APPLIED MICROBIOLOGY, Sept. 1971, p. 267-269

Vol. 22, No. 3
Printed in U.S.A.

Evaluation of Accuracy of Multitest Micromethod System for Identification of *Enterobacteriaceae*

JOHN A. WASHINGTON II, PAULINE K. W. YU, AND WILLIAM JEFFERY MARTIN
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 30 April 1971

The Analytab system of 20 biochemical tests for identification of *Enterobacteriaceae* was evaluated in parallel with conventional tests on 128 *Enterobacteriaceae*, 5 *Aeromonas*, and 1 *Yersinia enterocolitica*. The results of tests for H_2S and indole production, citrate utilization, lysine and ornithine decarboxylase, arginine dihydrolase, nitrate reduction, β-galactosidase, and fermentation of arabinose, rhamnose, mannitol, and glucose showed almost complete agreement between the two systems. Eighty-eight per cent of *Enterobacteriaceae* were correctly speciated with the Analytab system; on repeat testing with heavier inocula of organisms failing to ferment glucose initially, the proportion of *Enterobacteriaceae* correctly speciated became 93%.

Of the positive cultures encountered in the routine clinical bacteriology laboratory, approximately 90% have been found to contain gram-negative bacilli (1); in our own experience, approximately 95% of these are *Enterobacteriaceae*. The need for identification of members of this family of bacteria has resulted in a variety of approaches (1) utilizing techniques which have been well described (2–4). To simplify the problems of media production and storage and to decrease the number of tests required, a variety of test systems with variable degrees of accuracy have become available commercially (7–11). In this study, we evaluated a multitest micromethod system for identification of the *Enterobacteriaceae*.

MATERIALS AND METHODS

One hundred and twenty-eight *Enterobacteriaceae*, 5 *Aeromonas*, and 1 *Yersinia enterocolitica* (Table 1), representing 96 fresh clinical or autopsy isolates and 38 stock cultures (Table 2), were used in the study. Each strain was assigned a code number, but the identity of the strains was kept unknown until the study was completed. A single colony of each strain was emulsified in 4 ml of distilled water and inoculated with a Pasteur pipette into each of the 20 biochemical tests of the Analytab system (Analytab Products, New York, lot no. 126). Each test in this miniaturized system is performed within a sterile plastic tube which contains the appropriate substrate, has a capacity of 0.12 ml, and is affixed to an impermeable plastic backing. The 20 tests consist of the following: β-galactosidase (ONPG), arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate, H_2S, urease, deaminase, indole, acetoin, gelatin, and fermentation tests of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdaline, and arabinose. All tests were performed as recommended by the manufacturer and incubated for 18 to 24 hr at 37 C in a special box supplied by the manufacturer to prevent excessive evaporation of moisture. Some tests (excluding those for fermentation) were observed for longer periods. Nitrate reduction was determined at 24 or 48 hr by adding 1 drop each of sulfanilic acid (0.8% in 0.2 N acetic acid) and α-naphthylamine (0.5% in 0.2 N acetic acid) to the glucose tube; negative reactions were checked for complete nitrate reduction by addition of zinc dust.

Each test strain was inoculated into 0.2 ml of 0.85% NaCl to which an ONPG disc (Difco) was added to determine the presence of β-galactosidase. Also inoculated were: triple sugar iron agar (BBL); lysine iron agar (BBL); Simmons' citrate agar (BBL); Christensen's urea agar (BBL); phenylalanine deaminase agar (BBL); methyl red-Voges-Proskauer broth (BBL); and Trypticase peptone broth (BBL). Decarboxylase tests were carried out in Moeller decarboxylase base (BBL) to which 0.3% agar was added. Media for testing carbohydrate fermentation and gelatin liquefaction were prepared as described by Edwards and Ewing (3). Nitrate reduction was determined by a method described elsewhere (6). Tests were performed as described by Edwards and Ewing (3) and by Douglas and Washington (2), and organisms were identified by the taxonomic system of Ewing and associates (5).

RESULTS AND DISCUSSION

The results of the biochemical tests of the two systems are listed in Table 3. Agreement between

TABLE 1. *Organisms used in evaluation of Analytab system*

Organisms	No.	Organisms	No.
Escherichia coli	16	*P. morganii*	4
Klebsiella pneumoniae	17	*Providencia*	9
K. rhinoscleromatis	1	*Salmonella*	7
Enterobacter aerogenes	5	*Salmonella typhi*	2
E. cloacae	4	*Shigella sonnei*	4
E. hafniae	4	*S. flexneri*	1
E. liquefaciens	4	*Edwardsiella tarda*	2
Serratia marcescens	9	*Arizona hinshawii*	3
Atypical *E. cloacae*	5	*Erwinia ananas*	1
Citrobacter freundii	5	*E. amylovora*	1
H_2S-negative *C. freundii*	4	*Pectobacterium carotovorum*	3
Proteus mirabilis	8	*Herbicola-lathyri*	1
P. vulgaris	2	*Aeromonas* sp.	5
P. rettgeri	6	*Yersinia enterocolitica*	1

TABLE 2. *Sources of organisms*

Source	No.	Source	No.
Clinical		Autopsy	
Urine	41	Heart blood	2
Sputum	20	Common duct	1
Stool	9	Abdominal fluid	1
Throat	2	Spleen	1
Kidney	1	Kidney	1
Vagina	2	Total	96
Cyst, coccyx	1	Stock culture	
Anal sinus	1	Center for Disease Control	9
Bile duct	2	Mayo Clinic	25
Abdominal wound	1	College of American Pathologists	2
Thorax	1	Pasteur Institute	2
Rectal area	1	Total	38
Stump	2		
Ear	3		
Trachea	1		
Abscess, breast	1		
Groin	1		

the two systems was high in the following tests: H_2S production, citrate utilization, indole production, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, nitrate reduction, β-galactosidase (ONPG), and fermentation of arabinose, rhamnose, mannitol, and glucose. Nineteen of the 47 Analytab system-positive lysine decarboxylase tests were only slightly positive in 24 hr but became strongly positive at 48 hr. Somewhat less agreement was obtained in the fermentation tests with sorbitol and sucrose; however, of the falsely negative sucrose tests, seven were with strains of *Providencia* which fermented sucrose in the conventional system within or after 48 hr of incubation. The Analytab system yielded nine more positive inositol fermentation reactions than did the conventional system, of which five represented typical or atypical *Enterobacter cloacae* and four represented *Citrobacter freundii*. The Analytab system yielded five more positive tests for gelatin liquefaction than did the conventional system of which two strains were *Providencia* and one was *Escherichia coli*.

The conventional system detected 21 more instances of urease activity than did the Analytab system. All of these strains gave weakly alkaline slants on Christensen's urea agar—15 at 24 hr, 2 at 48 hr, and 1 after more than 48 hr of incubation. The fact that the Analytab system urea test is buffered to *p*H 6.3 probably accounts for this discrepancy. The conventional system yielded nine more positive Voges-Proskauer tests, four of which represented strains of *Proteus mirabilis* and five of which were in the tribe Klebsielleae. In the Analytab system, one strain each of *P. morganii* and *P. rettgeri* and two strains of *Aeromonas* failed to produce deaminase. Negative nitrate reduction tests were obtained in the Analytab system with one strain of each of the following: *E. coli*, *C. freundii*, *P. mirabilis*, *P. morganii*, *Serratia marcescens*, *Aeromonas*, and *Y. enterocolitica*.

Of the 134 strains tested, 116 (87%) were cor-

TABLE 3. *Comparison of positive tests in Analytab and conventional systems*[a]

Test	No. positive by Analytab			No. positive by conventional		
	24 hr	48 hr	>48 hr	24 hr	48 hr	>48 hr
H_2S	22	2		24	1	1
Citrate	68	8	4	70	8	3
Urea	37			51	3	4
Indole	52			51	1	
Voges-Proskauer	34			43		
Lysine decarboxylase	47	16	5	61	2	1
Arginine dihydrolase	9	17	5	6	19	4
Ornithine decarboxylase	60	4		63	2	1
Deaminase	27			31		
Gelatin	14	12	1	7	5	10
Arabinose	75			79	2	
Rhamnose	73			74	2	
Sucrose	58			58	9	1
Inositol	59			36	14	
Sorbitol	75			83		
Glucose	124			124		
Mannitol	93			96		
o-Nitrophenyl-β-D-galactopyranoside	77	6		83		
Nitrate reduction	102			106	1	

[a] Fermentation tests by conventional means were not performed on melibiose and amygdaline.

[b] One hundred-eleven tests performed in parallel.

rectly speciated with the Analytab system and 117 (87%) were correctly identified as to genus. Of the 128 *Enterobacteriaceae*, 113 (88%) were correctly speciated and 114 (89%) were correctly grouped with the Analytab system. There were 10 strains (1 *Salmonella typhi*, 1 *Klebsiella rhinoscleromatis*, 2 *Pectobacterium carotovorum*, 1 *E. liquefaciens*, 3 *Aeromonas*, 1 *Herbicola-lathryi*, and 1 *E. amylovora*) which failed to ferment glucose in the Analytab system. Since this reaction is used as a control, a negative result was interpreted to mean that the test strain did not belong in the family *Enterobacteriaceae*. Repeat testing of these strains with heavier inocula did permit their identification.

The following strains were incorrectly identified by the Analytab system because of discrepancies between results of one or more of its tests and those same tests in the conventional system: one H_2S-negative *C. freundii*, one *P. rettgeri*, two *Shigella sonnei*, three *E. liquefaciens*, and one *Arizona hinshawii*. Since the Analytab system yielded weakly positive lysine decarboxylase tests with the two *S. sonnei*, they were called *E. coli*. Two *E. liquefaciens* were misidentified as *S. marcescens* because they failed to ferment arabinose or rhamnose in the Analytab system (one fermented arabinose and the other raffinose in conventional tests). A third *E. liquefaciens* could not be identified by the Analytab system because its only two positive reactions were the fermentation of glucose and decarboxylation of ornithine, despite characteristic reactions in the conventional tests. The *A. hinshawii* was H_2S- and ONPG-negative in the Analytab system. The *P. rettgeri* was called *Providencia* in the Analytab system because of a negative urease test result. The H_2S-negative *C. freundii* was read as an indole-negative *E. coli* because of a negative citrate test result; however, the positive amygdaline test was inconsistent with this interpretation.

The Analytab system represents the most complete commercially available test series for identification of members of the family *Enterobacteriaceae*. It is unique in its capability to speciate *Enterobacteriaceae* and also to do so with an accuracy of nearly 90%. Repeat testing with a heavier inoculum of those strains failing to ferment glucose initially improved the accuracy of the system in identifying the *Enterobacteriaceae* to approximately 93%. Its principal disadvantage is the time required to prepare and inoculate the 20 tests (approximately 3 min) and the care required in the tedious task of filling each tube. Its principal advantages are not only those of considerable accuracy but also those afforded by the ready availability of a large number of biochemical tests which are ready for use, require minimal storage space, and are stable at either refrigerater or room temperatures for prolonged periods.

LITERATURE CITED

1. Borchardt, K. A. 1968. Simplified method for identification of enteric and other gram-negative bacteria using reagent-impregnated strips. Amer. J. Clin. Pathol. 49:748–750.
2. Douglas, G. W., and J. A. Washington II. 1969. Identification of *Enterobacteriaceae* in the clinical laboratory. U.S. Department of Health, Education, and Welfare, Public Health Service, National Communicable Disease Center, Atlanta, Ga.
3. Edwards, P. R., and W. H. Ewing. 1962. Identification of *Enterobacteriaceae*, 2nd ed. Burgess Publishing Co., Minneapolis.
4. Ewing, W. H. 1963. An outline of nomenclature for the family *Enterobacteriaceae*. Int. Bull. Bacteriol. Nomen. Taxon. 13:95–109.
5. Ewing, W. H., J. N. Wilfert, L. J. Kunz, M. Dumoff, and H. D. Isenberg. 1969. Roundtable: how far to go with *Enterobacteriaceae*? J. Infec. Dis. 119:197–213.
6. Grunberg, E., E. Titsworth, G. Beskid, R. Cleeland, Jr., and W. F. Delorenzo. 1969. Efficiency of a multitest system (Enterotube) for rapid identification of *Enterobacteriaceae*. Appl. Microbiol. 18:207–213.
7. 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.
8. Martin, W. J., S. F. Bartes, and M. M. Ball. 1971. Evaluation of reagent-impregnated strips for identification of *Enterobacteriaceae*. Amer. J. Med. Technol. 37:99–101.
9. 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.
10. Matsen, J. M., and J. C. Sherris. 1969. Comparative study of the efficacy of seven paper-reagent strips and conventional biochemical tests in identifying gram-negative organisms. Appl. Microbiol. 18:452–457.
11. Yu, P., R. J. Birk, and J. A. Washington II. 1969. Evaluation of a reagent-impregnated strip for detection of nitrate reduction by bacterial isolates. Amer. J. Clin. Pathol. 52: 791–793.