Comparison of Two Liquid Blood Culture Media Containing Sodium Polyanetholesulfonate: Tryptic Soy and Columbia

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In a comparison of tryptic soy broth and Columbia broth, two blood culture media containing sodium polyanetholesulfonate, there were 589 positive cultures (excluding presumed contaminants). The two media were equivalent in performance except for lower detection rates for Staphylococcus aureus (P < 0.01) and Pseudomonas aeruginosa (P = 0.05) and a higher detection rate for Bacillus (P < 0.01) in Columbia broth. No significant differences were noted in time intervals to detection of positivity. Routine subcultures on the 1st and 5th days of incubation provided the initial detection of 18.1% of the positive cultures.

Because of the frequency of bacteremia and the morbidity and mortality associated with it, its detection remains of paramount importance to the clinical laboratory. Many different types of liquid blood culture media containing sodium polyanetholesulfonate (SFS) are now commercially available; however, reports of their relative performances are few. We previously compared tryptic soy broth (TSB) and thiold broth, both containing SPS (4). We now report a study comparing TSB and Columbia broth.

MATERIALS AND METHODS

Blood was collected with a sterile needle and syringe, from patients suspected of having bacteremia, by members of a venipuncture team; the skin site was prepared with alcohol followed by povidone-iodine (Swabsticks, Purdue Frederick Co.). Blood samples were inoculated (10%, vol/vol) into one bottle each of TSB (Difco) and Columbia broth (Difco), both under vacuum with CO₂ atmosphere. Each bottle contained 100 ml of medium and 0.05% SPS, and neither bottle was vented during incubation. All units were incubated at 35°C and were inspected daily for 14 days.

All units without visible evidence of growth were subcultured routinely within 24 h of blood collection, by sampling with a sterile needle and syringe through the stopper, inoculation of chocolate blood agar plates, and incubation in an atmosphere of 10% CO₂ for 48 h. This measure is recommended for the prompt detection of Pseudomonas aeruginosa (5), Haemophilus influenzae, H. parainfluenzae, Moraxella, and Neisseria gonorrhoeae (1). An additional routine subculture of 7,460 units that were negative on gross inspection was carried out in the same manner on the 5th day after collection. Finally, 1,258 units were routinely Gram stained at the time of the initial subculture. No further stains or subcultures were carried out unless there was gross evidence of growth, in which case stains and appropriate subcultures were performed.

Methods of statistical analysis have been reported previously (8) and are based on those described by Cochran (2).

RESULTS

During the period of study (1 June 1973 to 1 October 1973), 6,904 blood samples were cultured. There were 797 sets of positive cultures (11.5%); however, cultures containing Bacillus, Corynebacterium (including Propionibacterium), and Staphylococcus epidermidis (in a single culture only) comprised 208 sets of these cultures. Excluding these presumed contaminants, therefore, there were 589 positive cultures (8.5%). The numbers of isolates in positive cultures, by medium, are listed in Table 1. The two media performed equally well with only three exceptions: Bacillus was isolated more frequently in Columbia broth than in TSB, and Staphylococcus aureus and P. aeruginosa were isolated more frequently in TSB than in Columbia broth.

The time intervals to detection of positivity in each medium are listed in Table 2. There were no statistically significant differences between the two media in this respect.

Between 19 June 1973 and 4 September 1973, 4,258 samples were cultured; of these, 531 (12.4%) became positive. Of these positive cultures, 61 (11.5%) were first detected in the initial 24-h subculture, and 35 (6.6%) were first detected in the 5-day subculture. The remainder (81.9%) were first detected by visual inspection. Most cultures with P. aeruginosa were
detected in the initial subculture. Potentially significant organisms detected in the 5-day subcultures were *P. aeruginosa* (five times), *Candida albicans* (seven times), *S. aureus* (three times), and *P. cepacia* (once). The remainder detected in 5-day subcultures were presumed contaminants.

There were 629 cultures, representing 1,258 bottles, that were negative on gross inspection within the first 24 h after collection. At the time of the initial routine subculture, a smear of each was prepared, Gram stained, and examined microscopically. The only positive findings were a gram-negative bacillus that ultimately proved to be *Bacteroides fragilis* in two bottles and a gram-negative bacillus that proved to be *P. aeruginosa* in one bottle. During this same period of study, there were seven cultures in which *P. aeruginosa* was first detected in the 24-h subculture, but smears had been negative.

**DISCUSSION**

Columbia broth was initially described by Morello and Ellner (6), who compared it in parallel with TSB in 1,019 blood cultures; both media were bottled in 50-ml volumes and contained SPS. Although not specified in the report, it is assumed that CO₂ was added to each bottle, in view of the authors' reference to their method as that described in an earlier article by Ellner (3). On the basis of 95 isolates, these authors concluded that, although isolation rates in the two media did not differ significantly, the time interval to detection of growth did and was shorter in Columbia broth.

| Organism               | TSB and Columbia | TSB only | Columbia only | Total positive | *P*   |
|------------------------|------------------|---------|----------------|----------------|------|
| *Bacillus*             | 0                | 7       | 24             | 31             | <0.01|
| *Clostridium*          | 6                | 0       | 2              | 8              | NS   |
| *Corynebacterium*      | 23               | 55      | 40             | 118            | NS   |
| *Lactobacillus*        | 2                | 0       | 1              | 3              | NS   |
| *Escherichia*          | 79               | 27      | 21             | 127            | NS   |
| *Salmonella*           | 0                | 0       | 1              | 1              | NS   |
| *Citrobacter*          | 2                | 0       | 0              | 2              | NS   |
| *Klebsiella*           | 37               | 5       | 5              | 47             | NS   |
| *Enterobacter*         | 2                | 2       | 1              | 5              | NS   |
| *Serratia*             | 10               | 1       | 0              | 11             | NS   |
| *Proteus*              | 11               | 8       | 7              | 26             | NS   |
| *Haemophilus*          | 10               | 1       | 0              | 11             | NS   |
| *Listeria*             | 2                | 0       | 2              | 4              | NS   |
| *Streptococcus*        |                   |         |                |                |      |
| *S. pneumoniae*        | 3                | 4       | 2              | 9              | NS   |
| Viridans               | 36               | 5       | 4              | 45             | NS   |
| Group A                | 0                | 0       | 1              | 1              | NS   |
| Group B                | 0                | 2       | 2              | 4              | NS   |
| Group D                | 19               | 5       | 1              | 25             | NS   |
| *Eubacterium*          | 0                | 1       | 0              | 1              | NS   |
| *Acinetobacter*        | 0                | 2       | 1              | 3              | NS   |
| *Alcaligenes*          | 0                | 1       | 3              | 4              | NS   |
| *Flavobacterium*       | 0                | 0       | 0              | 1              | NS   |
| *Bacteroidaceae*       | 18               | 11      | 15             | 44             | NS   |
| *Micrococcus*          | 0                | 0       | 1              | 1              | NS   |
| *Staphylococcus*       |                   |         |                |                |      |
| *S. aureus*            | 60               | 25      | 5              | 90             | <0.01|
| *S. epidermidis*       | 25               | 33      | 19             | 77             | NS   |
| *Peptostreptococcus*   | 3                | 1       | 0              | 4              | NS   |
| *Peptococcus*          | 0                | 0       | 1              | 1              | NS   |
| *Veillonella*          | 1                | 0       | 0              | 1              | NS   |
| *Pseudomonas*          | 35               | 15      | 6              | 56             | 0.05 |
| *Aeromonas*            | 3                | 1       | 1              | 5              | NS   |
| *Candida*              | 6                | 1       | 1              | 8              | NS   |
| *Torulopsis*           | 0                | 3       | 0              | 3              | NS   |

* For difference between media.

*NS, Not significant.

**Table 1. Numbers of isolates in positive cultures, by medium**
TABLE 2. Time intervals to detection of positivity

| Organism                      | TSB            | Columbia        |
|-------------------------------|---------------|----------------|
|                               | No. | Mean ± SD (days) | No. | Mean ± SD (days) |
| Bacillus                      | 7   | 3.9 ± 4.6        | 24  | 4.5 ± 2.7        |
| Clostridium                   | 6   | 1.0 ± 0.0        | 8   | 1.0 ± 0.0        |
| Corynebacterium               | 78  | 8.2 ± 3.1        | 63  | 9.3 ± 4.0        |
| Lactobacillus                 | 2   | 6.5 ± 0.7        | 3   | 5.0 ± 2.6        |
| Escherichia                   | 106 | 1.6 ± 1.6        | 100 | 1.6 ± 1.5        |
| Salmonella                    | 0   | 1.0             | 1   | 1.0             |
| Citrobacter                   | 2   | 1.0 ± 0.0        | 2   | 1.0 ± 0.0        |
| Klebsiella                    | 42  | 2.3 ± 2.7        | 42  | 1.9 ± 2.1        |
| Enterobacter                  | 4   | 1.0 ± 0.0        | 3   | 1.0 ± 0.0        |
| Serratia                      | 11  | 1.2 ± 0.4        | 10  | 1.5 ± 1.0        |
| Proteus                       | 19  | 1.9 ± 1.2        | 18  | 2.1 ± 1.6        |
| Haemophilus                   | 11  | 2.5 ± 1.5        | 10  | 3.7 ± 4.4        |
| Listeria                      | 2   | 2.0 ± 0.0        | 4   | 2.0 ± 0.0        |
| Streptococcus                 |     |                 |     |                 |
| *S. pneumoniae*               | 7   | 1.3 ± 0.5        | 5   | 1.6 ± 0.5        |
| Viridans                      | 41  | 2.1 ± 1.8        | 40  | 1.9 ± 1.3        |
| Group A                       | 0   | 1.0 ± 0.0        | 1   | 2.0             |
| Group B                       | 2   | 1.0 ± 0.0        | 2   | 1.5 ± 0.7        |
| Group D                       | 24  | 2.3 ± 1.6        | 20  | 2.4 ± 1.6        |
| Eubacterium                   | 1   | 7.0             | 0   | 2.0             |
| Actinobacter                  | 2   | 2.0 ± 0.0        | 1   | 2.0             |
| Alocigenes                    | 1   | 7.0             | 3   | 9.6 ± 5.7        |
| Flavobacterium                | 1   | 5.0             | 1   | 7.0             |
| Bacteroidaceae                | 29  | 4.5 ± 3.7        | 33  | 3.8 ± 3.5        |
| Micrococci                    | 0   | 1.0             | 5.0 | 8.0             |
| Staphylococcus                |     |                 |     |                 |
| *S. aureus*                   | 85  | 2.9 ± 2.9        | 65  | 1.9 ± 1.2        |
| *S. epidermidis*              | 58  | 3.8 ± 2.1        | 44  | 3.9 ± 2.2        |
| Peptostreptococcus            | 4   | 1.5 ± 0.6        | 3   | 1.3 ± 0.6        |
| Peptococcus                   | 0   | 1.0             | 1   | 7.0             |
| Veillonella                   | 1   | 3.0             | 1   | 3.0             |
| Pseudomonas                   | 50  | 3.5 ± 2.6        | 41  | 3.1 ± 1.7        |
| Aeromonas                     | 4   | 1.3 ± 0.5        | 4   | 1.5 ± 0.6        |
| Candida                       | 7   | 8.3 ± 2.6        | 7   | 7.3 ± 0.9        |
| Torulopsis                    | 3   | 4.3 ± 1.5        | 0   | 1.0             |

* SD, Standard deviation.

These results differ from our experience reported here, and there are several factors that may contribute to these differences. Morello and Ellner used a 10-ml sample of blood transported to the laboratory in Vacutainer tubes containing SPS, inoculating 5 ml into 50 ml of each of the two media, whereas in our study a member of a venipuncture team inoculated 10 ml of blood directly into 100 ml of each of the two media and these bottles were transported to the laboratory. It is not known how the performance of TSB manufactured by BBL might differ from that manufactured by Difco, so the significance of this difference between the two studies is not clear.

The optimal timing and frequency of routine subcultures and the value of routine Gram-stained smears of blood cultures are not known. Blazevic et al. (1) studied the role of routine staining on the 1st, 4th, and 7th days of incubation and of routine subcultures on the 1st and 4th days of incubation. They first detected positivity by visual inspection in 65%, by microscopy examination of the Gram-stained smear in 23%, and by subculture in 12% of their cultures. Their rate of positivity first detected by subculture is similar to that in our study (18.1%), but their rate of first detection by microscopy examination of the stained smear is substantially in excess of ours and cannot be explained satisfactorily. In our estimation, the yield from a routine Gram-stained smear is so small that its use, except to confirm suspicion of positivity, is not warranted, particularly considering the effort involved.

It appears that at least one routine ("blind")
subculture within 24 h after collection of the blood culture is mandatory and that a second routine subculture 3 or 4 days later is advisable. Furthermore, it appears, on the basis of our data and those of Blazevic et al. (1), that routine subcultures should be inoculated onto chocolate blood agar incubated in CO₂ and that routine anaerobic subcultures are not warranted. Most anaerobic bacteria are easily detectable by visual inspection after a short period of incubation in unvented blood culture bottles.

Previous studies in this laboratory have compared TSB in an unvented bottle containing 100 ml of medium with SPS under vacuum and CO₂ with (i) supplemented prereduced brain heart infusion broth with SPS (9), (ii) the aerobic and anaerobic culture vials used in a radiometric system (7), (iii) thiol broth (4), and (iv) aerobic and anaerobic Vacutainer tubes with supplemented peptone broth (4). Under our conditions, TSB with SPS has been the best general-purpose medium for detection of bacteremia. We prefer not to vent the bottles, so that we maintain a sufficiently low redox potential in the medium to favor growth and prompt detection of anaerobic bacteria; we depend on routine subcultures on the 1st and 5th days of incubation for detection of P. aeruginosa and other organisms whose growth may not be evident macroscopically.

Selection of microbiological media is complex because there are few standards against which to compare new products. Moreover, there are many individual differences in practices among laboratories, including number of specimens collected, methods of collection and transport, types and volumes of media, use of additives, atmosphere of incubation, frequency of examinations and subcultures, and duration of incubation. All of these variables need to be identified and controlled in comparative evaluations.

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