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Standardization of the Analytab Enteric (API 20E) System to Increase Accuracy and Reproducibility of the Test for Biotype Characterization of Bacteria

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Procedures employing the Analytab Enteric (API 20E) system were standardized to improve the accuracy and reproducibility of the individual biochemical tests so that the system could be used to biochemically characterize bacteria for epidemiological studies. The standardized method and the method recommended by the manufacturer (routine method) were tested in parallel with 130 clinical isolates. Tests with 100 randomly selected clinical isolates demonstrated that the standardized method was more accurate and reproducible than the routine method. In addition, the standardized method accurately identified 24 of 30 clinical isolates which could not be identified with the routine method.

The epidemiological investigation of hospital-associated infections often requires bacterial characterization at the subspecies level. This can be accomplished by determining the susceptibility of the isolate to lysis by bacteriophages, the production of or susceptibility to bacteriocins, serological typing, the analysis of the antimicrobial susceptibility pattern (antibiogram), or the determination of the biochemical reaction pattern (biotype). Bacteriophage or bacteriocin testing is generally performed only in research laboratories, and serological typing requires specific antisera and is primarily used to characterize only enteric pathogens. Analyses of antibiograms and biotype profiles are, however, commonly used in clinical laboratories. The usefulness of either antibiograms or biotype profiles is dependent on the number of tests performed and the reproducibility of the individual tests. The commercial development of compact biochemical systems for the identification of bacteria made the use of a large number of tests for biotyping financially feasible for diagnostic laboratories (7). One such identification system is the Analytab Enteric (API 20E) test system, which consists of a miniaturized set of 20 biochemical tests. With this system the individual biochemical test results and the overall identification of bacteria have been reported to be highly accurate (6, 10). Conflicting reports have, however, been published concerning the reproducibility of the individual biochemical tests and the biotype profiles (2-4, 8, 9). In the following study, it is reported that the API 20E test system, when used as recommended by the manufacturer, was not sufficiently reproducible for the biotype classification of organisms. Modifications of the recommended methods are described which increased the reproducibility and accuracy of the individual biochemical tests and the biotype profiles.

MATERIALS AND METHODS

API 20E test system. The API 20E test system consists of 20 cupules, each of which contains dehydrated medium, and includes the following tests: *o*-nitrophenyl-β-D-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization (CIT), hydrogen sulfide production, urease, tryptophan deaminase, indole production (IND), acetoin production (VP), gelatinase, and fermentation of glucose, mannose, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdaline, and arabinose. The medium was reconstituted with a suspension of bacteria, and the system was incubated in an air atmosphere for 18 to 24 h at 35°C. After the addition of the specified reagents tryptophan deaminase, IND, and VP cupules, the 20 reactions were interpreted according to the explicit directions of the manufacturer.

Preparation of inocula. Two methods were evaluated for the preparation of the inoculum. In the method recommended by the manufacturer (routine method), the inoculum was prepared by gently touching the top of an isolated colony with the tip of a wooden applicator stick and then suspending the bacteria in 5 ml of sterile, distilled water. This produced an inoculum of approximately 10^5 to 10^6 colony-forming units (CFU) per ml, which was determined with quantitative serial dilutions of the suspension.

In the second (standardized) method, a suspension of bacteria was prepared either with isolated colonies of bacteria collected directly from agar plates or with bacteria collected from plates and preincubated in tryptic soy broth (TSB). Bacteria from plates were suspended in sterile, distilled water and adjusted to

the desired concentration by comparing the turbidity of the suspension with McFarland barium sulfate nephelometer standards. The broth inoculum was prepared by touching the top of an isolated colony and transferring this to 10 ml of TSB in a 12-ml conical centrifuge tube. After 2 to 4 h of incubation at 35°C, the broth was centrifuged at 3,000 × *g* for 10 min, the supernatant was decanted, and the pellet of bacteria was resuspended in sterile, distilled water to the desired concentration. The API 20E test strips were then inoculated with either the routine or the standardized suspensions of bacteria.

Determination of optimum standardized inoculum for reproducibility. Clinical isolates from the Barnes Hospital diagnostic microbiology laboratory were used to determine the optimum inoculum for test reproducibility. The inoculum of bacteria was prepared with bacteria both preincubated in TSB and selected from agar plates as described above. The following inocula were tested: 2×10^8 CFU/ml (determined to be equivalent to the turbidity of a no. 1 McFarland standard), 10^8 CFU/ml (one-half of a no. 1 McFarland standard), 10^7 CFU/ml (1:10 dilution of 10^8 CFU/ml), and 10^6 CFU/ml (1:10 dilution of 10^7 CFU/ml). The different concentrations of each organism were independently tested by two technologists to determine the reproducibility between technologists for each biochemical test with each inoculum preparation.

Standardized test reproducibility. The reproducibility of the standardized testing method, using an inoculum of 2×10^8 CFU/ml prepared from TSB, was further evaluated with 100 clinical isolates. Each organism was independently tested by two technologists to determine the reproducibility of the testing method. In addition, each of the test strips was interpreted by the two technologists to determine the reproducibility of test readings.

Standardized test accuracy. The biochemical test results obtained with the standardized method with the 100 clinical isolates described above were compared with the results obtained with the routine method. The routine tests were performed by one of the two technologists who had performed the standardized tests. The biochemical reactions, for which the routine and the standardized testing methods were not in agreement, were retested with conventional biochemical tests as recommended by Edwards and Ewing (5). An additional 30 clinical isolates, for which an identification had not been obtained with duplicate tests in the clinical laboratory with the routine testing method, were tested with the standardized method. The identification obtained with the standardized method was compared with that obtained with conventional biochemical tests.

RESULTS

The optimum inoculum for reproducibility of the API 20E test system was initially determined. Table 1 shows the results of duplicate tests with 20 clinical isolates, including 5 *Escherichia coli*, 4 *Klebsiella pneumoniae*, 3 *Enterobacter aerogenes*, 2 *Enterobacter cloacae*, 4 *Proteus mirabilis*, and 2 *Proteus rettgeri*. A total of 58 discrepant biochemical reactions were recorded for the 20 isolates tested with the eight different inoculum preparations. The most reproducible tests were those in which the API 20E test strips were inoculated with bacteria preincubated in broth and adjusted to a concentration of 2×10^8 CFU/ml. With this inoculum, only one biochemical reaction (IND) with one organism (*E. cloacae*) was not reproducible with the duplicate tests. The individual biochemical reactions and the biotype profiles were less reproducible with both the smaller inocula and the inocula prepared directly from agar plates. An inoculum with 2×10^8 CFU/ml prepared from broth was used in the subsequent studies to further evaluate the reproducibility and the accuracy of the standardized testing method.

The reproducibility of the biochemical reactions obtained with the standardized testing method was determined with 100 randomly selected clinical isolates. Each isolate was tested in duplicate, and each test strip was independently read by two technologists. For each of the 100 isolates, there was complete agreement with the duplicate biochemical tests and with the test interpretations by the two technologists.

The 100 clinical isolates were then tested with the routine method to determine the concordance between the two testing methods. A comparison of the biotypes obtained with the routine and standardized API 20E testing methods is summarized in Table 2. The same biotype profile was obtained with the two methods with only 49 of the 100 isolates (Table 2), and a total of 72 discrepant biochemical reactions between the two testing methods were recorded (Table 3). A

TABLE 1. *Effect of different inoculum preparations on the reproducibility of the API 20E test system*

Inoculum tested (CFU/ml)	Inoculum from broth		Inoculum from agar	
	Organisms with same biotype/total tested	No. of discrepant individual tests	Organisms with same biotype/total tested	No. of discrepant individual tests
2×10^8	19/20 (95)[a]	1	15/20 (75)	6
10^8	17/20 (85)	4	12/20 (75)	6
10^7	14/20 (70)	7	12/20 (60)	10
10^6	12/20 (60)	10	13/20 (65)	14

[a] Number in parentheses indicates percentage of the total organisms tested with the same biotype profiles with duplicate testing.

TABLE 2. *Analysis of the identification agreement between the routine and the standardized API 20E testing methods*

Clinical isolate	No. tested	Biotype obtained with testing methods	
		Same	Different
Escherichia coli	53	31	22
Klebsiella pneumoniae	13	5	8
Enterobacter aerogenes	9	3	6
Enterobacter cloacae	5	3	2
Citrobacter freundii	2	1	1
Citrobacter diversus	1	0	1
Serratia marcescens	2	0	2
Proteus mirabilis	13	4	9
Proteus rettgeri	1	1	0
Providencia stuartii	1	1	0

TABLE 3. *Comparison of the biochemical testing accuracy of the routine and standardized API 20E testing methods*

Biochemical test[a]	No. of discrepancies between methods	No. of tests confirmed[b]	
		Standardized method	Routine method
ADH	4	4	0
LDC	3	3	0
ODC	3	3	0
CIT	12	12	0
H_2S	1	1	0
URE	7	7	0
IND	5	2	3
VP	12	6	6
GEL	1	1	0
INO	2	2	0
SAC	4	4	0
MEL	16	16	0
AMY	2[c]		
Total	72	61 (87)[d]	9 (13)

[a] ADH, Arginine dihydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; H_2S, hydrogen sulfide production; URE, urease; GEL, gelatinase; INO, inositol; SAC, sucrose; MEL, melibiose; AMY, amygdaline.

[b] Number of test results confirmed by conventional biochemical tests as accurate.

[c] Conventional biochemical tests were not performed.

[d] Number in parentheses indicates percentage of tests confirmed as accurate.

total of 70 of the 72 discrepant reactions were retested with conventional biochemical tests, and 61 of the reactions observed with the standardized method were confirmed. With the standardized method there were nine falsely positive reactions, including three IND reactions (two with *E. cloacae* and one with *E. aerogenes*) and six VP reactions (all with *P. mirabilis*). There were five discrepancies in identification of bacteria between the routine and standardized methods: (i) and (ii) *Enterobacter agglomerans* to *K. pneumoniae*, (iii) *K. pneumoniae* to *E. aerogenes*, (iv) no identification to *Citrobacter diversus*, and (v) *E. aerogenes* to no identification.

To further examine the accuracy of the standardized method, 30 clinical isolates which could not be identified with duplicate tests with the routine API 20E test method were selected from the diagnostic laboratory. All 30 isolates were identified by using conventional biochemical tests as described by Edwards and Ewing (5). In addition, the isolates were tested with the standardized testing method. An accurate identification was obtained with the standardized method for 24 of the 30 isolates (Table 4), and no identification was obtained for the other 6 isolates, which were biochemically nonreactive or weakly reactive in the API 20E test strips.

DISCUSSION

The usefulness of the API 20E test system as an aid in characterizing bacteria for epidemiological investigations is related to the reproducibility of the biotype profile. In the present study the same biotype profile was obtained for only 13 (65%) of the 20 organisms tested when the method recommended by the manufacturer for preparing the inoculum was used (Table 1). By increasing the inoculum concentration and using an inoculum of bacteria in the logarithmic phase of growth, more reproducible test results were obtained. The best results were with an inoculum of bacteria pregrown in broth and standardized to a concentration of 2×10^8 CFU/ml. With this inoculum there was only one nonreproducible biochemical reaction with duplicate testing of 20 organisms in the first part of the study and 100 organisms in the second part of the study. This represents one (0.04%) nonreproducible test out of a total of 2,400 individual reactions. The nonreproducibility of the routine testing method was due to difficulties encountered in interpreting weakly reactive tests. This was particularly true for CIT utilization and carbohy-

TABLE 4. *Performance of the standardized method with clinical isolates not identified with the routine method*

Isolate	No. tested	No. identified
Klebsiella pneumoniae	11	11
Escherichia coli	8	6
Citrobacter freundii	3	2
Citrobacter diversus	2	1
Enterobacter agglomerans	2	2
Enterobacter cloacae	2	0
Proteus mirabilis	2	2

drate fermentation tests, which resulted in 34 (59%) of the total 58 discrepancies recorded for the 20 isolates.

Although reading discrepancies were not recorded for 100 organisms tested with the standardized method, the CIT and VP reactions posed potential problems. The CIT reaction with *E. coli* turned a pale blue after incubation for 16 to 18 h, and a pale-pink-colored reaction was noted to develop with the VP test after incubation for 15 min. A positive test, therefore, was considered to be a royal-blue CIT reaction and a deep-pink or red VP reaction which developed within 5 to 10 min. It should also be noted that falsely positive CIT reactions were recorded with *E. coli* when the TSB was not adequately decanted from the pellet of bacteria. This same effect was observed with API 20E tests prepared directly from blood culture broths, although this was not reported by Blazevic et al. (1).

Although the data are not tabulated herein, the accuracy and the reproducibility of the standardized API 20E test method was determined for the 100 isolates after incubation for both 16 to 18 h and 22 to 24 h. Whereas the routine API 20E test method has been reported to be less reproducible at 16 to 18 h of incubation compared with 22 to 24 h (2), there was no difference between the readings at the two time intervals for test strips inoculated by the standardized method.

The test reactions obtained with the standardized API 20E testing method for 100 randomly selected clinical isolates were more accurate than those obtained with the routine API 20E testing method. There were 9 inaccurate reactions with the standardized test method and 63 inaccurate reactions with the routine test method, which represented 0.45 and 3.15%, respectively, of the total 2,000 individual biochemical tests performed with each method. The individual test accuracy with the routine test method is comparable with that reported elsewhere (2, 10). Whereas the inaccurate test reactions observed with the routine method were not associated with a particular species of bacteria, six false-positive VP reactions with *P. mirabilis* and three false-positive IND reactions with *Enterobacter* spp. were recorded with the standardized test method. Only one of the nine inaccurate reactions, the IND reaction with *E. aerogenes*, resulted in a change in identity to a nonexistent biochemical profile. The standardized test method was, therefore, able to identify 99 of the 100 clinical isolates tested, compared with 96 of 100 isolates tested with the routine method. The accuracy of the routine API 20E tests reported herein for identification of bacteria is comparable with that reported by other investigators (6, 10).

The standardized method accurately identified 24 of 30 selected clinical isolates which were not identified with the routine method. The difficulty encountered with the routine method was biochemical inactivity of these selected isolates, rather than the limitations of the biochemical tests available in the API 20E system.

The following can be concluded from these studies. First, the identification of isolates tested in this study at the species level was highly accurate with both the routine and the standardized API 20E testing methods. Although only a limited number of bacterial species were tested herein, it is likely that either method could be used for the identification of clinical isolates. In practice the routine method would, however, be preferred because there is less technologist time involved in specimen processing. Second, the individual test reactions are more accurate and reproducible with the standardized API 20E testing method compared with the routine API 20E testing method. The standardized method can, therefore, be used to characterize bacteria at a subspecies level for epidemiological studies.

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LITERATURE CITED

1. **Blazevic, D. J., C. M. Trombley, and M. E. Lund.** 1976. Inoculation of API-20E from positive blood cultures. J. Clin. Microbiol. **4**:522-523.
2. **Butler, D. A., C. M. Lobregat, and T. L. Gavan.** 1975. Reproducibility of the Analytab (API 20E) system. J. Clin. Microbiol. **2**:322-326.
3. **Davies, B. I.** 1977. Biochemical typing of urinary *Escherichia coli* strains by means of the API 20E Enterobacteriaceae system. J. Med. Microbiol. **10**:293-298.
4. **DeSilva, M. I., and S. J. Rubin.** 1977. Multiple biotypes of *Klebsiella pneumoniae* in single clinical specimens. J. Clin. Microbiol. **5**:62-65.
5. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.
6. **Nord, C. E., A. A. Lindberg, and A. Dahlback.** 1974. Evaluation of five test kits—API, Auxotab, Enterotube, PathoTec, and R/B—for identification of Enterobacteriaceae. Med. Microbiol. Immunol. **159**:211-220.
7. **Robertson, E. A., G. C. Macks, and J. D. MacLowry.** 1976. Analysis of cost and accuracy of alternative strategies for *Enterobacteriaceae* identification. J. Clin. Microbiol. **3**:421-424.
8. **Rubin, S. J., S. Brock, M. Chamberland, and R. W. Lyons.** 1976. Combined serotyping and biotyping of *Serratia marcescens*. J. Clin. Microbiol. **3**:582-585.
9. **Rutherford, I., V. Moody, T. L. Gavan, L. W. Ayres, and D. L. Taylor.** 1977. Comparative study of three methods of identification of *Enterobacteriaceae*. J. Clin. Microbiol. **5**:458-464.
10. **Smith, P. B., K. M. Tomfohrde, D. L. Rhoden, and A. Balows.** 1972. API system: a multiple micromethod for identification of *Enterobacteriaceae*. Appl. Microbiol. **24**:449-452.