

Evaluation of Modified R-B System for Identification of Members of the Family *Enterobacteriaceae*

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In a paired, double-blind study, the modified ("Beckford tube") R-B system was compared with conventional bacteriological procedures for the identification of members of the family *Enterobacteriaceae* from clinical isolates and stock cultures. The tests in the R-B system yielding positive reactions comparable to those predicted by Ewing's taxonomic classification of *Enterobacteriaceae* were production of hydrogen sulfide and presence of lysine and ornithine decarboxylase activities. The test reactions in the R-B system found to be comparable to those in the conventional method were fermentation of glucose, hydrogen sulfide production, and lysine and ornithine decarboxylase activities. The production of gas from glucose was positive in the R-B system more often than in the conventional method; however, the motility test and the production of indole were positive less often in the R-B system. Adequate preliminary identification of the *Enterobacteriaceae* with the R-B system is enhanced if Simmons' citrate and Christensen's urea tests are used concomitantly. These findings emphasize the manufacturer's instructions that, in interpretation of results, colonial morphology and biochemical reactions must be used concurrently to make an accurate identification.

Recently a two-tube composite test, designated the R-B system (Diagnostic Research, Inc., Roslyn, N.Y.), was made available for the identification of members of the family *Enterobacteriaceae*. Two previous reports (11, 12) indicated that this test was an acceptable alternative to conventional methods in identifying these bacteria. However, in an evaluation previously reported from this institution (9), it was concluded that the R-B system was difficult to read and to interpret. To obviate these difficulties, the R-B system has been modified to include a constriction at the base of both tubes. The constriction in the first tube separates the lysine decarboxylase test from the remainder of tests in that tube. In the second tube the constriction confines the indole-ornithine-motility medium which contains less agar than used previously.

Recent reports by Smith et al. (13) and by Isenberg and Painter (6) have shown the modified R-B systems to be more accurate than the original R-B system in the identification of *Enterobacteriaceae*. This report presents results of our evaluation of the modified R-B system.

MATERIALS AND METHODS

Ewing's (5) compilation of the biochemical reactions of members of the family *Enterobacteriaceae* was used to establish a comparison between results obtained by the modified R-B system and those obtained by the conventional system used in our laboratory. The latter system consists of the following tests: Triple Sugar Iron Agar (TSI), lysine iron agar, Simmons' citrate, Christensen's urea, ornithine decarboxylase (Moeller's base in 0.3% agar), and indole as described elsewhere (2, 4, 7, 8, 14, 15). One hundred thirty-seven strains of the bacteria tested were isolated from clinical specimens; the remainder represented stock cultures obtained from the Enteric Bacteriology Unit of the Center for Disease Control and the Proficiency Testing Program of the College of American Pathologists (Tables 1 and 2). Testing with the R-B system was not begun until a representative of the manufacturer was satisfied with our ability to use and interpret it properly.

Each isolate was inoculated onto two eosin-methylene blue (EMB) agar plates which were coded, randomly labeled, and incubated for 18 to 24 hr at 37 C. One of the two plates then was assigned for identification by the R-B system and the other, by the conventional method. Additional biochemical tests as described by Edwards and Ewing (3) were used when indicated. No serotyping was performed.

All test reactions were recorded as being negative [0], weakly positive [1], strongly positive [2], or positive after 24 hr [3]. To assess the role of experience in the identification process, all isolates used in both systems were identified by the least-experienced investigator and his results were referred to an impartial referee who returned each incorrectly identified isolate for one reevaluation. All data were transcribed on computer cards.

Cross-classification tables of matched pairs of tests were constructed for glucose, lactose, gas production from glucose, hydrogen sulfide, lysine and ornithine decarboxylases, indole, and motility by groups within the *Enterobacteriaceae* (Table 3). The phenylalanine deaminase in the R-B system was not compared to the lysine deaminase of the conventional method because the latter test is frequently negative with *Proteus morganii*. Any disagreement between the two methods was evaluated by Cochran's test for matched pairs (1).

RESULTS AND DISCUSSION

The percentages of positive reactions obtained from the tests for production of hydrogen sulfide (H₂S), presence of lysine and ornithine decarboxylases, production of indole, and utilization of urea and citrate in the conventional method, as well as those from the tests for production of H₂S and the presence of ornithine and lysine decarboxylases in the modified R-B system, showed no significant differences from those expected from Ewing's taxonomic classification of the *Enterobacteriaceae* (5) (Table 4). Percentages of positive reactions for glucose and lactose fermentation, gas production from glucose, and motility listed in Ewing's tables were not comparable

TABLE 1. *Organisms used in evaluation of modified R-B System*

Organism	No.
<i>Shigella</i>	20
<i>Escherichia coli</i>	21
<i>Edwardsiella tarda</i>	3
<i>Salmonella</i>	22
<i>Arizona hinshawii</i>	3
<i>Citrobacter freundii</i>	15
<i>Klebsiella</i>	25
<i>Enterobacter aerogenes</i>	10
<i>E. cloacae</i>	11
<i>E. hafniae</i>	6
<i>E. liquefaciens</i>	6
<i>Serratia</i>	11
<i>Proteus vulgaris</i>	9
<i>P. mirabilis</i>	10
<i>P. morganii</i>	10
<i>P. rettgeri</i>	9
<i>Providencia</i>	9
Total	200

TABLE 2. *Sources of organisms used in study of R-B System*

Source	No.
Stock cultures	63
Urine	60
Sputum	33
Wound	10
Ulcer	5
Stool	2
Throat	1
Blood	1
Abscess	1
Autopsy	1
Ear	2
Other	21
Total	200

^a Center for Disease Control, Atlanta, Ga.; Proficiency Testing Program, College of American Pathologists.

TABLE 3. *Representative cross-classification table: indole (Proteus-Providencia)*

R-B method	Conventional method				Total
	0	1	2	3	
0	10	1	8	0	19
1	0	0	10	0	10
2	0	0	18	0	18
3	0	0	0	0	0
Total	10	1	36	0	47

Cochran's test:

Total negative (N) versus total positive (P)
Conventional

$$\begin{array}{c}
 \begin{array}{cc}
 \text{N} & \text{P} \\
 \hline
 10 & 9
 \end{array} \\
 \chi_1^2 = \frac{(0 - 9)^2}{(0 + 9)} = 9.0 \\
 \text{R-B} \quad \begin{array}{cc}
 \text{N} & \text{P} \\
 \hline
 10 & 9
 \end{array} & P < 0.01
 \end{array}$$

Weakly positive (W) versus strongly positive (S)
Conventional

$$\begin{array}{c}
 \begin{array}{cc}
 \text{W} & \text{S} \\
 \hline
 0 & 10
 \end{array} \\
 \chi_1^2 = \frac{(10 - 0)^2}{(10 + 0)} = 10.0 \\
 \text{R-B} \quad \begin{array}{cc}
 \text{W} & \text{S} \\
 \hline
 0 & 10
 \end{array} & P < 0.01
 \end{array}$$

to those obtained in the R-B system or the conventional method because of differences in methods (3). Ewing used fermentation broths with Durham tubes to assess fermentation and gas production. Ewing's motility data were based on culture in a clear semisolid agar, whereas in both the R-B system and the conventional method, motility was determined in ornithine decarboxylase medium to which agar had been added.

The conventional method classified 93% of all organisms by genus and 45% by species

TABLE 4. Positive tests observed compared to prediction by Ewing's classification^a

Test	Percent positive		
	R-B	Ewing	Conventional
Glucose	88 ^b	c	100
Lactose	26	c	53
Gas	73	c	63
H ₂ S	28	30	27
Indole	23 ^d	36	33
Ornithine decarboxylase	62	62	63
Motility	55	76 ^e	60
Lysine decarboxylase	50	50	52
Urea		40	40
Citrate		60	63

^a See reference 5.

^b One hundred percent if adjusted to positive when H₂S obscured the test result.

^c Not comparable because of differences in methods.

^d Significantly lower than predicted.

^e Plain agar medium.

without the aid of additional tests, whereas the R-B system classified 90% by genus and 40% by species. The time required to inoculate media in the conventional and the R-B system was 5 hr and 2.5 hr, respectively.

On comparing total number of positive reactions—including weakly positive, strongly positive, and delayed positive reactions—by individual tests in the two systems, the R-B system produced a higher percentage of positive reactions for production of gas from glucose ($P < 0.01$) and these tests were easier to interpret, but the R-B system had significantly fewer positive reactions in both the motility test ($P < 0.01$) and the production of indole ($P < 0.05$) than the conventional method (Table 5). This was especially true for members of the tribe *Proteeae* with which the R-B indole test resulted not only in a larger number of false-negative reactions but also was judged to be more difficult to interpret. A dark brown fluid would sometimes adsorb onto the swab from the indole medium and partly obscure the test result. The R-B system had weaker reactions in the test for fermentation of glucose. The slightest change in color had to be recorded as definite evidence of fermentation of glucose; otherwise many false-negative reactions resulted. Since the sucrose present in the TSI may be fermented by some species of *Enterobacteriaceae*, it was not possible to compare accurately the fermentation of lactose in this

medium with that in the first tube of the R-B system.

The flow chart supplied by the manufacturer classifies the *Enterobacteriaceae* into four major "groups" as follows: (i) *Proteus-Providencia* group, phenylalanine deaminase-positive; (ii) *Edwardsiella-Salmonella-Arizona-Citrobacter* group, phenylalanine deaminase-negative and H₂S-positive; (iii) *Escherichia coli-Shigella* group, phenylalanine deaminase-negative, H₂S-negative, and indole-positive; and (iv) *Klebsiella-Enterobacter-Serratia* group, phenylalanine deaminase-negative, H₂S-negative, and indole-negative. In addition, the genera within each group are identified with the aid of other test reactions in the R-B system or additional tests recommended by the manufacturer.

Within the *Proteus-Providencia* group, one strain each of *Proteus morgani* and *Proteus vulgaris* were phenylalanine deaminase-negative by the R-B system. Moreover, false-negative tests for indole by the R-B system included 11 organisms that could not be classified. In the *Edwardsiella-Salmonella-Arizona-Citrobacter* group, the conventional method yielded four strains of *Citrobacter* that were H₂S-negative, whereas the R-B system yielded two strains of *Citrobacter* and one of *Edwardsiella* that failed to produce H₂S. Although considered infrequent in occurrence, H₂S-negative strains of *Citrobacter* have been encountered in clinical specimens (16). In general, however, organisms in these two groups were easier to speciate than those in the *E. coli-Shigella* and *Klebsiella-Enterobacter-Serratia* groups. Separation of the *E. coli-Shigella* group from the *Klebsiella-Enterobacter-Serratia* group on the basis of production of indole was of limited usefulness, and other tests were needed to make this distinction. The fermentation of mannitol, to separate *E. coli* from *Shigella*, as recommended by the manufacturer, appears to be of limited usefulness since this carbohydrate is fermented by *E. coli* and nearly all species of *Shigella* other than *S. dysenteriae* (3). The single nonmotile and lysine decarboxylase-negative strain in the *E. coli-Shigella* group proved to be a phenylalanine deaminase-negative *P. morgani*. There were, in addition, five strains of indole-positive *Klebsiella* which clearly required additional tests for differentiation from *E. coli*; there was one strain of H₂S-negative *Edwardsiella* and one strain of indole-positive *E. liquefaciens*. These strains of *Klebsiella* were correctly identified by the conventional method without

TABLE 5. Summary of statistically significant differences between R-B System and conventional method in glucose, gas, H₂S, indole, lysine and ornithine decarboxylases, and motility tests

Group	Tests	Comparison of reactions	Strength of reactions
<i>Proteus</i> <i>Providencia</i>	Glucose Gas production	More negatives in conventional	Weaker in R-B
	Indole Motility	More negatives in R-B More negatives in R-B	Weaker in R-B
<i>Edwardsiella</i> <i>Salmonella</i> <i>Arizona</i> <i>Citrobacter</i>	Gas production	More negatives in conventional	Stronger in R-B
<i>E. coli-Shigella</i>		No difference	No difference
<i>Klebsiella</i> <i>Enterobacter</i> <i>Serratia</i>	Glucose		Weaker in R-B

additional tests because it included tests for citrate utilization and urease production. Because the frequency of indole-positive strains of *Klebsiella* in this laboratory has been found to be 16.9% (10), users of the R-B system should be aware of this possibility.

By the R-B system, the *Klebsiella-Enterobacter-Serratia* group included four strains of H₂S-negative *Citrobacter*, and one strain of indole-negative *E. coli* as well as the *Klebsiellaeae*. One strain each of *E. cloacae*, *E. hafniae*, and *E. liquefaciens* was ornithine decarboxylase-negative in the R-B system. Their motility, lack of gas production, and colonial morphology prevented them from being classified as *Klebsiella*. Other than *E. cloacae*, certain members of the genera *Enterobacter* and *Serratia* could not be separated primarily on the basis of rhamnose utilization, as suggested by the R-B system directions. For adequate identification it is recommended that these organisms be inoculated routinely to deoxyribonucleic acid, sorbitol, raffinose, and rhamnose media as suggested by the manufacturer.

As the manufacturer points out, most strains of *Shigella* are indole-negative. Colonial morphology, negative lysine and motility reactions, and lack of gas formation should suggest *Shigella* rather than the *Klebsiella-Enterobacter-Serratia* group.

Analysis of the data shows a total of 26 isolates (21 in the R-B system and 5 in the conventional method; $P < 0.01$) that could not be classified on the basis of biochemical criteria alone. By correlating colonial morphology with the biochemical reactions, all 200 organisms

studied were correctly identified by the conventional method, and all but 2 were correctly identified by the R-B system. One incorrect identification was due to a negative phenylalanine deaminase reaction with *P. morganii* and the other to a positive indole reaction with *E. liquefaciens*. These findings emphasize the manufacturer's instructions that, in interpretation of results, colonial morphology and biochemical reactions must be used concurrently to make an accurate identification. It is our opinion, however, that the concomitant use of Simmons' citrate and Christensen's urea with the R-B system would overcome this potential difficulty in laboratories with less experience in the identification of the *Enterobacteriaceae*. The addition of these two tests allows all *Proteus-Providencia* species to be identified with the basic R-B system and the *E. coli-Shigella* group to be separated more adequately from the other groups of the family *Enterobacteriaceae*.

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