extraction-ELISA detection systems and enzyme extraction-slide agglutination systems were higher than those for chemical extraction-slide agglutination systems (Table 1).

The test results of five products designed to detect the presence of PYRase are shown in Table 2. Only one of these products (Strep-A-Fluor) is marketed as a direct test for GAS. Of the streptococci possessing group A antigen, only *S. pyogenes* strains possess PYRase. Strains of *S. anginosus* and *S. intermedius* did not react with any of the products for detection of PYRase. PYRase was present in all *Enterococcus* sp. strains and several of the *Staphylococcus* sp. strains. Strains of *S. haemolyticus*, *S. saprophyticus*, and *Stomatococcus mucilaginosus* were consistently positive for PYRase in all products. *S. aureus* strains were negative in all

TABLE 2. Percent positive reactions of test strains in tests designed to detect PYRase

Test	Time	Lot no.	Positive reactions (%) for:				
			S. pyogenes	Other streptococci	Enterococci	Viridans group strepto-cocci	Staphylo-cocci and stomato-cocci
PYR-broth	4 h	063272	100	–[a]	100	–	48
Strep-A-Fluor	5 min	A454	100	–	100	–	NT[b]
		021186	100	–	100	–	50
Strep-A-Chek	10 min	032203	100	–	100	–	33
Identicult-AE	10 min	No lot no.	100	–	100	–	33
Minitek-PYR Disc	10 min	255758	100	–	100	–	48

[a] –, No reaction (all strains).
[b] NT, Not tested.

tococcus mucilaginosus were consistently positive for PYRase in all products. *S. aureus* strains were negative in all tests except for PYR-broth, in which two of four strains were positive. Strains of *S. epidermidis* and *S. warneri* were consistently negative for PYRase, whereas strains of *S. simulans* and *S. cohnii* gave variable results with the PYRase tests.

DISCUSSION

These results indicate that the rapid antigen detection devices are very specific. The specificities of these products are dependent upon the strains chosen for the study. Do they represent a sample of oral bacteria that may be found in high enough concentrations in the human oral cavity to cause cross-reactions? The true test of specificity must be obtained

TABLE 3. Specificity and sensitivity of direct identification of GAS from throat swabs

Reference or source	No. of swabs processed/no. with GAS	Selective media	Incu ATM[a]	Sensitivity (%)	Specificity (%)
A. Culturette 10-min GAS ID					
Campos and Charilaou (3)	415/150	Yes	AN	62.0	99.6
Chang and Mohla (4)	435/70	No	A	90.0	99.2
Gerber (8)	339/108	No	CO_2	83.0	99.0
Gerber et al. (9)	313/257	No	A	88.0	96.0
Kellogg and Manzella (14)	905/170	No	AN	63.0	?
Miceika et al. (17)	813/92	No	AN	92.4	92.8
Roddey et al. (21)[b]	512/201	No	AN	72.0	98.0
Schwartz et al. (22)[b]	425/211	No	AN	93.4	90.2
Shriner et al. (24)[b,c]	400/238	No	?	97.8	100.0
Slifkin and Gil (25)	557/82	No	AN	95.1	100.0
Venezia et al. (26)	64/26	No	CO_2	100.0	97.0
Wagener and Remington (27)	722/105	No	AN	89.5	95.5
White et al. (28)	589/84	No	CO_2	78.0	88.0
B. Directigen					
Berkowitz et al. (2)	1,044/214	No	A	88.3	98.0
Gerber et al. (11)	263/85	No	CO_2	84.0	99.0
Kamm and Bille (13)	229/46	No	CO_2	93.0	98.0
Matteson and Anhalt (15)	964/93	No	?	61.0	99.0
McCusker et al. (16)	500/144	No	AN	90.9	99.2
Miller et al. (18)	149/23	Yes	CO_2	91.0	98.0
S. Redd, R. Facklam, and M. Cohen, unpublished data	251/85	No	AN	90.6	94.0
C. Detect-A-Strep					
Campos and Charilaou (3)	539/135	Yes	AN	64.4	96.5
Wagener and Remington (27)	744/109	Yes	AN	83.5	98.6
D. Others					
DAI, Difco Venezia et al. (26)	196/54	No	CO_2	83.0	99.0
Phadirect Ogay and Bille (19)	307/66	No	CO_2	86.4	90.0
Streptex Petts and Mantell (20)	265/43	No	CO_2	87.8	100.0
Strep-A-Fluor Gerber et al. (10)	118/45	No	AN	44.0	78.0
Directigen, Rapid Group A Strep Test Redd et al., unpublished data	249/86	No	AN	62.8	96.9

[a] Incu ATM, Incubation atmosphere; A, aerobic; AN, anaerobic; CO_2, increased CO_2 in atmosphere or CO_2 incubator.
[b] Office setting; all others, laboratory setting.
[c] Positive culture only when blood agar plate contains 100 colonies.

from comparing the antigen detection test results with GAS recovery by conventional techniques. However, there is an unfortunate problem with this comparison. There is no conventional standard procedure that is universally accepted. There are a wide variety of techniques available to both microbiologists and practicing physicians who perform throat cultures. The use of selective media or atmospheres of culture incubation (or both) is recommended by some but not by others. The recent statement of Gerber (8) that if you asked 10 people how they do throat cultures, you would probably get 10 different answers is a disconcerting but accurate assessment of the current situation.

To help validate the conclusions of this study that the antigen detection systems are specific, I compared the results of this study to the results of other studies for which the sources of the throat swabs were patients with upper-respiratory-tract illness. The results of several studies are summarized in Table 3. Note that the authors of some of these reports used selective media, whereas others did not. Also note that the authors of these reports used three different incubation atmospheres for their "conventional" isolation procedures.

All the reports in part A of Table 3 used the Culturette 10-Minute GAS identification procedure. The specificities ranged from 88 to 100%, but most (10 of 12 reports) were between 95.5 and 99.0% specific. My results with two lots of this product were 97.8 and 98.7% specific.

There are seven reports about the Directigen GAS detection system. The unpublished report is a study performed by a colleague. The specificities mentioned in the published reports were 98 to 99%. The specificity of the one lot of Directigen GAS reagents evaluated in this study was 100%.

Two studies have evaluated the Detect-A-Strep GAS kit. The specificity of the Detect-A-Strep product evaluated in this study was 100% (two lots). The specificities reported by the other authors were 96.5 and 98.6% (Table 3, part C).

Three of the five studies, each using a different product, summarized in Table 3, part D, reported specificities nearly identical to those observed in this study. The specificity of the Phadirect Strep A test reported by Ogay and Bille (19) was lower than I observed, but these authors delayed processing some of the swabs. This could have altered both the conventional and direct antigen test results. The specificity of the Strep-A-Fluor test was also low (78%). I did not calculate the specificity of the Strep-A-Fluor test in the present study, because the data could be easily manipulated by the choice of test strains. The Strep-A-Fluor test is based on detection of the enzyme PYRase, which is known to occur not only in group A *Streptococcus* but in *Enterococcus* and *Staphylococcus* species as well. The results of Gerber et al. (10) indicate that either enterococci or staphylococci are among the bacteria found in the oral cavity that may give positive PYRase reactions. It is dubious whether this test should be used as a direct test for the identification of GAS.

I have also listed the sensitivities reported by the authors of these studies in Table 3. There is a wide range of results, 62 to 100% sensitive. Even studies using the same product with the same conventional procedure have a difference of 20% in sensitivity (Roddey et al. [21] versus Schwartz et al. [22]). There is a general trend that the products using enzymes for extraction (Directigen, DAI, and Streptex) have sensitivities near or at 90%. Also note how one group of authors improved the sensitivity of one product to 97.8% by disregarding the cultures with fewer than 100 beta-hemolytic colonies (24). There is a controversial theory held by some streptococcologists that if the conventional throat culture has between 1 and 10 colonies, then the patient is a probable carrier and does not have streptococcal pharyngitis. This controversy has not been settled.

In summary, I believe that the results of these studies have been substantially validated. This representative collection of bacteria appears to be useful in predicting potential cross-reactions and, thus, specificities of products.

Users of these products may encounter some difficulty in reading slide agglutination tests unless they have some experience. I generally found from my personal experience and from what I have observed firsthand that after reading about 30 reactions the difficulties disappeared. The most difficult reactions to interpret were those in which nonspecific agglutination occurred, that is, when both the group A and control reagents were positive. These reactions should be interpreted as negative, but the tendency is to read them as positive. These difficulties were not encountered in interpreting the modified ELISAs.

The antigen detection devices offer identification of the majority of patients with GAS in much less time than that required for conventional culturing. Therefore, they can and should be put into use. The high specificity allows for the immediate treatment of patients with positive test results. The somewhat-lower-than-desired sensitivity does not allow us to discontinue the use of conventional culturing techniques when a negative antigen test is observed. The best scenario at this time is for physicians to obtain a pair of swab samples from their patients; use a direct antigen test for one swab and, if the direct antigen test is negative, prepare a culture from the second swab. Physicians should prescribe antimicrobial therapy for all patients with positive direct antigen tests, prescribe antimicrobial therapy for those patients who give the clinical impression of having strep throat even in view of a negative test result, and withhold antimicrobial therapy from all patients with negative direct antigen test results and for whom there is any reasonable doubt about clinical impressions. More than likely, this scenario would still probably result in the overtreatment of patients, as is currently being done, but it should decrease the indiscriminate distribution of antimicrobial agents.

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