JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1993, p. 2996–3000
0095-1137/93/112996-05$02.00/0

Vol. 31, No. 11

Rapid Detection of *Streptococcus pyogenes* in Pediatric Patient Specimens by DNA Probe

LISA L. STEED,[1]† E. KENT KORGENSKI,[2] AND JUDY A. DALY[1,2]*

Primary Children's Medical Center[2] and Department of Pathology,[1] University of Utah School of Medicine, Salt Lake City, Utah 84132

Received 17 May 1993/Returned for modification 4 July 1993/Accepted 17 August 1993

A chemiluminescent DNA probe test (Group A Streptococcus Direct Test; Gen-Probe, Inc., San Diego, Calif.) for rapid, direct detection of cRNA of *Streptococcus pyogenes* in throat swabs was compared with conventional culture and identification techniques. Throat swabs from 277 patients suspected of having streptococcal pharyngitis were examined. By DNA probe alone, 10 specimens were positive, 51 were positive by both assays, and 8 were positive by culture alone. Thus, DNA probe sensitivity, specificity, and positive and negative predictive values were 86, 95, 84, and 96%, respectively. Including an indeterminate category, sensitivity, specificity, and positive and negative predictive values were 89, 96, 86, and 97%, respectively. After discrepancy testing, these values for the raw data improved to 90, 98, 93, and 97%, respectively. None of the 24 specimens that grew non-*S. pyogenes* beta-hemolytic streptococci in culture were positive by the DNA probe. Because mucoid *S. pyogenes* strains are more virulent than nonmucoid strains, 24 isolates were retrospectively tested with the DNA probe to ensure that both types would be detected equally well. Isolates were examined in pure cultures as well as mixed with representative normal oral flora. There was no statistical difference in detection of any of the four groups. Group A Streptococcus Direct Test is a rapid, sensitive, and specific test for *S. pyogenes*.

Streptococcus pyogenes causes 15 to 30% of all pharyngitis cases and may cause up to 50% of pediatric pharyngitis during late winter and early spring (10, 19). While other bacteria and viruses will also cause pharyngitis, *S. pyogenes* has been associated with invasive disease and serious sequelae in healthy individuals. Diagnosis of streptococcal pharyngitis is crucial to ensure adequate treatment of the infected individual and avoid the development of rheumatic fever (10, 19). The rheumatic fever outbreak that began in 1985 in the intermountain West coupled with an increasing incidence of toxic streptococcal syndrome nationwide has heightened our interest in more efficient methods of identification of *S. pyogenes* (2, 20, 21).

Throat culture is currently the definitive method for identification of streptococcal pharyngitis. However, results are not available until 18 to 72 h after specimen collection. Rapid testing methods have been developed to reduce the time to results to less than 1 h. Currently, there are numerous commercially available rapid testing methods: fluorescent antibody (6), latex agglutination (8), and enzyme immunoassay (18). Each method requires approximately 2 to 5 min of hands-on time. The sensitivity and specificity range from 60 to 85% and 95 to 99%, respectively (7, 11, 14, 17, 18). The American Heart Association requires that culture be performed only if the rapid streptococcal test is negative to ensure detection and treatment of all *S. pyogenes* infections in order to prevent nonsuppurative complications (4).

In order to eliminate throat cultures entirely, a rapid method for detecting *S. pyogenes* in patient specimens must be able to identify the equivalent of 1 CFU. An acridinium ester-labelled chemiluminescent DNA probe test (Group A Streptococcus Direct Test [GASD]; Gen-Probe, San Diego, Calif.) has been developed recently to directly detect cRNA of *S. pyogenes* in throat swabs. The purpose of this study was to evaluate GASD in comparison with conventional culture during the clinical-trial phase of development. Because of the association of virulence with capsule production, we also evaluated the ability of GASD to detect mucoid and nonmucoid isolates in pure culture and in normal oral flora.

MATERIALS AND METHODS

Specimen collection and cultivation. Throat swabs from a total of 277 patients suspected of having streptococcal pharyngitis were examined. Each swab was plated onto sheep blood agar with sulfamethoxazole (BBL Microbiology Systems, Cockeysville, Md.), and the plate was streaked for isolation and stabbed. After incubation in CO_2 at 37°C, the plates were examined for beta-hemolytic colonies daily for a total of 3 days. If no beta-hemolytic colonies were seen after overnight incubation, a streak of growth from the primary quadrant was subcultured onto selective streptococcal agar (BBL). Any beta-hemolytic colonies were screened for *S. pyogenes* with susceptibility to bacitracin (Taxo A, BBL) and sulfamethoxazole (BBL) or with detection of pyrrolidonyl arylamidase activity by the filter paper substrate test (E-Y Laboratories, San Mateo, Calif.). Presumptive *S. pyogenes* isolates were confirmed serologically with the PathoDx Strep Grouping Kit (Diagnostic Products Corp., Los Angeles, Calif.) according to the manufacturer's instructions.

Gen-Probe GASD. After culture inoculation was completed, throat swabs were allowed to accumulate up to 3 days before testing with GASD. Our batch size was limited to 20 specimens, and so GASD was usually run 3 times weekly. Specimens were submitted on four types of swabs: wooden-shafted Dacron swabs (Pur-Wraps; Harwood Products, Guilford, Maine), plastic-shafted Dacron swabs

* Corresponding author.
† Present address: Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425.

(Pur-Wraps), Culturette (Becton Dickinson Microbiology Systems), and Cellmatics (Difco, Detroit, Mich.). A representative sterile swab was used as a control for each swab type in a batch of specimens. The GASD procedure used during clinical trials was as follows. Viable *S. pyogenes* and *Streptococcus agalactiae* cell controls and a patient swab(s) were placed in polypropylene tubes containing lysing reagent. Positive and negative kit controls were placed into polypropylene tubes without lysing reagent. The tubes were incubated in a 95°C heating block for 10 min and then cooled at room temperature for 5 min. Extracts were expressed from all swabs. Swabs were returned to their containers and were stored at −70°C in case they were needed for discrepancy testing. Fifty microliters of both control and patient extracts was transferred to new polypropylene tubes. The remaining lysates were stored at −70°C in case they were needed for discrepancy testing. Fifty microliters of probe was added to each tube; all tubes were covered and shaken gently. The tubes were incubated in a 60°C water bath for exactly 30 min. After hybridization, 300 μl of selection reagent was added to each tube; the tubes were covered and vortexed thoroughly. The tubes were incubated in a 60°C water bath for exactly 7 min. After selection, the tubes were uncovered and allowed to cool at room temperature for 5 min. Results were reported as relative light units (RLU) by a Leader 50 luminometer within 1 h. Specimen results were calculated as the difference between the luminometer RLU and the mean RLU of the sterile swab control for that swab type. RLU are reported here as RLU (in thousands). Adjusted RLU above the cutoff value of 3.2×10^3 were considered positive for this study.

Discrepancy testing. If the culture was positive and GASD was negative, the lysate was retested and an isolate from the plate was tested by GASD. If neither the lysate nor the isolate was positive, the isolate was retested by susceptibility to bacitracin and sulfamethoxazole or by pyrrolidonyl arylamidase activity and confirmed by serotyping. If the culture was negative and GASD was positive, growth from the inoculation area of both plates was subcultured onto another set of plates, incubated for up to 72 h, and examined for beta-hemolytic and nonhemolytic streptococcal colonies. In addition, the lysate was retested by GASD. If the discrepancy was not resolved, the white plug from the Culturette transport system or the envelope from the Dacron swabs was placed in a tube of Todd-Hewitt broth with sulfamethoxazole. After incubating overnight, the broth was subcultured onto a set of plates, incubated for up to 72 h, and examined for beta-hemolytic colonies.

Comparison of mucoid and nonmucoid *S. pyogenes* by GASD. Mucoid and nonmucoid *S. pyogenes* strains were obtained from clinical specimens submitted to the clinical microbiology laboratory of Primary Children's Medical Center. The isolates had been frozen at −70°C in skim milk. Subcultures of these isolates were passed twice on sheep blood agar (BBL) with incubation as described above. Isolates were confirmed as being mucoid or nonmucoid. A sterile swab was touched to one isolated colony of each isolate and tested as described above. A second sterile swab was thoroughly moistened in fresh saliva and then touched to one isolated colony of approximately the same size and tested as before.

RESULTS

Effect of swab type on GASD results. Specimens in our hospital are submitted on four types of swabs: wooden-shafted Dacron swabs, plastic-shafted Dacron swabs, Culturettes, and Cellmatics swabs. To determine the background RLU for these swab types, a sterile swab was used as a negative control for each swab type present in a batch of specimens. A total of 16 control wooden-shafted swabs were tested by GASD, with a mean RLU of 2.5 ± 0.8 (range, 1.4 to 4.3); 1 control swab was discounted for very high RLU (32.6). Thirteen control Culturettes gave a mean RLU of 2.2 ± 0.4 (range, 1.8 to 3.1), and six control plastic-shafted swabs gave a mean RLU of 1.6 ± 0.4 (range, 1.3 to 2.2). There were only two control Cellmatics swabs tested (RLU of 2.6 and 3.3).

A major problem with the wooden-shafted swabs was that the wood absorbed much of the lysing reagent and expressing liquid back out of the swab was very difficult. For this reason, several patient specimens were tested with less than the 50 μl of lysate required. In most wooden-shafted swab specimens, there was sufficient lysate for the initial testing but insufficient lysate remaining for discrepancy testing.

Comparison of culture with GASD. Of a total of 277 specimens tested, 51 were positive by culture and GASD (18%), 8 were positive by culture only (21% isolation rate overall), and 10 were positive by GASD only (22% detection rate overall).

As shown in Table 1, culture results of 3+ or 4+ for *S. pyogenes* isolated correlated well with an RLU value of ≥100 (see Table 1 for explanation of 1+ to 4+ scale). Culture results of ≤5 CFU or 1+ for *S. pyogenes* isolated were predominantly in the RLU range of <2 (technically negative) to 109, with one 1+ culture having an RLU value of 861. Interestingly, of the 10 cultures reported as having ≤5 CFU of *S. pyogenes*, 7 were positive and 3 were negative. The GASD RLU for these cultures were between 0.4 and 109, and there was no correlation between number of CFU and RLU (data not shown). The sensitivity for these 10 cultures

TABLE 1. Direct detection of *S. pyogenes* by GASD compared with that by culture

GASD RLU (10^3)	No. of cultures with result:						
	No GAS[a]	? CFU[b]	≤5 CFU[c]	1+[d]	2+[d]	3+[d]	4+[d]
<2.2	198	1	1	1	2		1
2.2–3.2	10		2				
3.2–4.2	2	2					
4.2–4.5				1			
4.5–10.0	5	1	2	2			
10–20	3		3	1			
20–50		1	1	1			
50–100	1	1			1		
100–200			1			3	1
200–300						2	2
300–400						1	
400–500					1		
500–600					1	1	
600–700							
700–800		2				1	2
800–900				1			
900–1,000							2
1,000–1,500					1		1
1,500–2,000					2	1	2
>2,000		1				1	3

[a] GAS, group A streptococci.
[b] Amounts of *S. pyogenes* isolated were not specified on the worksheets.
[c] Amounts of *S. pyogenes* isolated were given in number of CFU present.
[d] 1+, <10 CFU; 2+, 10 to 30 CFU; 3+, 30 CFU or growth in the third quadrant; 4+, growth in the fourth quadrant.

TABLE 2. Sensitivity, specificity, and predictive values for GASD versus culture

GASD[a] result	Culture result		Total
	No. positive	No. negative	
No. positive	51	10	61
No. negative	8	208	216
Total	59	218	

[a] Sensitivity, 86%; specificity, 95%. Predictive values: positive, 84%; negative, 96%.

alone was 70% but improved to 75% for those cultures with ≤5 CFU or 1+ for *S. pyogenes*. GASD was initially negative for a culture with 4+ for *S. pyogenes* isolated but was positive upon retesting. Sensitivity, specificity, and positive and negative predictive values overall were 86, 95, 84, and 96%, respectively (Table 2). If we established a zone of indeterminate results—those results between 2.2 and 4.2—the sensitivity, specificity, and predictive values were 89, 96, 86, and 97%, respectively (Table 3). Of the 19 discrepancies between GASD and culture, one culture was shown to be falsely negative (GASD RLU for that specimen were 81.2 and 65.5). Six GASD positive results were negative upon repeat GASD testing; initial RLU were between 7 and 14 and were ≤2.2 upon retesting. Three GASD negative results were positive upon repeat GASD testing, and five GASD results did not change positive/negative results. Additional testing was unable to resolve four discrepancies. Revised correlations of GASD and culture are shown in Table 4. Sensitivity, specificity, and positive and negative predictive values after discrepancy testing were 90, 98, 93, and 97%, respectively (Table 5).

After the study was completed, we reassessed our data in view of the 4.5 cutoff to be used in the marketed GASD kit. The sensitivity, specificity, and positive and negative predictive values of the raw data (without the subtraction of the swab controls) were 90, 89, 70, and 97%, respectively. There were 29 specimens for which the results became discrepant with the 4.5 cutoff. We were unable to resolve 7 of these 29 discrepancies. After discrepancy testing, these values improved to 93, 94, 82, and 98%, respectively.

A total of 24 non-*S. pyogenes* streptococcal isolates were grown in culture. These included 12 group B, 5 group C, 2 group F, and 5 group G streptococci. All but one of these isolates were clearly negative by GASD. This one culture isolate was confirmed with serotyping as group B while the swab lysate was strongly positive (over 20 RLU) by GASD. Additional testing was unable to resolve this discrepancy.

Detection of mucoid and nonmucoid *S. pyogenes* by GASD. No mucoid strains of *S. pyogenes* were isolated from any of the test specimens. Therefore, we tested 24 previously isolated mucoid strains and 24 nonmucoid strains of *S. pyogenes*. In order to more closely approximate the conditions of a pharyngeal specimen, a duplicate set of strains was combined with normal oral flora prior to testing. There was no significant difference between the RLU of mucoid isolates and those of nonmucoid isolates or between the RLU of pure cultures and the RLU of those with normal oral flora (data not shown).

TABLE 4. Direct detection of *S. pyogenes* by GASD compared with that by culture (after discrepancy testing)

GASD RLU (10^3)	No. of cultures with result:						
	No GAS[a]	? CFU[b]	≤5 CFU[c]	1+[d]	2+[d]	3+[d]	4+[d]
<2.2	205	1		1	2		
2.2–3.2	9		2				
3.2–4.2		2					1
4.2–4.5				1			
4.5–10.0	2	1	2	2			
10–20	2		4	1			
20–50		1	1	1			
50–100		2			1		
100–200			1			3	1
200–300						2	2
300–400						1	
400–500					1		
500–600					1	1	
600–700							
700–800		2				1	2
800–900				1			
900–1,000							2
1,000–1,500					1		1
1,500–2,000					2	1	2
>2,000		1				1	3

[a] GAS, group A streptococci.
[b] Amounts of *S. pyogenes* isolated were not specified on the worksheets.
[c] Amounts of *S. pyogenes* isolated were given in number of CFU present.
[d] For definitions, see Table 1, footnote *d*.

DISCUSSION

We participated in clinical trials of the Gen-Probe GASD, which directly detects rRNA of *S. pyogenes* on pharyngeal swabs. A total of 277 patient specimens were tested with GASD in comparison with culture. Sensitivity, specificity, and positive and negative predictive values were 86, 95, 84, and 96%, respectively. Use of an indeterminate category produced sensitivity, specificity, and positive and negative predictive values of 89, 96, 86, and 97%, respectively. After discrepancy testing, these values for the raw data improved to 90, 98, 93, and 97%, respectively. In addition, GASD can detect mucoid *S. pyogenes* strains as effectively as it can detect nonmucoid strains. Thus, our data show that GASD is a rapid, sensitive, and specific test for *S. pyogenes*. In our

TABLE 3. Sensitivity, specificity, and predictive values for GASD without indeterminate results versus culture

GASD[a] result	Culture result		Total
	No. positive	No. negative	
No. positive	49	8	57
No. negative	6	198	204
Total	55	206	

[a] Sensitivity, 89%; specificity, 96%. Predictive values: positive, 86%; negative, 97%.

TABLE 5. Sensitivity, specificity, and predictive values for GASD versus culture (after discrepancy testing)

GASD[a] result	Culture result		Total
	No. positive	No. negative	
No. positive	53	4	57
No. negative	6	214	220
Total	59	218	

[a] Sensitivity, 90%; specificity, 98%. Predictive values: positive, 93%; negative, 97%.

study, the PathoDx rapid streptococcal latex agglutination test resulted in a sensitivity and specificity of 59 and 96%, respectively (data not shown). While low, these values are within the published range of sensitivities and specificities for rapid tests (7, 11, 14, 17, 18). In comparison with this rapid streptococcal test, GASD is more sensitive and equally specific.

Although GASD was positive on 10 occasions when there were ≤5 CFU and detected just 1 CFU from three of four specimens, culture is still considered to be the "gold standard" to detect small numbers of CFU of *S. pyogenes*. GASD was falsely negative for three cultures with ≤5 CFU, one culture with 1+, and two cultures with 2+. Sensitivity of the 10 cultures with ≤5 CFU alone was 70% but improved to 75% for those cultures with ≤5 CFU or 1+ for *S. pyogenes*. However, GASD was performed on the same swab previously used to inoculate the culture medium. If there are few organisms present on a swab, they might be removed by the process of inoculation and thus no cells would remain for detection by GASD. Still, the important issue is the clinical significance of small numbers of *S. pyogenes* isolates. Without clinical correlation and serological studies, we cannot determine whether these cultures represent streptococcal carriage or true infection (1). About 25 to 30% of all children with sore throat have a positive *S. pyogenes* throat culture, one-half of whom will develop significantly increased antistreptococcal antibody titers. Asymptomatic carriage occurs in about 15% of school-age children during the winter months; these children are not at risk of developing sequelae, nor do they need antibiotic therapy (1). Several reports in the literature correlate positivity of streptococcal culture results with patient symptomatology (16). Other reports contradict this position (12). The American Heart Association has stated that small numbers of *S. pyogenes* isolates do not differentiate a carrier from an acutely infected individual (4). There are many factors that affect the positivity of streptococcal cultures: antibiotic use prior to culture, duration of symptoms, specimen collection, culture medium, atmosphere of incubation, and duration of incubation (7, 12). In view of this information, culture remains the gold standard for detection of *S. pyogenes*.

Because background RLU vary greatly among swabs from different manufacturers and because of the potential of lot-to-lot variation in background RLU within a swab brand, Gen-Probe plans to recommend the use of standardized Culturette-type swabs which can be purchased separately from Gen-Probe or another distributor. Use of recommended swabs will alleviate the need for the luminometer to calculate adjusted RLU prior to categorizing the result as positive or negative. A simple cutoff of 4.5 is recommended for use with the recommended swabs. The GASD kit will have one kit positive control and one kit negative control to be run with each batch of specimens (personal communication).

We reassessed our data in view of the 4.5 cutoff. The sensitivity, specificity, and positive and negative predictive values of the raw data (without the subtraction of the swab controls) were 90, 89, 70, and 97%, respectively. There were 29 discrepancies between GASD and culture. Because the reassessment was conducted after the study was completed, we were unable to resolve 7 of the 29 discrepancies. After discrepancy testing, these values improved to 93, 94, 82, and 98%, respectively.

Practical considerations of GASD include cost and work flow analyses. The procedure calls for incubations at 95°C and at 60°C; thus, a heating block or water bath or both are necessary. The Leader 50 luminometer is required for the addition of detection reagents and to measure and report the intensity of the chemiluminescence reaction. The kit price will depend on order volume and has not been determined at the time of this writing. On the other hand, the kit testing procedure is very simple to perform and does not require the expertise necessary for culture interpretation, and the results are easily reproducible. The major work flow consideration is that the required incubation periods total 57 min. Only one incubation period is over 10 min long, and there are two critical incubation periods: 30 and 7 min. Thus, batching of patient specimens may be required for cost-effective use of GASD. Our batch size was limited to no more than 20 specimens and required no more than 2 h to complete. Considering these issues, GASD appears to be most valuable in the setting of a reference laboratory or a laboratory with a large number of outpatient specimens to be tested and staffing in multiple shifts. In this setting, one or two batches of 10 to 200 specimens each could be tested daily. However, if a laboratory is already using other Gen-Probe tests, incorporation of GASD into the testing regimen will probably be quite simple.

Gen-Probe tests other than GASD belong to either AccuProbe (5, 9), PACE (13, 15), or AccuPace (3). GASD is the first of a new generation of DNA probes which are capable of directly detecting pathogens in patient specimens without using a magnetic sphere separation step. If the utility of GASD proves as valuable to laboratory practice as the Gen-Probe assays previously mentioned, GASD will be a welcome addition to clinical microbiology.

ACKNOWLEDGMENTS

We gratefully thank the excellent staff of the Primary Children's Medical Center Microbiology Laboratory for their technical assistance and Lisa Anderson for her valued secretarial assistance. In addition, we thank Kathleen Clark and Diana Kobrin at Gen-Probe for their support of this evaluation.

This evaluation was supported in part by a grant from Gen-Probe, Inc.

REFERENCES

1. **Bisno, A. L.** 1990. *Streptococcus pyogenes*, p. 1519–1528. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, Inc., New York.
2. **Bisno, A. L.** 1991. Group A streptococcal infections and acute rheumatic fever. N. Engl. J. Med. **325**:783–793.
3. **Campos, J. M., and C. Mohla.** 1992. Detection of pharyngeal group A streptococci by DNA probe, abstr. 1036, p. 283. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother.
4. **Dajani, A. S., A. L. Bisno, K. J. Chung, D. T. Durack, M. A. Gerber, E. L. Kaplan, H. D. Millard, M. F. Randolph, S. T. Shulman, and C. Watanakunakorn.** 1988. Prevention of rheumatic fever. A statement for health professionals by the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, the American Heart Association. Circulation **78**:1082–1086.
5. **Daly, J. A., N. L. Clifton, K. C. Seskin, and W. M. Gooch III.** 1991. Use of rapid, nonradioactive DNA probes in culture confirmation tests to detect *Streptococcus agalactiae*, *Haemophilus influenzae*, and *Enterococcus* spp. from pediatric patients with significant infections. J. Clin. Microbiol. **29**:80–82.
6. **Facklam, R. R., and R. B. Carey.** 1985. Streptococci and aerococci, p. 154–175. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

7. **Gerber, M. A.** 1989. Comparison of throat cultures and rapid strep tests for diagnosis of streptococcal pharyngitis. Pediatr. Infect. Dis. J. **8**:820–824.
8. **Gerber, M. A., L. J. Spadaccini, L. L. Wright, and L. Deutsch.** 1984. Latex agglutination tests for rapid identification of group A streptococci directly from throat swabs. J. Pediatr. **105**:702–705.
9. **Goto, M., S. Oka, K. Okuzumi, S. Kimura, and K. Shimada.** 1991. Evaluation of acridinium-ester-labelled DNA probes for identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-Mycobacterium intracellulare* complex in culture. J. Clin. Microbiol. **29**:2473–2476.
10. **Gwaltney, J. M., Jr.** 1990. Pharyngitis, p. 493–498. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, Inc., New York.
11. **Hoffmann, S.** 1990. Detection of group A streptococcal antigen from throat swabs with five diagnostic kits in general practice. Diagn. Microbiol. Infect. Dis. **13**:209–215.
12. **Kellogg, J. A.** 1990. Suitability of throat culture procedures for detection of group A streptococci and as reference standards for evaluation of streptococcal antigen detection kits. J. Clin. Microbiol. **28**:165–169.
13. **Lees, M. I., D. M. Newnan, and S. M. Garland.** 1990. Comparison of a DNA probe assay with culture for the detection of *Chlamydia trachomatis*. J. Med. Microbiol. **35**:159–161.
14. **Mackenzie, A. M. R., M. M. Li, and F. T. H. Chan.** 1988. Evaluation of a kit for rapid detection of group A streptococci in a pediatric emergency department. Can. Med. Assoc. J. **138**: 917–919.
15. **Panke, E. S., L. I. Yang, P. A. Leist, P. Magevney, R. J. Fry, and R. F. Lee.** 1991. Comparison of Gen-Probe DNA probe test and culture for the detection of *Neisseria gonorrhoeae* in endocervical specimens. J. Clin. Microbiol. **29**:883–888.
16. **Roddey, O. F., Jr.** 1991. Quantitation of group A beta-hemolytic streptococci in throat cultures. J. Clin. Microbiol. **29**:1279–1280.
17. **Roddey, O. F., Jr., H. W. Clegg, L. T. Clardy, E. S. Martin, and R. L. Swetenburg.** 1986. Comparison of a latex agglutination test and four culture methods for identification of group A streptococci in a pediatric office laboratory. J. Pediatr. **108**:347–351.
18. **Schwabe, L. D., A. F. Gobbo, R. L. Gottschall, and E. L. Randall.** 1991. Comparison of TestPack Plus Strep A with selective and nonselective culture media for detection of group A streptococci. Diagn. Microbiol. Infect. Dis. **14**:367–372.
19. **Shulman, S. T.** 1989. Streptococcal pharyngitis: clinical and epidemiologic factors. Pediatr. Infect. Dis. J. **8**:816–819.
20. **Stevens, D. L., M. H. Tanner, J. Winship, R. Swarts, K. M. Reis, P. M. Schlievert, and E. Kaplan.** 1989. Reappearance of scarlet fever toxin A among streptococci in the Rocky Mountain West: severe group A streptococcal infections associated with a toxic shock-like syndrome. N. Engl. J. Med. **321**:1–7.
21. **Veasy, L. G., S. E. Wiedmeier, G. S. Orsmond, H. D. Ruttenberg, M. M. Boucek, S. J. Roth, V. F. Tait, J. A. Thompson, J. A. Daly, E. L. Kaplan, and H. R. Hill.** 1987. Resurgence of acute rheumatic fever in the intermountain area of the United States. N. Engl. J. Med. **316**:421–427.