

Clinical Trial Comparing Bacitracin with Strep-A-Chek for Accuracy and Turnaround Time in the Presumptive Identification of *Streptococcus pyogenes*

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In a clinical trial, Strep-A-Chek (a 10-min chromogenic test) was compared with the bacitracin disk susceptibility test for accuracy and turnaround time in the presumptive identification of *Streptococcus pyogenes*. Among 461 isolates of beta-hemolytic streptococci (344 throat isolates and 117 isolates from other sites), 303 group A *S. pyogenes* isolates were found. The sensitivities of the Strep-A-Chek and bacitracin tests were high (96.4 and 100%, respectively), but the bacitracin test had a lower specificity (84.2%) than the Strep-A-Chek test (98.7%). The predictive values for positive and negative test results were 99.3 and 93.4%, respectively, for Strep-A-Chek and 92.4 and 100%, respectively, for bacitracin. Strep-A-Chek correctly identified all isolates upon repeat testing. All bacitracin tests were performed on subcultures of isolates from the primary plate. Strep-A-Chek testing was performed on colonies from the primary plate when isolated colonies were available. This shortened the turnaround time for Strep-A-Chek compared with bacitracin by at least 24 h on nearly one-half (45%) of the isolates. A peripheral finding of this study was that sulfamethoxazole-trimethoprim blood agar offered no advantage over conventional blood agar with regard to the number of false-positive bacitracin tests obtained from each medium.

The bacitracin disk susceptibility test is commonly used to presumptively identify *Streptococcus pyogenes* (group A streptococci). The test is highly sensitive but not specific. It is estimated that 6 to 12% of non-group A beta-hemolytic streptococci are sensitive to bacitracin (2, 4, 7). If a laboratory identifies a presumptive positive group A streptococcus on the basis of the bacitracin disk susceptibility test, a significant number of patients may receive antibiotic therapy for a presumed group A streptococcal infection although no group A streptococci are present. Bacitracin susceptibility is especially prevalent among streptococci with group C antigen (4, 9). In a previous study, we found bacitracin susceptibility in 33% of our group C *Streptococcus equisimilis* isolates and in 56% of our beta-hemolytic group C *Streptococcus milleri* isolates (8). A further drawback of the bacitracin disk susceptibility test is the length of time required to obtain a final result. Although many laboratories place a bacitracin disk on the primary blood agar plate and make a final report after 24 h of incubation, this is not the recommended procedure (1). The procedure for bacitracin disk susceptibility testing recommended in the fourth edition of the *Manual of Clinical Microbiology* requires a heavy inoculum of a pure culture (1). This requirement usually necessitates a subculture and an additional 24 h of incubation. Our laboratory routinely uses a selective medium containing sulfamethoxazole-trimethoprim (SXT) on throat cultures and performs a bacitracin disk susceptibility test on a purified subculture.

These considerations prompted us to evaluate Strep-A-Chek (E-Y Laboratories, San Mateo, Calif.) in a clinical trial. Strep-A-Chek is a new, rapid, chromogenic substrate test which is based upon hydrolysis of a beta-naphthyl

derivative of pyroglutamic acid (PYR). PYR is reported to be hydrolyzed by 100% of *S. pyogenes* strains and by the enterococci (3; J. Lawrence, D. M. Yajko, and W. K. Hadley, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 19, 1984). The test can be performed on as few as two colonies taken from the primary isolation plate. We compared Strep-A-Chek with the bacitracin disk susceptibility test for sensitivity, specificity, and turnaround time in the presumptive identification of *S. pyogenes*.

MATERIALS AND METHODS

All cultures received by the clinical microbiology laboratory, San Francisco General Hospital Medical Center, between February and May 1985 from which beta-hemolytic streptococci were found are included in this study. These included throat cultures and cultures from other sites which were examined for the presence of beta-hemolytic streptococci. Specimens which were to be screened for group A streptococci only were plated on SXT-blood agar (SXT-BA) plates. Routine throat culture specimens were plated on both SXT-BA and conventional blood agar plates. Specimens from other body sites were plated on blood agar but not on SXT-BA. Conventional blood agar plates were incubated in 3 to 8% CO₂ at 35°C. SXT-BA plates were incubated anaerobically at 35°C. All plates were held for 48 h before being discarded.

Medical technologists were asked to record on the protocol worksheet (i) the date the specimen was received, (ii) whether tests were performed from an SXT-BA or conventional blood agar plate, and (iii) the dates tests were completed. This was done so that the turnaround time for the bacitracin disk susceptibility and Strep-A-Chek tests could be compared. The data could also be analyzed to determine

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TABLE 1. Comparison of the Strep-A-Chek and bacitracin disk susceptibility tests in the presumptive identification of group A and non-group A streptococci

Test result	No. of tests	
	Strep-A-Chek	Bacitracin
True-positive (group A) ($n = 303$)	292	303
True-negative (non-group A) ($n = 158$)	156	133
False-positive	2	25
False-negative	11	0

whether the use of SXT-BA offered any advantages over the use of conventional blood agar plates.

Media. Trypticase soy blood agar plates were prepared with Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) and 6 to 7% defibrinated sheep blood. Stock solutions of trimethoprim (Burroughs Wellcome Co., Research Triangle Park, N.C.) and sulfamethoxazole (Burroughs Wellcome Co.) were prepared and stored as previously described (5). SXT-BA was prepared by adding 1.25 ml of a 0.1% filter-sterilized solution of trimethoprim and 1.25 ml of a 1.9% filter-sterilized solution of sulfamethoxazole per liter of Trypticase soy blood agar medium. The final concentration of SXT was 25 μ g/ml. Each batch of SXT-BA was quality assured by testing for the inhibition of growth of *Escherichia coli* and an alpha-hemolytic streptococcus. *S. pyogenes* served as the positive control for both growth and beta-hemolysis. The SXT-BA plates were given an expiration date of 30 days after preparation, but most plates were used within 2 weeks of preparation.

The ability of SXT-BA to suppress the growth of non-group A and non-group B beta-hemolytic streptococci was determined by comparing the rate of recovery of non-group A and non-group B beta-hemolytic streptococci isolated on conventional blood agar versus on SXT-BA.

Bacitracin. Colonies of beta-hemolytic streptococci which grew on the primary plate were tested for bacitracin susceptibility by subculturing the isolates to a blood agar plate and placing a 0.04-U bacitracin disk (Taxo A disk; BBL Microbiology Systems) in the area of the initial streak. If the subculture was not pure, the test was repeated with a pure culture. After 24 h of incubation in CO₂, plates were examined for inhibition of growth around the bacitracin disk. Any zone of inhibition was considered to be a positive test (1, 2).

Strep-A-Chek. Whenever the primary plate had more than two isolated colonies of beta-hemolytic streptococci, medical technologists were instructed to perform the Strep-A-Chek test directly from the primary plate. When this was not possible, the Strep-A-Chek test was performed by using growth from the same subculture plate that was used for the bacitracin disk susceptibility test. Occasionally, the primary SXT-BA plate showed a light haze of pure or apparently pure growth after 24 h of incubation at 35°C. In this case, a scoop of the hazy growth was used for the Strep-A-Chek test instead of reincubating until individual colonies grew out. Since such an inoculum might contain contaminants which could give a false-positive test result, a record was kept of cultures which were tested in this manner.

The Strep-A-Chek test was performed by using a wire loop or wooden stick to pick colonies for transfer to the test strip. The inoculum was rubbed onto the end of the paper strip

containing the PYR substrate, and a drop of reconstituted EY-20 dye (E-Y Laboratories) was added to develop the color. The reconstituted EY-20 dye was used within 30 min according to the manufacturer's directions. Positive results usually occurred within 2 min, but strips were held for 10 min before being discarded. We found the test easier to perform in a petri dish lid than in a test tube, as is recommended in the product insert.

Streptex. To resolve discrepancies between the bacitracin disk susceptibility and Strep-A-Chek test results, all isolates were also tested for the presence of group A streptococcus antigen with the Streptex latex agglutination test (Wellcome Reagents, Div. Burroughs Wellcome Co.). All isolates which were finally identified as group A streptococci were shown to possess group A antigen with the Streptex test. Most of the isolates which were bacitracin susceptible but not group A were further tested for the presence of group B, C, D, F, and G antigen with the Streptex test kit.

RESULTS

A total of 461 isolates of beta-hemolytic streptococci were found during the 3 months of this study. These included 344 (75%) isolates from throats and 117 (25%) from other sites. A total of 303 *S. pyogenes* (group A) isolates were identified, of which 243 (80%) were from throats and 60 (20%) were from other sites.

The number of true-positive and true-negative test results given by Strep-A-Chek and bacitracin on initial testing of group A and non-group A beta-hemolytic streptococci is given in Table 1. The corresponding values for the sensitivity and specificity of each test are given in Table 2. The 11 group A isolates which were false-negative by Strep-A-Chek were retested. All 11 isolates gave a positive test result on repeat tests. Thus, the sensitivity of both the bacitracin disk susceptibility and Strep-A-Chek tests is 100% if repeat test results are included.

The two non-group A cultures scored as positive by Strep-A-Chek were subsequently found to be mixed cultures. Pure cultures of the non-group A streptococci which grew from these cultures gave negative Strep-A-Chek results. The bacteria which caused the positive Strep-A-Chek test result were not isolated in pure culture. They failed to grow on Trypticase soy blood agar in the absence of the beta-hemolytic streptococci. These organisms were thought to be *Haemophilus* species. However, negative Strep-A-Chek test results were obtained upon testing 10 strains of *Haemophilus influenzae*, 1 strain of *Haemophilus parainfluenzae*, and 1 strain of *Haemophilus parahaemolyticus*. A total of 39 respiratory specimens were also tested by using a scoop of young hazy growth from SXT-BA plates. None of these yielded a false-positive reaction by Strep-A-Chek.

TABLE 2. Sensitivity, specificity, and predictive values of Strep-A-Chek and bacitracin disk susceptibility tests in the presumptive identification of *S. pyogenes*

Parameter	% Correspondence of:	
	Strep-A-Chek	Bacitracin
Sensitivity	96.4	100
Specificity	98.7	84.2
Predictive value, positive test	99.3	92.4
Predictive value, negative test	93.4	100

The 25 non-group A isolates which gave false-positive results by the bacitracin disk susceptibility test were group B, group C, and group G streptococci which exhibited a zone of inhibition of growth around the bacitracin disk. All 25 isolates gave a negative test result on Strep-A-Chek strips.

The turnaround time required to produce a final report from respiratory specimens was compared for bacitracin disk susceptibility and Strep-A-Chek tests. The Strep-A-Chek test was completed sooner than the bacitracin disk susceptibility test on 95 (28%) of the 344 respiratory isolates. However, during the last month of the study, after medical technologists had become more familiar with the test and the procedures of the study, the Strep-A-Chek test was completed sooner than the bacitracin disk susceptibility test for 44 (45%) of the 98 isolates tested. On the average, the bacitracin disk susceptibility test took 0.6 day (14.4 h) longer to complete than the Strep-A-Chek test.

An analysis of the data from the 344 respiratory cultures showed that the 194 isolates from SXT-BA plates included 48 beta-hemolytic non-group A streptococci, whereas the 150 isolates from conventional blood agar plates included 53 beta-hemolytic non-group A streptococci. There were 19 false-positive bacitracin disk susceptibility test results among these 101 non-group A isolates (overall false-positive rate of 19%). The 48 non-group A isolates recovered from SXT-BA included 9 (19%) isolates which were false-positive in the bacitracin test. Among the 53 non-group A isolates recovered from conventional blood agar plates, 10 (19%) were false-positive in the bacitracin disk susceptibility test. Most of the false-positive isolates were group C streptococci (12 isolates: 7 from blood agar and 5 from SXT-BA) and group G streptococci (4 isolates: 1 from blood agar and 3 from SXT-BA). Two false-positive isolates were group B streptococci (one each from conventional blood agar and SXT-BA). The remaining false-positive isolate was characterized only as being not group A or group B.

An analysis of the ability of SXT-BA to suppress the growth of non-group A and non-group B beta-hemolytic streptococci showed that the recovery rate of non-group A and non-group B beta-hemolytic streptococci among SXT-BA isolates (19 out of 194; 9.8%) was approximately one-half the recovery rate found among conventional blood agar isolates (30 out of 150; 20%).

DISCUSSION

The high sensitivity and relatively low specificity of the bacitracin disk susceptibility test reported by many others (2, 4, 7, 9) were obtained in this study as well. In contrast to the bacitracin disk susceptibility test, the new Strep-A-Chek test was both sensitive and specific. The false-negative test results obtained with Strep-A-Chek were not due to the absence of pyro-glutamyl aminopeptidase activity in these strains of *S. pyogenes* because all 11 strains which gave false-negative test results were positive on retesting. The most likely cause of the initial false-negative test result was insufficient inoculum, although other causes (e.g., masking of enzyme activity by glycocalyx) cannot be ruled out. We also found that the use of a wire loop is preferable to the use of a wooden stick when only a small number of colonies is available for the Strep-A-Chek test. Presumably, bacteria can be trapped in the crevices of wooden sticks. Our data suggest that when a negative test result is obtained with a small inoculum, the test should be repeated with a heavier inoculum.

The two false-positive test results obtained with Strep-A-Chek were caused by nonstreptococcal contaminants, not by

the beta-hemolytic streptococci in the inoculum. This demonstrates the importance of the manufacturer's instructions that a pure culture be used when performing the Strep-A-Chek test.

It has been reported that SXT inhibits the growth of beta-hemolytic group C, F, G, and nongroupable streptococci while permitting the growth of group A streptococci (4, 6). Group B, C, G, and nongroupable streptococci are the organisms which have been reported to on occasion give false-positive results in the bacitracin disk susceptibility test (9). The specificity of this test should therefore be better with isolates obtained from SXT-BA than with isolates obtained from conventional blood agar plates since the growth of organisms most likely to give a false-positive bacitracin disk susceptibility test result should be inhibited on the SXT-BA plate. Our finding that the rate of false-positive bacitracin disk susceptibility test results among non-group A beta-hemolytic streptococci isolated from SXT-BA was equal to the rate obtained from conventional blood agar was therefore somewhat surprising. In studies by Gunn et al. (5), 83 to 90% of non-group A and non-group B beta-hemolytic streptococci failed to grow on SXT agar. Our data are more in agreement with those of Kurzynski and Meise (6), who found that conventional blood agar plates yielded about twice as many non-group A beta-hemolytic streptococci as did SXT-BA plates.

Since our laboratory does not use a bacitracin disk on primary plates, the Strep-A-Chek test offers a means of speeding up the reporting of cultures which are to be screened for group A streptococci. Nearly half of the cultures examined during the last month of this study had a sufficient number of isolated colonies to permit testing with Strep-A-Chek from the primary plate. This number is probably lower than what other laboratories might find because many throat cultures are received in our laboratory preplated by clinic personnel who are unfamiliar with good isolation technique. Even so, the bacitracin disk susceptibility test took an average of 14.4 h longer than the Strep-A-Chek test to complete.

The cost of the Strep-A-Chek test is \$0.35 per strip. The cost of the bacitracin disk plus a blood agar subculture plate is also approximately \$0.35. Strep-A-Chek approaches the sensitivity of the bacitracin disk susceptibility test but is more specific than this test and can yield results sooner in laboratories which use a subculture to perform the bacitracin disk susceptibility test.

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