Detection of Group D and Viridans Streptococci in Blood by Radiometric Methods

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A prospective study was conducted to evaluate the radiometric detection of group D and viridans streptococci in blood, using three media preparations, Bactec 6A and 6B isotonic media and 8B hypertonic medium. All enterococci tested were detected by the 6A and 6B media. However, the 6A medium failed to detect 76% of the *Streptococcus bovis* isolates and 57% of the viridans streptococci, whereas all *S. bovis* isolates and 95% of the viridans streptococci were detected with the 6B formulation. No improvement in detection was noted in comparing the 6B and the 8B hypertonic media. The importance of adequate detection of this group of organisms, especially in patients with endocarditis, is discussed.

Alpha-hemolytic streptococci are often the etiology of natural valve-infective endocarditis. Garvey and Neu (5) in their 5-year review of endocarditis at Columbia-Presbyterian Medical Center indicate that 62% of infective endocarditis is caused by streptococci, 54% of which are viridans streptococci and 21% of which are Lancefield group D streptococci. In the community hospital, natural valve endocarditis is the most commonly encountered type of endocarditis. Optimal therapy depends on prompt and accurate identification of the etiological agent since different species of streptococci may be treated with different antibiotic regimens.

One effective and rapid method of detecting bacteremia is by radiometric detection of bacterial metabolism. Although the efficacy and rapidity of the method has been documented for most commonly encountered microorganisms (8), Strauss et al. (9) indicated that alpha-hemolytic streptococci were detected poorly or only on subculture. These reports used a formulation of medium (6A) that has subsequently been superseded by one modified to give purported better detection. Because clinical microbiology laboratories should be able to detect efficiently the etiological agents of endocarditis, the efficacy of this new medium formulation (6B) in detecting viridans and group D streptococci was investigated.

MATERIALS AND METHODS

Bacterial strains. Enterococci and viridans streptococci were obtained from patient specimens. *Streptococcus bovis* isolates were kindly provided by R. Facklam, Center for Disease Control (nine isolates); Johnston Laboratories, Cockeysville, Md. (four iso-

lates); and St. Luke's Hospital (four isolates). Group D streptococci were distinguished from viridans streptococci by their ability to hydrolyze esculin in the presence of 40% bile. Enterococci were distinguished from non-enterococcal group D streptococci by their tolerance to 6.5% sodium chloride and hydrolysis of arginine. Confirmation of isolates as *S. bovis* was accomplished by using standard methods (3, 6).

Media. Two formulations of media were originally compared, the Bactec 6A and 6B aerobic media. The 6A formulation contains a basal medium of tryptic soy broth, hemin, menadione, sodium polyanetholesulfonate, purified water, and ^{14}C -labeled substrates, with a total radioactivity of 1.5 μCi /vial. The 6B bottle contains an additional 0.5 μCi of ^{14}C -labeled substrate per vial and 0.25% sucrose. The 8B formulation is identical to the 6B formulation, with the exception that the sucrose content is 10%.

Assay. Radiometric detection was assessed with a 0.1-ml inoculum of between 7 and 105 colony-forming units (CFU). Three milliliters of donor blood collected in Vacutainer tubes containing sodium polyanetholesulfonate was then added. The actual number of CFU added per bottle was determined by pour plate. The bottles, incubated at 35°C, were agitated immediately after inoculation on a New Brunswick Reciprocator at 250 rpm for a minimum of 24 h, after which they were incubated in a stationary position. After incubation for 8 h, the bottles were removed to a Bactec 225 unit every 2 h for a minimum of 24 h and the release of $^{14}\text{CO}_2$ was determined. Negative bottles were then assayed daily for a total of 7 days. Bottles were subcultured at 7 days or when (i) they exceeded a threshold reading of 30 growth index units (GIU) for the 6A and 6B bottles and 20 for the 8B bottle; (ii) they appeared visually positive (i.e., the blood became discolored a brownish-red or hemolyzed); or (iii) a jump of 10 GIU was observed between consecutive readings. Bottles were subcultured at 24 h and 7 days when the hypertonic bottle was evaluated. Subcultures were used to confirm both the viability of the organisms and the purity of the inoculum.

RESULTS

Radiometric detection of 19 strains of viridans streptococci, 15 strains of enterococci, and 17 strains of *S. bovis* were compared in 6A and 6B aerobic bottles (Table 1).

All enterococci grew and were detected in both formulations with a median detection time of 8 h in the 6A bottle and 10 h in the 6B bottle. No significant difference in performance between the two isotonic formulations was noted. The ability to detect *S. bovis* and viridans streptococci, on the other hand, was significantly improved in the 6B bottle when compared with the 6A ($P = 0.001$) (1). All *S. bovis* isolates were detected by the 6B bottle, whereas only 24% (4 of 17) were detected in the 6A. Likewise, the viridans streptococci were better detected in the 6B bottle, i.e., 43% (9 of 20) in the 6A and 95% (19 of 20) in the 6B. Of importance, not only did the 6A bottles not exceed a 30-GIU threshold when positive on subculture, but frequently they neither appeared visually positive nor demonstrated an increment of 10 GIU between consecutive readings.

No advantage was noted in this experimental protocol by using a hypertonic formulation (8B) instead of, or simultaneously with, the isotonic formula (6B) (Table 2).

Two isolates of viridans streptococci were not detected in the hypertonic bottle by using a 20-GIU threshold, although they were detected in the isotonic bottle with a 30-GIU threshold. With one isolate the hypertonic bottle was considered visually positive when the isotonic bottle exceeded the threshold. The other isolate met none of the three criteria in the hypertonic bottle but was detected in the isotonic bottle and was positive on subculture from both bottles.

The single isolate of viridans streptococcus that was not detected in either the 6B or the 6A bottle (CFU/vial = 7) was tested in triplicate in

TABLE 2. Comparison of alpha-streptococci detection in Bactec media 6B and 8B

Bacterial designation (no. tested)	Mean CFU/ml	Detection of organisms incubated in Bactec medium:				<i>P</i>
		6B ^a		8B ^b		
		%	Median time (h)	%	Median time (h)	
<i>S. bovis</i> (8)	17	100	14	100	14	NS ^c
Viridans streptococci (9)	9	100	16	88	18	0.5

^a 6B threshold = 30 GIU.

^b 8B threshold = 20 GIU.

^c NS, Not significant.

the comparison of 6B and 8B bottles (CFU/vial = 17, 26, and 28, respectively). In this instance it was detected in all the 6B vials with 30 GIU as the threshold but not in the hypertonic vials with 20 GIU as the threshold. The hypertonic vials did become visually positive, however, and all vials were positive on subculture.

DISCUSSION

Use of a blood culture system that efficiently and accurately detects group D and viridans streptococcal bacteremia is mandatory in any clinical microbiology laboratory. Initial reports of equivalent detection rates for most bacteria, using radiometric methods compared with conventional techniques, were challenged for this group of organisms by Strauss et al. (9). In a retrospective study, they indicated that 67% of isolates that did not radiometrically exceed a growth index threshold of 30 or appear visually positive were enterococci and non-enterococcal group D streptococci. The medium formula utilized in their study was the 6A aerobic bottle, and 12 of 17 enterococcal isolates were from one patient and all the non-enterococcal group D streptococci were from another single patient.

Detection of bacterial growth by radiometric methods depends on adequate evolution of radioactively labeled carbon dioxide sufficient to exceed the growth index threshold. A new formulation of aerobic broth (6B) containing 0.5 μ Ci of additional labeled substrate as well as 0.25% sucrose was prepared by Johnston Laboratories. They claim that the added sucrose should stimulate metabolism of streptococci and the added radioactive label should assist in exceeding the threshold.

Streptococci, in particular, non-enterococcal group D and viridans streptococci, are homofermentative, with lactic acid as the end product of fermentation. Using conventional techniques, no

TABLE 1. Comparison of alpha-streptococci detection in Bactec media 6A and 6B

Bacterial designation (no. tested)	Mean CFU/ml	Detection of organisms incubated in Bactec medium:				P
		6A		6B		
		%	Median time (h)	%	Median time (h)	
Enterococci (15)	23	100	8	100	10	NS ^a
<i>S. bovis</i> (17)	17	24	11	100	14	0.001
Viridans streptococci (19)	13	43	14.5	95	14.5	0.001

^a NS, Not significant.

CO₂ is evolved. The radiometric method of CO₂ detection, however, is considerably more sensitive than conventional techniques and has been reported to detect CO₂ evolution in media containing ¹⁴C-labeled carbohydrates from organisms classically considered nonfermenters (10). The medium also contains sources of ¹⁴C other than carbohydrates, and a portion of the CO₂ evolution may be attributable to catabolism of these substrates.

Finegold et al. (4) demonstrated that the median colony count per milliliter in gram-positive bacteremia was 8 CFU/ml, with an average count of 313 CFU/ml. The experimental design of this study used inocula ranging from 3 to 35 CFU/ml of whole blood.

Equivalent or better detection of alpha-streptococci occurred when using the new 6B medium compared with the 6A. Most importantly, the new medium detected all isolates if the criteria of visual positivity and 10-GIU increments were applied as well as the ability to exceed a growth index threshold. This was not true of the 6A medium. Frequently, bottles that were positive on subculture showed no indication of their positivity by application of any of the above three criteria.

The fact that Strauss et al. (9) had 17 isolates of enterococci that were not detected may have been due to the medium formulation used. There are other variables which cannot be duplicated in a prospective study of this nature which might have also interfered with the detection of these streptococci from blood, including antibody titers and antibiotic therapy. The results of this study suggest that the original formulation (6A) of the medium alone can account for the inability to detect certain alpha-streptococci and that the new formulation (6B) improves detection irrespective of individual patient variables.

In actual practice three patients were admitted to St. Luke's Hospital during the course of the study with natural valve endocarditis from whose blood cultures viridans streptococci were isolated in the 6B bottles. The median detection time was 18 h (average, 24 h). An isolate from one of the three patients was tested in the experimental protocol and was not detected in the 6A bottle. The other isolates were not tested in this study.

Coleman et al. (2) reported superior radiometric detection of gram-positive cocci in hypertonic medium (8B) compared with isotonic medium (6A). The antibiotic status of the patients was not reported, nor were subcultures performed of bottles which did not exceed the threshold.

Use of the experimental protocol, in our hands, to compare detection of *S. bovis* (8

strains) and viridans streptococci (10 strains) in hypertonic versus isotonic media demonstrated no significant difference in detection. In order for the hypertonic medium to perform as efficiently as the isotonic medium, it is essential that the hypertonic threshold be set at 20 rather than 30 GIU. If 30 GIU is considered the hypertonic threshold, the viridans streptococci do demonstrate significantly better detection ($P = 0.05$) in the isotonic medium. It should also be pointed out that 30% of the viridans streptococci were only detected by visual examination of the hypertonic bottles. They did not exceed the 20-GIU threshold.

Because the 6B isotonic medium detects group D and viridans streptococci as efficiently as the hypertonic formulation, selective application of the hypertonic broth as advocated by Louria et al. (7) can be reserved for those unusual instances of suspected sepsis or endocarditis where an etiological agent has not been isolated by using isotonic media.

The results of this study indicate that the new (6B) aerobic medium used with Bactec 225 offers a significant advantage over the previous formulation in the detection of group D and viridans streptococci from blood. Because this study uses a small number of known organisms, observation of actual clinical specimens should be evaluated to confirm the advantage noted. This advantage should also apply when the medium is used with other Bactec instruments.

LITERATURE CITED

1. Cochran, W. G. 1950. Comparison of percentages in matched samples. *Biometrika* 37:256-266.
2. Coleman, R. M., W. W. Laslie, and D. W. Lambe. 1976. Clinical comparison of aerobic, hypertonic, and anaerobic culture media for the radiometric detection of bacteremia. *J. Clin. Microbiol.* 3:281-286.
3. Facklam, R. R. 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. *Appl. Microbiol.* 23:1131-1139.
4. Finegold, S. M., M. L. White, I. Ziment, and W. R. Winn. 1969. Rapid diagnosis of bacteremia. *Appl. Microbiol.* 18:458-463.
5. Garvey, G. J., and H. C. Neu. 1978. Infective endocarditis—an evolving disease. *Medicine* 57:105-127.
6. Gross, K. C., M. P. Houghton, and L. B. Senterfit. 1975. Presumptive speciation of *Streptococcus bovis* and other group D streptococci from human sources by using arginine and pyruvate tests. *J. Clin. Microbiol.* 1:54-60.
7. Louria, D. B., T. Kaminski, R. Kapila, F. Tecson, and L. Smith. 1976. Study on the usefulness of hypertonic culture media. *J. Clin. Microbiol.* 4:208-213.
8. Randall, E. L. 1975. Long-term evaluation of a system for radiometric detection of bacteremia, p. 39-44. In D. Schlessinger (ed.), *Microbiology—1975*. American Society for Microbiology, Washington, D. C.
9. Strauss, R. R., R. Thom, and H. Friedman. 1977. Radiometric detection of bacteremia: requirement for terminal subcultures. *J. Clin. Microbiol.* 5:145-148.
10. Waters, J. R., and A. A. Zwarun. 1973. Results of an automated sterility test as applied to clinical blood cultures. *Dev. Ind. Microbiol.* 14:80-89.