

R/B Enteric Differential System for Identification of *Enterobacteriaceae*

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The R/B Enteric Differential System for identifying enteric bacteria has been evaluated with 451 "unknown" cultures from the stock culture collection of the Center for Disease Control. An average of 89.6% of these cultures were correctly identified by the R/B system, when used as recommended by the manufacturer but without the assistance of serology. This percentage ranged, however, from 47% for *Klebsiella* to 100% for *Serratia* and *Providencia*. Of 11 groups or genera of *Enterobacteriaceae* tested, only three (*Enterobacter*, *Serratia*, and *Providencia*) were identified with 95% or better accuracy. Four groups (*Arizona*, *Citrobacter*, *Escherichia*, and *Salmonella*) attained 90 to 95% accuracy of identification, and three groups (*Edwardsiella*, *Proteus*, and *Shigella*) scored between 85 and 90% accuracy. We recommend the R/B system as a screening device which is reasonably successful in grouping bacteria but not as a substitute for more exacting conventional procedures.

Within the past few years, increasingly varied products for the "simplified" isolation or identification, or both, of enteric bacteria have become available. One of these is the R/B Enteric Differential System, manufactured by Diagnostic Research, Inc.; it has been widely used by clinical laboratories. This is a two-tube system which incorporates into one tube the tests for hydrogen sulfide production, phenylalanine deaminase, lysine decarboxylase, lactose utilization, and gas production from glucose and, in the other tube, the tests for indole production, ornithine decarboxylase, and motility. In 1969, Sellers et al. (*Bacteriol. Proc.*, p. 97, 1969) concluded that this system provided essentially the same answers as a very abbreviated conventional system and within 24 hr usually provided a genus identification and often a species identification. In 1970, O'Donnell et al. (5) evaluated this system with 408 clinical isolates, the vast majority of which (398) were *Escherichia coli*, *Klebsiella*, *Enterobacter-Serratia*, and *Proteus mirabilis*. They concluded that the R/B system provided an acceptable alternative to conventional techniques, even though in the R/B system they found the motility reaction unreliable, the indole reaction less sensitive than their method, and the ability to distinguish *Salmonella* from *Arizona* and *Enterobacter* from non-pigmented *Serratia* lacking. More recently, Martin and co-workers (4) have also evaluated this product. They too used primary clinical speci-

mens but a somewhat more limited number of isolates (179). This study, in contrast to the other two, employed much more extensive "conventional" procedures. In direct contrast with earlier studies, Martin et al. obtained very poor results with the R/B system: 44% of all cultures tested could not be accurately identified with the R/B system because half were atypical or produced conflicting reactions in the R/B system and half did not ferment glucose. They concluded that the disadvantages of the R/B system outweighed its advantages and did not recommend its use. The present study is a controlled evaluation of the R/B system with only thoroughly documented cultures of *Enterobacteriaceae*. We hope it will help resolve apparent discrepancies in previous reports on the efficacy of this product.

MATERIALS AND METHODS

All bacterial cultures were provided by the Enteric Bacteriology Laboratory, Laboratory Division, Center for Disease Control (CDC) and had been received at the CDC for identification.

The R/B media, generously supplied by Diagnostic Research, Inc., Roslyn, N.Y., were used exactly as the manufacturer recommended. We paid strict attention to details of inoculation, incubation, and recording of results as well as to the shelf life of media. Other media used were prepared in a central media kitchen from commercially available dehydrated media (Difco, BBL). Each batch of these media was tested for appropriate reactions with a collection of

stock cultures of known biochemical reactions. The tests or media used in the R/B system and in our conventional procedures are as follows. The eight tests routinely incorporated into the two tubes of the R/B system are H₂S production, indole, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, motility, gas from glucose, and acid from lactose; additional media or tests employed whenever necessary are β -D-galactosidase, gelatin hydrolysis, deoxyribonuclease, urease, acid from dulcitol, sorbitol, arabinose, rhamnose, and raffinose, and malonate utilization. The 18 tests used routinely in our conventional procedure are triple sugar iron agar, H₂S production, urease, indole, methyl red, acetoin, citrate, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, phenylalanine deaminase, KCN, motility, and acid from glucose, lactose, arabinose, rhamnose, and raffinose; the 11 tests used as required are acid from dulcitol, inositol, sorbitol, adonitol, gelatin hydrolysis, β -D-galactosidase, Jordan's tartrate, deoxyribonuclease, mucate, sodium acetate, and malonate utilization. In general, the media employed for the conventional and extra tests were those recommended by Edwards and Ewing (1), by Ewing (2), and by Martin (3). Serology was not employed in this study, although most cultures had been serologically identified by the Enteric Bacteriology Laboratory, CDC.

Cultures were provided as coded unknowns on plain agar slants. One technician transferred each culture to the media used in our conventional procedure. Another technician then took the unknown culture and streaked it onto a MacConkey agar plate to simulate primary isolation techniques and inoculated the two tubes of the R/B system. Each worker proceeded independently, using whatever tests were required, except for serology, to arrive at an identification. All data were compiled and compared by a third party, and any discrepancies in test results were resolved by repeating tests. Where such procedures still did not resolve differences, the Enteric Bacteriology Laboratory was consulted as a reference laboratory. All nomenclature and taxonomic identifications were based on the recommendations of Ewing (2).

RESULTS

The data obtained have been analyzed in two ways. In one, individual test results obtained in the R/B system were compared with corresponding results obtained by our conventional procedures. In Table 1, the seven tests listed in decreasing order of correlation show this comparison. Lactose utilization was not included in this tabulation after the manufacturer advised us that it was of no real significance in identification procedures. Hydrogen sulfide production, phenylalanine deaminase, and ornithine decarboxylase reactions showed better than 95% correlation in the 451 parallel tests, indole production correlated at nearly 95%, and motility, lysine decarboxylase, and gas from glucose correlated at slightly under 90%. The overall correlation for all

TABLE 1. Correlation of biochemical tests with the R/B system

Test	No. agree	Disagree	Per cent agree
H ₂ S production	448	3(6) ^a	99.3
Phenylalanine deaminase . . .	446	5	98.9
Ornithine decarboxylase	435	16	96.4
Indole production	426	25	94.4
Motility	400	51	88.7
Lysine decarboxylase	396	55	87.8
Gas from glucose	390	61	86.4

^a Six cultures gave delayed reactions.

tests was 93.2%. Our experiences with reading motility and lysine decarboxylase tests were similar to those of others: both were difficult to read, even after we had gained some experience. Tests for gas production from glucose were sometimes difficult to read, and our data demonstrate a very poor correlation with conventional procedures. This was due, however, to false-negative reactions in the R/B system.

The second type of data analysis concerns the ability of the R/B system to identify correctly an unknown member of the *Enterobacteriaceae*. Table 2 shows each of the genera tested and certain species which, according to R/B literature, can be identified with the system. Because serology was not used for identifying cultures in either the conventional system or the R/B system, results are entered for both identification systems. The R/B system accurately identified 404 of 451, or 89.6%, of the cultures tested, whereas our conventional procedures accurately identified 443 of 451, or 98.2%. Of the various genera tested, the R/B system was quite accurate for identifying *Serratia*, *Providencia*, *Enterobacter*, *Arizona*, *Citrobacter*, and *Escherichia* species, only moderately successful in identifying *Proteus*, *Salmonella*, *Shigella*, and *Edwardsiella* species, and very poor for identifying *Klebsiella*. The manufacturer claims that the R/B system can distinguish *Enterobacter hafniae* from other *Enterobacter* species, and it did this with reasonably high accuracy. The system is also said to be able to speciate members of the genus *Proteus*, but only *P. morganii* was identified correctly in more than 90% of the *Proteus* strains tested. The very poor results obtained with *Klebsiella* strains were mainly due to the combination of inaccurate results of lysine decarboxylase, motility, and indole tests in the R/B system. For comparison, it should be noted that our conventional procedures also missed some *Arizona*, *Klebsiella*, and *Shigella* strains and one *Enterobacter* strain.

TABLE 2. Diagnostic correlation—comparison of R/B and conventional systems

Organism	No. tested	No. identified			
		R/B	Per cent	Conventional	Per cent
<i>Arizona</i>	30	28	93.4	28	93.4
<i>Citrobacter</i>	30	28	93.4	30	100
<i>Edwardsiella</i>	30	26	86.7	30	100
<i>Enterobacter</i> (all)	102	97	95.1	100	99.0
<i>E. hafniae</i>	26	24	92.3	26	100
<i>Escherichia</i>	30	28	93.4	30	100
<i>Klebsiella</i>	30	14	46.7	27	90.0
<i>Proteus</i> (all)	78	69	88.5	78	100
<i>P. mirabilis</i>	26	23	88.5		
<i>P. rettgeri</i>	20	17	85.0		
<i>P. morgani</i>	20	19	95.0		
<i>P. vulgaris</i>	12	10	83.3		
<i>Providencia</i>	30	30	100	30	100
<i>Salmonella</i>	30	27	90.0	30	100
<i>Serratia</i>	31	31	100	31	100
<i>Shigella</i>	30	26	86.7	28	93.4
Totals	451	404	89.6 ^a	443	98.2 ^a

^a Average.

The types of identification errors occurring with the R/B system are shown in Table 3. The reasons for these errors were either erroneous results in the R/B system or the occurrence of atypical reactions. The *Arizona* culture which was called a *Citrobacter* was lysine decarboxylase-negative in the R/B system. One *Citrobacter* culture was called *Enterobacter* because it was H₂S-negative in the R/B system; another was called *Proteus vulgaris* because it was phenylalanine deaminase-positive in the R/B system. At first, the latter reaction was considered an error in reading the test, but it was subsequently confirmed as being correct. Two *Edwardsiella* strains which were indole-negative by R/B were called *Salmonella* and *Arizona*, the latter also being malonate-negative. The two strains of *Escherichia* which were called *Shigella* were both anaerogenic, nonmotile cultures and were therefore easily misidentified. The one *Klebsiella* which was called a *Shigella* was later found to be a strain of *K. ozaenae* which was lysine decarboxylase-negative, lactose-negative, and anaerogenic. Thus, it was easily misidentified. Two *Salmonella* were misidentified as *Enterobacter* and *Klebsiella* because they failed to produce H₂S in the R/B system, and a lysine-negative *Salmonella typhi* was called *Citrobacter*. Errors in identification of *Shigella* strains were related to gas production and a positive ornithine reaction (*Enterobacter*), a positive lysine decarboxylase test (*Klebsiella*), and weakly positive indole and motility reactions (*E. coli*) in the R/B system.

The misidentifications made with our conven-

TABLE 3. Identification errors with the R/B system

Organism	Erroneous identifications
<i>Arizona</i>	<i>Citrobacter</i> (1), <i>Salmonella</i> (1)
<i>Citrobacter</i>	<i>Enterobacter</i> (1), <i>Proteus vulgaris</i> (1)
<i>Edwardsiella</i>	<i>Salmonella</i> (1), <i>Arizona</i> (1), <i>Escherichia coli</i> (2)
<i>Enterobacter</i>	<i>E. hafniae</i> (3), <i>Enterobacter miscel-laneus</i> (2)
<i>Escherichia</i>	<i>Shigella</i> (2)
<i>Klebsiella</i>	<i>Enterobacter</i> (11), <i>E. coli</i> (4), <i>Shigella</i> (1)
<i>Proteus</i>	<i>Citrobacter</i> (1), <i>Providencia</i> (1), species errors (7)
<i>Providencia</i>	None
<i>Salmonella</i>	<i>Citrobacter</i> (1), <i>Enterobacter</i> (1), <i>Klebsiella</i> (1)
<i>Serratia</i>	None
<i>Shigella</i>	<i>Enterobacter</i> (1), <i>Klebsiella</i> (1), <i>E. coli</i> (2)

TABLE 4. Identification errors with the conventional system

Organism	Erroneous identification
<i>Arizona</i>	<i>Salmonella</i> (2)
<i>Enterobacter</i>	<i>Serratia</i>
<i>Klebsiella</i>	<i>Shigella</i> (2), <i>Enterobacter</i> (1)
<i>Shigella</i>	<i>Escherichia coli</i> (2)

tional procedures are shown in Table 4. Two *Arizona* cultures were called *Salmonella* on the basis of atypical malonate and dulcitol tests. The *Enterobacter* which was called *Serratia* did not ferment arabinose or raffinose. All three of the *Klebsiella* strains which were missed were *K. ozaenae*. The two *Shigella* cultures which were called *E. coli* were strains of *S. flexneri* 2b which produced gas from glucose.

DISCUSSION

The data presented indicate that the R/B system is not an alternative to our conventional system but that it should perform reasonably well with typical enteric bacteria, provided the manufacturer's instructions are followed precisely and the user does not attempt to make the system perform beyond its capabilities. Additional biochemical tests will often be required, and some organisms cannot be differentiated without serology. In the present study, antisera were not employed; if they had been used, identifications would have been more accurate. The R/B system must not be considered a substitute for classical methods but rather an initial step in identification which may save the careful user both time and money. In most cases, the R/B system was quite accurate in placing one of the test cultures in the proper general group, and, by intelligently using this in-

formation, the laboratorian should be able to arrive at an accurate final identification.

On the other hand, the poor performance of lysine decarboxylase, gas from glucose, and motility tests in the R/B system can easily lead to identification errors. In this study, a number of *Klebsiella* strains were mistakenly called *Enterobacter* because of faulty lysine decarboxylase reactions. In the R/B identification key supplied to us, a culture had to be positive for lysine decarboxylase, negative for ornithine decarboxylase, and nonmotile to be called a *Klebsiella*. No exceptions were mentioned. In the manufacturer's more recent instructions, some of the possible variations from these reactions are mentioned. It is also true, however, that the R/B system is structured so that some atypical reactions can be encountered and a correct identification can still be made. The key factor is whether the aberrant reaction is a critical one, such as H₂S production, lysine decarboxylase, or indole production. Thus, a nonmotile *E. coli* should be easily identifiable if its other characteristics are typical.

It might be argued that the R/B system was not properly evaluated in this study because freshly isolated cultures from clinical specimens were not used and because the cultures actually tested may have changed somewhat during storage. The authors believe, however, that new techniques in

clinical bacteriology should be evaluated with both freshly isolated bacterial cultures and well-documented cultures from stock collections. The three previous studies already referred to have satisfied the criterion of using freshly isolated test strains, but reference cultures were not used extensively in any of them. We have attempted to do this. In addition, none of the previous studies has been able to present statistically significant numbers of certain organisms, especially those in the pathogenic groups. We have attempted to do this by testing at least 30 strains of each group or genus.

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