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NOTES

Inoculation of API-20E from Positive Blood Cultures

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The API-20E system (Analytab Products, Inc., Plainview, N.Y.) was inoculated from 4- to 6-h tryptic soy broth cultures that had been inoculated from positive blood cultures containing gram-negative bacilli. This method gave the same genus and species identification for 139 of 140 organisms (47 patient and 93 simulated positive cultures) when compared to the Analytab Products, Inc., recommended method of inoculation.

The API-20E (Analytab Products, Inc., Plainview, N.Y.) system for identification of enteric gram-negative rods has been shown to be accurate and comparable to conventional biochemical testing (2). The system is designed to utilize an inoculum prepared from an isolated colony on solid media. This method is satisfactory for organisms growing on primary plates but is not useful with growth in broth, such as in a positive blood culture. To avoid the 24-h delay necessitated by an intervening subculture to plate media, we evaluated a method for inoculating API-20E strips from positive blood culture broths on the same day growth was detected.

When gram-negative rods of one morphological type were found in a blood culture (Columbia broth with 0.03% sodium polyanethol sulfonate [BBL]), a few drops of the blood culture broth were inoculated into 5 ml of tryptic soy broth (GIBCO Diagnostics, Madison, Wis.). The tryptic soy broth was incubated at 35°C until visual growth was achieved and the erythrocytes had settled to the bottom of the tube (usually 4 to 6 h). At this time one drop of the tryptic soy broth culture was added to 5 ml of saline (pH 6.0 ± 0.5), and this saline suspension was used to inoculate the API-20E system. At the same time, the blood culture broth was subcultured to 5% sheep blood agar and MacConkey agar plates; after overnight incubation at 35°C, if the plates showed a pure culture, another API-20E was inoculated from the MacConkey plate according to the manufacturer's directions. If the culture was not pure, it was not included in the study. Forty-seven positive blood cultures and ninety-three simulated positive blood cultures (negative blood cultures into which a drop of a faintly turbid suspension of selected gram-negative rods was inoculated and which were incubated overnight) were tested.

All the gram-negative rods isolated from the positive blood cultures were identified as the same genus and species by both methods of inoculation. The organisms are shown in Table 1. Of these 47 organisms, the API biotype number was identical in 34 (72%), whereas the number varied by one digit in twelve and by two digits in one. With the 93 simulated positive cultures, 92 of the organisms were identified as the same genus and species by both methods. One organism was identified as *Serratia liquefaciens* from the tryptic soy broth and as *Serratia marcescens* from the MacConkey plate. The other organisms used are shown in Table 2. Of the 92 organisms, 43 (47%) gave an identical biotype number. The API tests responsible for the differences in biotype numbers included all except ornithine decarboxylase, H_2S, mannitol, and rhamnose. No one test predominated as a cause of the discrepancies.

Overall, of the 140 cultures, the organisms were identified as the same genus and species

TABLE 1. *Identification results with positive blood cultures using inoculum from broth or MacConkey agar*

Organism	No. with identical biotype	No. with different biotype
Alcaligenes	2	0
Citrobacter diversus	2	0
Enterobacter cloacae	2	0
Escherichia coli	21	4
Klebsiella pneumoniae	3	3
Proteus mirabilis	1	0
Proteus morganii	0	2
Pseudomonas aeruginosa	3	4
Total	34	13

TABLE 2. *Identification results with simulated positive blood cultures using inoculum from broth or MacConkey agar*

Organism	No. with identical biotypes	No. with different biotypes
Citrobacter diversus	4	1
Citrobacter freundii	2	8
Citrobacter species	0	5
Enterobacter aerogenes	8	0
Enterobacter cloacae	8	2
Escherichia coli	4	6
Klebsiella pneumoniae	4	8
Proteus mirabilis	2	8
Pseudomonas aeruginosa	2	3
Salmonella typhi	1	0
Salmonella enteritidis	5	4
Serratia marcescens	3	6
Total	43	50

by both methods 99.9% of the time. The biotype numbers were identical 55% of the time. This latter percentage is almost identical to the reproducibility of the API biotype numbers as shown by Butler et al. (1), who used only the inoculation method suggested by the manufacturer. However, our reproducibility of identification was somewhat better than theirs. On the basis of these results, the method used here for inoculation of API from positive blood cultures can be recommended to avoid delays in the identification of gram-negative rods.

LITERATURE CITED

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