

# Auxotab—a Device for Identifying Enteric Bacteria

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A multitest system called the Auxotab that uses ten dehydrated reagents on a paper card has been evaluated with 417 known stock cultures of *Enterobacteriaceae*. In double-blind studies with the Auxotab, 87% of the strains tested were correctly identified. Results of this study indicate that there is a need for modification of the product in regard to ease of handling, time required for use, and accuracy of identification of enteric bacteria.

Many products have become available in recent years for use in the rapid and simplified identification of enteric bacteria. This report deals with the evaluation of one of these products, the Auxotab Enteric 1 Card, which is manufactured by Colab Laboratories, Inc., a division of Wilson Pharmaceutical and Chemical Corp. The product consists of a card of 10 capillary units, each containing a specific biochemical test; a viability control (which is resazurin reduction); and tests for malonate utilization, phenylalanine deaminase, hydrogen-sulfide production, sucrose fermentation, *o*-nitrophenyl- $\beta$ -D-galactopyranoside, lysine decarboxylase, ornithine decarboxylase, urease, and tryptophan.

In 1972, Washington et al. (3) evaluated this product with 160 freshly isolated and stock cultures of *Enterobacteriaceae*. Use of the product was found to be laborious and a potential hazard to those working with it. The percentage of correct identification was 83.8% at the species level and 90% at the generic level. This study was undertaken with stock cultures instead of fresh isolates to test the validity of the system when it is used with controlled organisms.

## MATERIALS AND METHODS

All bacterial cultures were provided by the Enterobacteriology Unit, Laboratory Division, Center for Disease Control (CDC), as coded unknowns in plain agar deeps. All cultures had been received at the CDC for confirmation or identification. A MacConkey agar plate was streaked from the agar deep to serve as a primary isolation medium. One microbiologist identified each culture by our conventional system, while another microbiologist, working independently, used the Auxotab on the same culture.

A third microbiologist compared the results obtained and indicated whether tests should be repeated.

The Auxotab Enteric 1 cards were supplied by Colab Laboratories, Inc., Glenwood, Ill., and were used exactly as the manufacturer recommended. The Auxotab system was used by selecting an 18- to 24-hr colony from a MacConkey agar plate and subculturing it in 5 ml of brain heart infusion broth. The broth was incubated for 3.5 hr at 35 C and then centrifuged for 15 min at 3,500 rev/min. The supernatant fluid was discarded, and the cells were suspended in 1.8 ml of distilled water with a pH between 5.5 and 6.7. The Auxotab card was slightly tilted, and the suspended cells were dispensed by a Pasteur pipette into the upper opening of each capillary unit until a convex drop appeared at the bottom.

The card was then placed in an Auxochamber, a molded plastic container containing a moist sponge. The sliding door of the chamber was closed, and the chamber was placed in a 35 C incubator for 1 hr. At this time, the viability control was checked. If the control had turned pink, the chamber would have been returned to the incubator for an additional 2 hr. After the incubation period, the test results were read according to the manufacturer's instructions. The manufacturer suggested that a transfer of the selected colony be made in case the culture failed to react, or for additional testing later.

Media used in our conventional system for authentication of stock cultures were prepared in a central media kitchen from commercially available dehydrated media (BBL, Difco). The quality of each batch of this medium was controlled with a collection of stock cultures of known biochemical reactions. Our system consists of the following biochemical tests: triple sugar iron, urea, indole, methyl red, acetoin, Simmon's citrate, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, motility, arabinose, raffinose, and rhamnose fermentation. Additional biochemical tests were used as needed. The media used for the conventional and extra tests were those recommended by Edwards and Ewing (1) and

by Ewing (2). Serology was not used in this study, although the Enterobacteriology Unit, CDC, did serologically test some of the cultures.

### RESULTS

The capability of the Auxotab system in correctly identifying an unknown from the *Enterobacteriaceae* family was examined. Table 1 shows the number and percentage of each genus or species that was correctly identified by the Auxotab system. In each case, the result obtained with our conventional system was accepted as the correct one. The total accuracy for all specimens with the Auxotab system was 87%, or 363 of 417 specimens. Identification by the Auxotab was correct at least 95% of the time for *Citrobacter freundii*, *Klebsiella pneumoniae*, *Providencia*, *Proteus vulgaris*, *Salmonella*, *Shigella*, *Escherichia coli*, and *Proteus morgani*. However, *Enterobacter hafniae*, *Proteus mirabilis*, *Edwardsiella tarda*, *Proteus rettgeri*, *Arizona hinshawii*, *Enterobacter cloacae*, and the *Serratia marcescens-Enterobacter liquefaciens-Enterobacter aerogenes* group were correctly identified only 68 to 89% of the time. The latter three organisms were considered as an *Enterobacter-Serratia* group because the manufacturer states that these organisms cannot be fully differentiated without using an additional card, the Auxotab Enteric Card 2.

In Table 2, the number and percentage of times that *S. marcescens*, *E. liquefaciens*, and *E. aerogenes* were correctly identified as be-

TABLE 1. Accuracy of identification with the Auxotab system

Organism	No. correct/no. tested	% Correct
<i>Citrobacter</i> .....	23/23	100
<i>Klebsiella</i> .....	29/29	100
<i>Providencia</i> .....	28/28	100
<i>P. vulgaris</i> .....	11/11	100
<i>Salmonella</i> .....	28/28	100
<i>Shigella</i> .....	19/19	100
<i>E. coli</i> .....	27/28	96.4
<i>P. morgani</i> .....	19/20	95.0
<i>E. hafniae</i> .....	26/29	89.7
<i>P. mirabilis</i> .....	23/26	88.5
<i>E. tarda</i> .....	14/17	82.3
<i>P. rettgeri</i> .....	18/22	81.8
<i>Arizona</i> .....	23/29	79.3
<i>E. cloacae</i> .....	21/29	72.4
<i>Serratia, E. liquefaciens, E. aerogenes</i> .....	54/79	68.4
Total	363/417	Avg 87.1%

TABLE 2. Identification of *Enterobacter-Serratia* group using the Auxotab system

Organism	No. <sup>a</sup>	%
<i>Enterobacter aerogenes</i> .....	27/29	93.1
<i>Serratia marcescens</i> .....	20/26	76.9
<i>Enterobacter liquefaciens</i> .....	7/24	29.2

<sup>a</sup> Number correct/number tested.

longing to the *Enterobacter-Serratia* group have been tabulated. These three organisms can produce the same reactions in the Auxotab system, except for malonate utilization by *E. aerogenes*. Organisms that were malonate positive were *E. aerogenes*. It is apparent from Table 2 that *E. liquefaciens* was the least accurately identified member of this group.

Individual test results of the Auxotab and conventional systems were also compared and examined. Table 3 lists the eight tests in ascending order of agreement of results from the two systems. The agreement between the results of the urease tests was the poorest; that between the other seven tests was better than 94%. The urease test was not sensitive enough to detect the weakly positive reaction of *E. cloacae*, *K. pneumoniae*, *C. freundii*, and some *P. rettgeri*. Malonate, lysine, and hydrogen sulfide tests caused problems. The hydrogen sulfide test was too sensitive for some organisms, causing two *E. liquefaciens*, two *E. aerogenes*, and eight *E. cloacae* to be called positive. The Auxotab system also had problems with some strong hydrogen sulfide producers, by failing to detect three *E. tarda* and two *A. hinshawii*. There was difficulty in the interpretation of the malonate, lysine, and in some instances, the ornithine tests. The malonate was not sensitive enough and in most cases was interpreted as negative in the Auxotab system, whereas by the conventional system, interpretation was not a problem. Many false negatives occurred in the lysine test, especially with the *E. liquefaciens* and *S. marcescens*.

### DISCUSSION

The results of this study indicate that the Auxotab system, although offering the user the advantages of same-day identification and moderate to good identification potential, has several disadvantages which make it a poor alternative to our conventional system or to other similar products on the market. These disadvantages are: (i) the potential danger of infection of laboratory personnel when using the card, (ii) long preparation time, (iii) variability of test sensitivity, and (iv) subjective

TABLE 3. Agreement of biochemical test results from the conventional and Auxotab systems

Test	Agreement	
	No. <sup>a</sup>	%
Urease .....	328/417	78.7
H <sub>2</sub> S .....	393/417	94.2
Lysine .....	396/417	95.0
Malonate .....	399/417	95.7
Ornithine .....	403/417	96.6
Indole .....	406/417	97.4
Sucrose .....	408/417	97.8
Phenylalanine .....	417/417	100.0

<sup>a</sup> Number agreeing/number tested.

interpretation of results.

Although the manufacturer has made available individual plastic incubation chambers, the potential for exposure to infectious material still exists because of the exposed drops on the surface of the card. The preparation time is such that all cultures must be picked very early in the day in order to get an answer within the normal 8-hr workday. The sensitivity of tests is

not standardized. The urease, malonate, lysine, and ornithine tests are not sensitive enough, and the hydrogen sulfide test is too sensitive. A constant problem throughout the study was interpretation of test results. The inability of the system to produce consistently strong positive or negative reactions often made it necessary to make a subjective decision as to whether the reaction was negative or positive. In most cases, this represents a real problem in the clinical laboratory. The possibility of using this system as a screening device might be explored when only one or two organisms are under consideration.

#### LITERATURE CITED

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