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Modified Inoculum for the Enteric Minitek System from Positive Blood Cultures

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A modified Minitek inoculum procedure for the identification of *Enterobacteriaceae* from positive blood cultures was shown to be reliable. The method consisted of inoculating the Minitek enteric and nonfermenter broth with blood culture fluid and incubating the inoculum for 4 h before use.

The Minitek test system (BBL Microbiology Systems, Cockeysville, Md.) is a miniaturized tube system for the identification of microorganisms. Disks impregnated with various chemical substrates are used to test for the biochemical activity of microorganisms. The recommended inoculum consists of suspending an isolated colony from plated medium into the appropriate inoculum broth. This test system for the identification of *Enterobacteriaceae* (1, 2) has been shown to be accurate and comparable to standard biochemical testing. To avoid delays in the identification of organisms present in blood culture specimens, we evaluated a method which allows for the preparation of an inoculum suspension from the positive blood culture fluid. The results from the modified inoculum procedure were compared with results obtained by the recommended inoculum procedure, using isolated colonies from the subcultures of the positive blood samples.

Blood culture bottles (brain heart infusion with *p*-aminobenzoic acid, 0.1% agar, 0.5 μg of hemin per ml, 10 μg of vitamin K_1 per ml, 0.03% sodium polyanethol sulfonate, and 5% CO_2 [BBL]) were visually screened for the presence of growth. Gram stain smears were prepared from suspected positive blood culture bottles. If gram-negative rods were visualized on the Gram stain, 1 to 2 drops of the blood culture fluid were added to the Minitek enteric and nonfermenter broth. The inoculum broth was incubated for 4 h at 37°C, after which it was used to inoculate the Minitek differentiation system. All Minitek plates were prepared and incubated as per the manufacturer's instructions. In addition to the recommended substrate disks for the differentiation of *Enterobacteriaceae*, we used additional disks to aid in the differentiation of the nonfermentative bacteria: dextrose without nitrate, maltose, xylose, sucrose, arginine, and nitrate reductase.

The blood culture fluid was also inoculated to a chocolate agar plate, 5% sheep blood agar plate, MacConkey agar plate, broth medium, and a Mueller-Hinton agar plate for a preliminary antibiotic susceptibility test. After overnight incubation, the plates were inspected to insure that the cultures were pure. If the culture was mixed, it was not included in the study. Organisms isolated on the plated medium were then used to prepare the standard inoculum in the Minitek system.

A total of 106 positive blood cultures from 61 patients were tested by the two Minitek procedures for the identification of *Enterobacteriaceae*. Nonfermentative organisms were present in eight positive blood cultures from seven patients. The identification results among the *En-*

TABLE 1. *Minitek identification results with a direct broth inoculum and the standard inoculum*

Organism	No. with identical profile	No. with different profile	
		Correct organism identification	Additional biochemical testing necessary
Escherichia coli	43	6	1
Klebsiella pneumoniae	18	1	2
Enterobacter cloacae	4	0	1
Enterobacter aerogenes	6	1	0
Serratia marcescens	4	0	2
Proteus mirabilis	10	0	0
Proteus rettgeri	1	0	0
Salmonella enteritidis	6	0	0

terobacteriaceae are shown in Table 1. In over 94% of the cases, the gram-negative rods isolated from the blood cultures were confidently identified to the same genus and species by both methods of inoculation. A breakdown of all *Enterobacteriaceae* organisms with conflicting biochemical profiles from the two methods is shown in Table 2. Six of seven positive cultures for *Escherichia coli* had minor biochemical variations which accounted for the different profile. In one case, a *Shigella* species (Minitek profile no. 32102) was identified by the Minitek plate inoculated by the direct blood culture fluid to broth procedure. A direct wet mount motility prepared at the time of inoculation demonstrated that the organism was motile; therefore, the organism would have required further biochemical testing to confirm the identification. Additionally, this organism utilized lactose in the MacConkey agar plate. *Klebsiella pneumoniae* identification in two of the three positive blood samples would have required additional biochemical testing for confirmation because the confidence level was low. Likewise, the two *Serratia marcescens* isolates required additional testing due to the low confidence level in their biochemical profile. An *Enterobacter cloacae* was erroneously identified as *K. pneumoniae* by the direct blood culture fluid to broth procedure; however, it was negative for motility on the wet mount preparation. Additional biochemical tests were required to confirm the identification.

Eight nonfermenting organisms were examined by the direct blood culture fluid to broth inoculum procedure. In this study, we found that the abbreviated biochemical test system and the shortened incubation time limit the usefulness of the modified inoculum procedure for nonfermenter identification. Five of eight nonfermenters recovered from blood cultures were *Pseudomonas aeruginosa*. All of those isolates produced pyocyanin on the Mueller-Hinton susceptibility plate prepared from the positive blood culture, which obviated the need for further identification procedures.

The direct broth inoculum procedure with the *Enterobacteriaceae* is a reliable means of identifying organisms present in blood cultures. The

TABLE 2. *Identification profile of organisms which gave conflicting results by the direct broth inoculum versus the standard inoculum procedure*

Organism	Identification based on inoculum procedure		Biochemical difference
	Direct broth	Colony	
Escherichia coli	*E. coli* (99.97)[a]	*E. coli* (97.89)	Lysine negative
E. coli	*E. coli* (99.97)	*E. coli* (97.89)	Lysine negative
E. coli[b]	*E. coli* (99.82)	*E. coli* (99.98)	Rhamnose negative
E. coli[b]	*E. coli* (99.82)	*E. coli* (90.28)	ONPG[c] negative
E. coli[b]	*E. coli* (99.96)	*E. coli* (99.97)	Ornithine negative
E. coli	*E. coli* (99.67)	*E. coli* (99.97)	Urea negative
E. coli	*Shigella* (88.9) *E. coli* (7.3)	*E. coli* (99.82)	Lysine negative
Klebsiella pneumoniae[b]	*K. pneumoniae* (93.8)	*K. pneumoniae* (99.9)	Malonate negative
K. pneumoniae[b]	*Klebsiella ozaenae* (70) *Enterobacter agglomerans* (15.6) *K. pneumoniae* (8.8)	*K. pneumoniae* (93.8)	Lysine negative
K. pneumoniae[b]	*E. agglomerans* (30.7) *K. pneumoniae* (23.6) *Citrobacter freundii* (23.6)	*K. pneumoniae* (99.9)	Urea negative, lysine negative
Serratia marcescens	*S. marcescens* (68.5)	*S. marcescens* (97.8)	Ornithine negative
S. marcescens	*S. marcescens* (51.6) *S. liquefaciens* (47)	*S. marcescens* (97.8)	ONPG negative
Enterobacter cloacae[b]	*K. pneumoniae* (98.8)	*E. cloacae* (93.8)	Nitrate negative, urea positive, lysine positive
E. aerogenes[b]	*E. aerogenes* (95.2)	*E. aerogenes* (97.4)	Citrate negative

[a] Numbers within parentheses represent the percent confidence of identification as found in the Minitek profile book.

[b] A separate blood culture from the same patient correlated by the direct broth inoculum procedure and the standard colony procedure.

[c] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

wet mount motility preparation from the inoculum broth is a useful adjunct to the test system. In the present study, no organisms would have been misidentified with these biochemical and microscopic procedures, and only 6 of 106 isolates would have required further biochemical testing for confirmation of species. The identification of nonfermenting gram-negative bacteria is best performed by using the manufacturer's recommendations.

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