APPLIED MICROBIOLOGY, Feb. 1972, p. 298-300
Vol. 23, No. 2

Printed in U.S.A.

Evaluation of the Auxotab Enteric 1 System for Identification of *Enterobacteriaceae*

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Received for publication 15 November 1971

An evaluation of the accuracy and convenience of the Auxotab Enteric 1 System for identification of *Enterobacteriaceae* was performed with 160 bacteria. Identification at the species level was correct in 134 (83.8%) instances and at the generic level in 144 (90%) instances. Sixty strains failed to achieve the minimal concentration of organisms required to complete the identification process within 7 hr. The system was judged to be laborious and to present a potential hazard to those working with it.

Previous reports (6-8) from this laboratory have described evaluations of several test systems designed to provide rapid or convenient identification of *Enterobacteriaceae*. This study was performed to assess the convenience and accuracy of the Auxotab Enteric 1 System for rapid identification of *Enterobacteriaceae*.

MATERIALS AND METHODS

One hundred sixty bacterial cultures belonging to the family *Enterobacteriaceae* were studied (Table 1); 122 strains had been recently isolated from clinical material, and 38 represented stock cultures. The organisms were identified by using conventional procedures, described elsewhere (2), according to the taxonomic system of Ewing (4).

The Auxotab Enteric 1 System (kindly supplied by Colab Laboratories, Inc., Glenwood, Ill.) consists of a card with 10 capillary units as described by Buissière and Nardon (1). The 10 capillaries contain the following reagents: viability control (resazurin reduction), malonate, phenylalanine deaminase, H_2S, sucrose, *o*-nitrophenyl-β-D-galactopyranoside (β-galactosidase), lysine decarboxylase, ornithine decarboxylase, urease, and tryptophan (indole). Each capillary is inoculated with a bacterial suspension prepared by subculturing a single suspected colony from a differential agar medium to 5 ml of brain-heart infusion broth (BHI) which is incubated at 35 C for 3.5 hr. After incubation, the broth is centrifuged at 1,000 to 2,000 × *g* for 15 min, the supernatant fluid is discarded, and the cell sediment is suspended in 1.8 ml of distilled or deionized water (*p*H 5.5 to 6.7 recommended) which need not be sterile. The aqueous suspension should have a bacterial concentration of at least 1.5×10^9/ml, which is comparable in turbidity to a McFarland no. 5 barium sulfate standard. If this concentration is not attained, the original colony must be inoculated onto an agar slant which is incubated overnight and from which several loopfuls of growth are then transferred to distilled or deionized water. The aqueous suspension is inoculated into the upper opening of each capillary by use of a Pasteur pipette, and each capillary must be filled so that a convex droplet appears in both upper and lower openings. The card is then incubated at 35 C for 3 hr in a special chamber provided by the manufacturer.

RESULTS

Sixty (37.5%) of the 160 strains failed to attain the desired turbidity in aqueous solution after 3.5 hr of incubation in BHI. Of these 60 strains, 36 belonged to either the *Proteus* (21 strains) or *Providencia* (15 strains) genera. In such instances, subcultures to agar slants had to be made, and the Auxotab process was started the next day, further delaying identification. The *p*H requirement of 5.5 to 6.7 for the distilled or deionized water posed a minor problem, because the *p*H of such water in our laboratory generally exceeded 7.0 and therefore required acidification.

Biochemical reactions obtained by conventional means are compared with those obtained in the Auxotab System in Table 2. Indeterminate reactions in the Auxotab System generally became clearly positive after overnight incubation. Poor agreement was noted between the Auxotab urease test and its conventional counterpart (Christensen's). Seven strains of *Proteus* failed to hydrolyze urea in the test system; the remainder of the falsely negative urease tests occurred with members of the tribe *Klebsielleae*. There was 79 and 90% agreement of the Auxotab lysine and ornithine decarboxylase tests, respectively, with

TABLE 1. *Members of the family Enterobacteriaceae used to evaluate the Auxotab Enteric 1 System*

Organism	No.
Escherichia coli	15
Shigella sp.	13
Edwardsiella tarda	2
Citrobacter freundii	14
Salmonella sp.	11
Arizona hinshawii	4
Klebsiella pneumoniae	18
Enterobacter aerogenes	11
E. cloacae	12
E. liquefaciens	5
E. hafniae	2
Serratia marcesens	5
Proteus mirabilis	12
P. vulgaris	6
P. morganii	7
P. rettgeri	7
Providencia sp.	16
Total	160

their conventional counterparts. The agreement between the Auxotab sucrose fermentation test and its conventional counterpart was 80%; however, since sucrose is not particularly useful in the differentiation of *Enterobacter hafniae* from *E. aerogenes*, *E. liquefaciens*, and *Serratia* (3, 5), as recommended by the manufacturer, this test was not considered to be especially important. The remainder of the tests, allowing for delayed reactions, agreed well.

Identification by means of the Auxotab System was correct in 118 instances. In 16 additional instances, identification could be made, despite negativity of one test reaction characteristic of that species, by relying on the organism's morphological features on eosin-methylene blue-agar (EMB). For instance, two strains of *P. vulgaris* failed to produce H_2S, and three strains of *Proteus* (one *P. vulgaris* and two *P. mirabilis*) failed to hydrolyze urea; however, swarming of the colonies on EMB

TABLE 2. *Comparison of biochemical reactions in conventional and Auxotab Enteric 1 Systems*

Tests	Conventional			Auxotab		
	Positive	Delayed positive[a]	Negative	Positive	Negative	Indeterminate
H_2S	48	0	112	36	118	6
Urea	72	5	83	25	133	2
Indole	61	0	99	64	96	0
Lysine decarboxylase	65	6	89	55	104	1
Ornithine decarboxylase	94	2	64	81	74	5
Phenylalanine deaminase	48	0	112	48	112	0
Malonate	41	4	115	44	115	1
Sucrose	76	11	73	60	90	10
o-Nitrophenyl-β-D-galactopyranoside	96	0	64	94	66	0
Resazurin (control)				160		

[a] Requiring 2 or more days for completion.

TABLE 3. *Organisms incorrectly identified by the Auxotab Enteric 1 System*

No.	Identification		Key test resulting in incorrect identification
	Conventional	Auxotab	
1	*Citrobacter*	?	H_2S, negative
1	*Arizona*	*Enterobacter*	H_2S, negative
1	*Arizona*	*Citrobacter*	Lysine decarboxylase, negative
1	*Salmonella*	*Citrobacter*	Lysine decarboxylase, negative
1	*Klebsiella*	*Enterobacter*	Ornithine decarboxylase, positive
1	*E. cloacae*	?	Lysine and ornithine decarboxylase, negative
1	*E. hafniae*	*Arizona*	H_2S, positive
2	*E. liquefaciens*	?	Lysine and ornithine decarboxylase, negative
2	*Serratia*	*E. cloacae*	Lysine decarboxylase, negative
1	*Serratia*	*Klebsiella*	Ornithine decarboxylase, negative
4	*P. rettgeri*	*Providencia*	Urease, negative

provided an important clue to their correct identification. In nine other instances (six *Salmonella*, one *Arizona*, and two *Citrobacter*), H_2S production was negative (four strains) or indeterminate (five strains) after the recommended 3-hr incubation of the Auxotab card; however, colonial morphological characteristics and other biochemical reactions permitted correct identification.

In 10 additional instances, grouping was correct but speciation was not. Three strains of *E. liquefaciens* and one strain of *E. aerogenes* failed to decarboxylate lysine and were therefore called *E. cloacae*. Two strains of *P. morganii* were identified as *P. rettgeri* because of failure to decarboxylate ornithine. Four strains of *P. mirabilis* failed to decarboxylate ornithine or produce indole, thereby creating uncertainty as to their correct speciation.

In 16 instances, generic identification by the Auxotab System was incorrect (12 strains) or inconclusive (4 strains) because of an erroneous key reaction (Table 3).

Therefore, 118 (73.8%) strains could clearly be speciated within the limitations of the 10 Auxotab tests. In 16 additional instances in which a biochemical reaction was incorrect, colonial morphology on EMB was used to assist in identification, resulting in a total of 134 (83.8%) strains being correctly speciated. Identification at the generic level was accomplished for 144 (90%) strains.

DISCUSSION

The Auxotab System was considered to be tedious or inconvenient. A minimum of 7 hr was required for completion of identification, so that the process had to be started early in the morning in order to complete it within an 8-hr day. Because of this temporal requirement, which would make it difficult for many laboratories to start and complete the Auxotab process within an 8-hr day, and because more than one-third of the strains tested in our study failed to attain the concentration required to complete the process within 1 day, it is doubtful that "rapid" features of the Auxotab System will present any significant advantages over the other systems we have evaluated (6–8).

Each broth culture in BHI required centrifugation, so that maximal efficiency in the processing of many strains necessitated the use of many centrifuges. Finally, contamination of laboratory benches or technicians was judged to be a significant hazard, because it was difficult to avoid spillage of filled capillaries on the Auxotab card, and the special incubation chamber precluded the use of a suitable disposable container into which to place the card.

Accuracy of identification of *Enterobacteriaceae* was adequate in the Auxotab System but was contingent in many instances upon technical and microbiological experience in correctly assessing morphological features of the colony being identified.

LITERATURE CITED

1. Buissière, J., and P. Nardon. 1968. Microméthode d'identification des bactéries. I. Intérêt de la quantification des caractèrs biochimiques. Ann. Inst. Pasteur (Paris) **115**:218–231.
2. Douglas, G. W., and J. A. Washington II. 1969. Identification of Enterobacteriaceae in the clinical laboratory. Public Health Service. National Communicable Disease Center, Atlanta, Ga.
3. Edwards, P. R., and W. H. Ewing. 1962. Identification of Enterobacteriaceae, 2nd ed., p. 209. Burgess Publishing Co., Minneapolis.
4. Ewing, W. H. 1968. Differentiation of Enterobacteriaceae by biochemical reactions. Public Health Service. National Communicable Disease Center, Atlanta, Ga.
5. Martin, W. J. 1970. *Enterobacteriaceae*, p. 151–174. *In* J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
6. Martin, W. J., R. J. Birk, P. K. W. Yu, and J. A. Washington II. 1970. Identification of members of the family *Enterobacteriaceae* by the R-B system. Appl. Microbiol. **20**:880–883.
7. Martin, W. J., P. K. W. Yu, and J. A. Washington II. 1971. Evaluation of the enterotube system for identification of members of the family *Enterobacteriaceae*. Appl. Microbiol. **22**:96–99.
8. Washington, J. A., II, P. K. W. Yu, and W. J. Martin. 1971. Evaluation of accuracy of multitest micromethod system for identification of *Enterobacteriaceae*. Appl. Microbiol. **22**:267–269.