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Comparison of Two Commercially Available Media for Detection of Bacteremia

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An analysis of 3,795 positive blood cultures obtained from 1,718 patients in a 2.5-year evaluation of Tryptic Soy Broth (TSB) and Thiol Broth is reported. Isolation rates of *Actinobacillus* and *Pseudomonas* were significantly greater in TSB, whereas isolation rates of *Streptococcus* and *Corynebacterium* (aerobic and anaerobic) were significantly greater in Thiol. Otherwise, the two media were similar. Disregarding contaminants, anaerobic bacteria represented 11% of positive cultures and 20% of patients with bacteremia. Eleven per cent of the patients had polymicrobial bacteremia.

Although recent reports have cited recovery rates of bacteria from blood culture media containing sodium polyanetholesulfonate (5, 8, 13) or sucrose (14), few data are available comparing isolation rates from different broth media. This report presents such data accumulated between July 1968 and December 1970 by using two commonly used and commercially available broth media; sodium polyanetholesulfonate was not used.

MATERIALS AND METHODS

Between July 1968 and April 1969, all blood for routine cultures at the Mayo Clinic and affiliated hospitals was collected with a sterile needle and syringe, after skin preparation with 2% aqueous iodine and 70% alcohol, by venipuncture teams and inoculated (10% v/v) at bedside into one bottle of Thiol and one bottle of Tryptic Soy Broth (TSB; Aloe Scientific); each bottle contained 50 ml of medium under vacuum with added CO_2. In April 1969, this system was replaced by one using two bottles (Difco) each containing 100 ml of one of these media under vacuum with added CO_2. As before, blood was collected and inoculated aseptically on a 10% (v/v) basis. Bottles were incubated unvented at 35 C and examined daily for 14 days. Subcultures were routinely made of all bottles without visible evidence of growth at 48 hr to enriched chocolate-blood-agar plates (BioQuest) which were incubated at 35 C in an atmosphere of 10% CO_2 for 48 hr.

Identification of isolates was generally accomplished by conventional procedures as described by Sonnenwirth (16), with the following significant qualifications. Group D streptococci were presumptively identified by using the bile-esculin test (17). β-Hemolytic streptococci were not grouped in the first year of the study. Anaerobic gram-negative bacilli all were called *Bacteroidaceae* until the latter part of the study when speciation was started. *Enterobacteriaceae* were classified by the taxonomic system of Ewing (6) and procedures described by Douglas and Washington (3).

Each positive culture was encoded on punch cards by patient number, laboratory accession number, date of accession, date of positivity in each medium, and identification of organism(s) isolated. Mean and median times for detection of positivity in each organism group were calculated. Cochran's chi-square (χ^2) test (1) was applied to determine significance of differences in isolation rates between the two media. Significance of differences in time intervals to positivity when both media became positive were determined by using the paired t test (2).

RESULTS

Between July 1968 and December 1970, 38,847 cultures were submitted to the microbiology laboratory. There were 3,795 positive cultures (Table 1), defined by positivity in one or both bottles in one set, representing 1,718 patients (Table 2). Of the 3,795 positive cultures, 692 represented isolates in a single culture only of *Corynebacterium* (aerobic and anaerobic), *Bacillus*, or *Staphylococcus epidermidis*, which were used to calculate the overall contamination rate. This approach to the determination of contamination rates of blood cultures is of necessity an arbitrary one, especially since it is difficult to differentiate between contaminating and significant isolates clinically and also since an appreciable number of positive cultures may be obtained from persons without a demonstrable focus of infection (12). Nevertheless, by using this criterion, the contamination rate during this study was 1.8% of the total number

TABLE 1. *Number of isolates in positive cultures by media*

Organism	No. positive with			Total positive	P (χ^2 analysis)
	TSB[a] and Thiol	TSB	Thiol		
Actinobacillus	14	7	1	22	<0.05
Bifidobacterium eriksonii	2	0	3	5	NS[b]
Clostridium	22	8	14	44	NS
Bacillus	16	65	82	163	NS
Corynebacterium	66	126	203	395	<0.001
Escherichia coli	302	120	111	533	NS
Shigella	0	0	1	1	NS
Salmonella	6	4	3	13	NS
Citrobacter	6	0	0	6	NS
Klebsiella	140	54	51	245	NS
Enterobacter	55	17	18	90	NS
Serratia marcescens	37	8	9	54	NS
Proteus	43	23	22	88	NS
Providencia	2	0	0	2	NS
Haemophilus	12	8	11	31	NS
Diplococcus pneumoniae	35	16	9	60	NS
Listeria monocytogenes	6	4	4	14	NS
Streptococcus	519	99	148	766	<0.005
Eubacterium lentum	1	0	2	3	NS
Herellea vaginicola	4	7	3	14	NS
Alcaligenes faecalis	6	7	4	17	NS
Mima polymorpha	0	2	0	2	NS
Pasteurella multocida	1	0	0	1	NS
Neisseria	0	5	0	5	NS
Bacteroidaceae	182	74	76	332	NS
Micrococcus	2	1	2	5	NS
Staphylococcus aureus	258	83	93	434	NS
Staphylococcus epidermidis	80	105	116	301	NS
Peptostreptococcus	13	5	9	27	NS
Peptococcus	4	2	7	13	NS
Veillonella	0	1	0	1	NS
Pseudomonas	57	131	25	213	<0.001
Aeromonas	0	1	1	2	NS
Candida	9	9	6	24	NS
Torulopsis glabrata	0	0	4	4	NS

[a] TSB, Tryptic Soy Broth.
[b] NS, not significant.

TABLE 2. *Number of patients positive by organism group*

Organism	No.	Organism	No.
Streptococcus, unspecified	4	*Bifidobacterium eriksonii*	4
Streptococcus, viridans group	141	*Clostridium*	30
Streptococcus, β-hemolytic	18	*Corynebacterium hemolyticum*	1
Streptococcus, group A	32	*Listeria monocytogenes*	6
Streptococcus, group B	5	*Eubacterium lentum*	2
Streptococcus, group C	2	*Escherichia coli*	245
Streptococcus, group D	90	*Shigella*	1
Streptococcus, group F	2	*Salmonella*	7
Streptococcus, other specified groups	5	*Citrobacter*	2
Diplococcus pneumoniae	35	*Klebsiella*	127
Staphylococcus aureus	159	*Enterobacter*	43
S. epidermidis	237	*Serratia marcescens*	25
Micrococcus	5	*Proteus*	53
Peptostreptococcus	16	*Providencia*	2
Peptococcus	12	*Haemophilus*	20
Neisseria	3	*Brucella*	1
Veillonella	1	*Actinobacillus*	3
Bacteroidaceae	145	*A. actinomycetemcomitans*	2
Bacillus	146	*A. lignieresi*	1
Corynebacterium (aerobic and anaerobic)	359	*Pasteurella multocida*	1
Candida	11	*Pseudomonas*	124
Torulopsis glabrata	2	*Aeromonas hydrophila*	2

of cultures. The overall rate of positivity, excluding contaminants, was 8.0%; however, this rate was 6.5% during the period up to April 1969 when bottles containing 50 ml of broth were replaced by bottles containing 100 ml of broth.

There were 27 patients in the study with multiple positive blood cultures containing *S. epidermidis*, which were thought clinically to be significant. One patient, described elsewhere (18), with a fatal brain abscess due to *Corynebacterium hemolyticum* had multiple positive blood cultures containing this organism. One patient with endocarditis had multiple positive cultures with *C. acnes* (*Propionibacterium acnes*). The remainder of the patients, 713, with positive blood cultures containing *S. epidermidis*, *Corynebacterium*, or *Bacillus* were presumed to have contaminated cultures.

Of the 3,795 positive cultures, 200 represented mixed cultures with two organisms and 9 represented mixed cultures with three organisms. In 55 of the cultures with two organisms and in 5 of those with three organisms, *S. epidermidis*, *Bacillus*, or *Corynebacterium* represented one of the isolates.

By Cochran's χ^2 test, two groups of organisms, *Actinobacillus* and *Pseudomonas*, were isolated in significantly greater numbers from TSB than from Thiol, whereas *Streptococcus* and *Corynebacterium* (aerobic and anaerobic) were isolated from Thiol to a significantly greater extent than from TSB (Table 1).

In those instances in which both media became positive, analysis as to the time interval required for positivity in each medium showed statistically significant differences ($P < 0.05$) with TSB as the best medium for *Actinobacillus*, *Enterobacter*, and *Pseudomonas* and Thiol as the best medium for *Proteus* and *Streptococcus*; however, only in the cases of *Actinobacillus* and *Proteus* did these differences exceed 1 day (2.1 and 1.3 days, respectively; Table 3).

TABLE 3. *Time interval to positivity*

Organism	Tryptic Soy Broth			Thiol		
	No.	Days		No.	Days	
		Mean	Median		Mean	Median
Streptococcus	618	2.9	2	667	2.7	2
Diplococcus pneumoniae	51	1.6	6	44	1.7	5
Staphylococcus aureus	341	3.7	2	351	3.6	2
S. epidermidis	185	6.0	4	196	6.2	5
Micrococcus	3	11.3		4	8.3	
Peptostreptococcus	18	5.5	4	22	3.9	3
Peptococcus	6	5.2		11	6.6	6
Neisseria	5	4.0		0		
Veillonella	1	14.0	14	0		
Bifidobacterium	2	6.0		5	3.8	
Clostridium	30	2.2	1	36	1.9	1
Listeria	11	3.8	2	10	2.7	2
Eubacterium	1	2.0		3	3.7	
Escherichia	422	2.3	1	413	2.1	1
Shigella	0			1	4.0	
Salmonella	10	2.1	2	9	1.9	2
Citrobacter	6	1.7		6	1.7	
Klebsiella	194	1.9	1	191	1.9	1
Enterobacter	72	2.2	1	73	2.1	1
Serratia	45	2.4	1	46	2.3	1
Proteus	66	3.9	2	65	3.5	2
Providencia	2	1.0		2	1.0	
Haemophilus	20	3.7	2	23	3.3	2
Brucella	1	7.0		0		
Actinobacillus	21	5.4	5	15	7.1	6
Pasteurella	1	3.0		1	3.0	
Pseudomonas	187	4.1	3	82	4.4	3
Aeromonas	1	1.0		1	1.0	
Bacteroidaceae	255	3.8	2	258	4.2	2
Candida	18	5.1	3	15	4.5	4
Torulopsis	0			4	10.3	

Of the 1,718 patients, 935 had single positive cultures, 378 had two positive cultures, and 164 had three positive cultures. The remainder had four or more positive cultures with two patients having as many as 19.

DISCUSSION

The blood culture positivity rate, excluding probable contaminants, found in this study is in close agreement with positivity rates reported by Scott (15), Ellner (4), and Morello and Ellner (11) using broth culture techniques. Substantially higher rates (17 and 12% in broth with and without added sodium polyanetholesulfonate, respectively) have been reported by Finegold et al. (8). In a more recent report by these same investigators (7), approximately 20% of broth cultures became positive. The explanation for the significantly higher positivity rates encountered by Finegold et al. is not readily apparent, unless perhaps their total number of cultures represented multiple replicate samples from a relatively smaller number of selected patients.

It is surprising that, in the studies just cited, there were few anaerobic bacterial isolates reported. In the present study, disregarding contaminants, anaerobic bacteria represented 11% of the positive cultures and 20% of the patients with bacteremia. The *Bacteroidaceae* represented 78% of the positive cultures with anaerobes and 69% of patients with bacteremias due to anaerobes.

During the entire period of study, again disregarding contaminants, approximately 5% of the cultures contained more than one organism, and nearly 11% of patients had more than one organism isolated from one culture. This proportion is higher than that reported earlier by Hermans and Washington (9) and probably reflects not only a greater awareness of the problem in the laboratory but also the greater accuracy of the machine data-processing system (10) used to compile the information for this study over the human system used in the earlier study.

Thiol, which according to the manufacturer (Difco Supplementary Literature, 1968) has the capacity to neutralize bacteriostatic and bactericidal properties of streptomycin, penicillins, and sulfonamides, was able to recover streptococci significantly more frequently ($P < 0.005$) and more rapidly ($P < 0.001$) than TSB. The latter difference, however, was only 0.33 day which, for all practical purposes, is of little or no importance. Since Werner et al. (19) reported a statistically significant difference in positivity rates of blood cultures from patients with bacterial endocarditis according to whether antimicrobial agents had been given within 2 weeks of the blood cultures, the blood cultures of 70 patients with streptococcal endocarditis were examined to see if positivity in Thiol and negativity or delayed positivity in TSB reflected recent prior antimicrobial therapy. No such relationship was found. Indeed, in a few patients with multiple positive cultures of *S. aureus* in Thiol only, there was no relationship between this microbiological characteristic and the frequency of recent prior antimicrobial therapy. It appears likely, therefore, that Thiol represents a somewhat better growth medium for streptococci than TSB and that its higher positivity rate reflects that characteristic rather than that of antibiotic neutralization. Conversely, *Pseudomonas* was not only more frequently ($P < 0.001$) but also more rapidly ($P < 0.05$) isolated from TSB than from Thiol. There were significantly more contaminants in the anaerobic and aerobic *Corynebacterium* groups recovered

from Thiol than in those from TSB ($P < 0.001$). With these few notable exceptions, these media were remarkably similar.

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LITERATURE CITED

1. Cochran, W. G. 1950. The comparison of percentages in matched samples. Biometrika 37:256–266.
2. Dixon, W. J., and F. J. Massey, Jr. 1969. Introduction to statistical analysis, 3rd ed., p. 119–122. McGraw-Hill Book Co., Inc., New York.
3. Douglas, G. W., and J. A. Washington II. 1969. Identification of Enterobacteriaceae in the clinical laboratory. National Communicable Disease Center, Atlanta.
4. Ellner, P. D. 1968. System for inoculation of blood in the laboratory. Appl. Microbiol. 16:1892–1894.
5. Ellner, P. D., and C. J. Stoessel. 1966. The role of temperature and anticoagulant on the in vitro survival of bacteria in blood. J. Infec. Dis. 116:238–242.
6. Ewing, W. H. 1963. An outline of nomenclature for the family Enterobacteriaceae. Int. Bull. Bacteriol. Nomencl. Taxon. 13:95–110.
7. Finegold, S. M., M. L. White, I. Ziment, and W. R. Winn. 1969. Rapid diagnosis of bacteremia. Appl. Microbiol. 18:458–463.
8. Finegold, S. M., I. Ziment, M. L. White, W. R. Winn, and W. T. Carter. 1968. Evaluation of polyanethol sulfonate (Liquoid) in blood cultures. Antimicrob. Ag. Chemother. 1967, p. 692–696.
9. Hermans, P. E., and J. A. Washington II. 1970. Polymicrobial bacteremia. Ann. Intern. Med. 73:387–392.
10. Krieg, A. F., T. J. Johnson, C. McDonald, and E. Cotlove. 1971. Clinical laboratory computerization, p. 8. University Park Press, Baltimore.
11. Morello, J. A., and P. D. Ellner. 1969. New medium for blood cultures. Appl. Microbiol. 17:68–70.
12. Reith, A. F., and T. L. Squier. 1932. Blood cultures of apparently healthy persons. J. Infec. Dis. 51:336–343.
13. Rosner, R. 1968. Effect of various anticoagulants and no anticoagulant on ability to isolate bacteria directly from parallel clinical blood specimens. Amer. J. Clin. Pathol. 49:216–219.
14. Rosner, R. 1970. Comparison of a blood culture system containing Liquoid and sucrose with systems containing either reagent alone. Appl. Microbiol. 19:281–282.
15. Scott, E. G. 1951. A practical blood culture procedure. Amer. J. Clin. Pathol. 21:290–294.
16. Sonnenwirth, A. C. 1970. Collection and culture of specimens and guides for bacterial identification, p. 1160–1169. *In* S. Frankel, S. Reitman, and A. C. Sonnenwirth (ed.), Gradwohl's clinical laboratory methods and diagnosis: a textbook on laboratory procedures and their interpretation, 7th ed., vol. 2, part VII. C. V. Mosby Co., St. Louis.
17. Swan, A. 1954. The use of a bile-aesculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group *D* streptococci). J. Clin. Pathol. 7:160–163.
18. Washington, J. A., II, W. J. Martin, and R. E. Spiekerman. 1971. Brain abscess with *Corynebacterium hemolyticum*: report of a case. Amer. J. Clin. Pathol. 56:212–215.
19. Werner, A. S., C. G. Cobbs, D. Kaye, and E. W. Hook. 1967. Studies on the bacteremia of bacterial endocarditis. J. Amer. Med. Ass. 202:199–203.