

## r/b Expanders: Their Use in Identifying Routinely and Unusually Reacting Members of *Enterobacteriaceae*

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Received for publication 5 December 1973

This cooperative study between a large clinical laboratory and a reference laboratory evaluated the performance of the expanded r/b system for identifying *Enterobacteriaceae*. The 2,200 cultures isolated in the normal hospital routine presented no problem of identification to the r/b system. About 250 "atypical" cultures which were exchanged between the collaborating laboratories were also identified accurately. The expanded r/b system was found to perform as well as most biochemical-physiological diagnostic identification systems, and when used appropriately was highly satisfactory as a system for identification of *Enterobacteriaceae*.

There can be little challenge to the statement that many members of the *Enterobacteriaceae* hitherto regarded as commensal bacteria are being recognized with increasing frequency in their role as etiological agents of infectious diseases. Epidemiological considerations, changing patterns of antimicrobial susceptibility, and the vexing presence of plasmid-mediated alterations in a number of characteristics are but a few reasons why more laboratories are attempting to identify significant clinical isolates to the generic or species level, or both. The means by which this plateau of identification is achieved varies widely from one laboratory to another. Prime consideration has been given to the time required, cost involved, the number and level of competence of technical personnel, and the general operating policy of any given clinical laboratory as to how far they will identify clinical isolates and what general methods will be used. The advent of a number of commercially available kits or systems has been regarded as a significant contribution toward meeting the objective of correct identification while still retaining practicability when properly used and interpreted by trained technical personnel. As these systems have appeared on the market they have been evaluated and reported by a number of laboratories. These reports have concerned the usefulness, reliability, and accuracy of the systems (3, 4, 6-12). Recently, one of these, the r/b system (Diagnostic Research, Inc., Division of Corning, N.Y.), enlarged the capabilities of its original two-tube system by adding two additional tubes

of differential media—the Cit/Rham and Soranase tubes. The two new tubes consist of six additional substrates or characteristics which expand the original number of characteristics that can be measured to a total of 14. A joint study by a clinical and federal reference laboratory was undertaken to assess the benefits which these additional tests would provide to the user in a clinical laboratory setting and to measure the capability of the expanded r/b system to recognize members of the *Enterobacteriaceae* which did not react in a routine fashion. The results of this study are contained in this report.

### MATERIALS AND METHODS

#### **Bacteria: organisms from clinical sources.**

The microorganisms used in this study were isolated in the manner reported earlier (5) from a variety of clinical specimens which included urines, sputa, post-operative and traumatic wounds, blood, etc.

**Special study organisms.** These organisms were submitted on agar slants by a reference laboratory to the Clinical Bacteriology Section, Bacteriology Branch at the Center for Disease Control (CDC) and to the Microbiology Section of the Long Island Jewish-Hillside Medical Center (LIJ-HMC). Subcultures were made on either MacConkey or eosin-methylene blue agar, and the organisms were treated in the same fashion as specimens isolated from clinical sources.

One hundred of the clinically isolated organisms were exchanged between the two laboratories as well. A simple system was used to code all bacterial cultures before they were exchanged. The identities of all of the organisms in the study were never revealed until the study had been concluded.

**Use of the r/b system.** Both laboratories used the

r/b system in the precise fashion prescribed by the manufacturer. The basic r/b system, supplemented by the Cit/Rham tube, a Beckford or pinched tube containing citrate in the slant portion and rhamnose in the butt portion, was inoculated from a single colony with a modified loop. After incubation and initial reading, all microorganisms requiring the second expander of the r/b system were so treated. The second expander (Soranas) tube consists of a Beckford tube which carries deoxyribonucleic acid, raffinose, and sorbitol in the slant portion. The pinched-off butt of the tube contains arabinose.

**Conventional systems: Long Island Jewish-Hillside Medical Center.** The methods used for identification of the *Enterobacteriaceae* have been described previously (6). When additional fermentation tests were set up, they were prepared in Trypticase agar base (Bio-Quest). The carbohydrates were added in the form of Taxos carbohydrate disks (Bio-Quest). When the carbohydrates were not available in the form of commercially available paper-impregnated disks, they were added to the Trypticase agar base to a final concentration of 1%.

**Clinical Bacteriology Section, CDC.** All methods used in this laboratory were according to the directions of Edwards and Ewing (1); all media were prepared as described therein.

## RESULTS

The 2,454 organisms isolated from clinical specimens reacted as shown in Table 1. The reactions shown are those elicited with the

Cit/Rham tube. The percentages obtained with these organisms are compared with those reported by Edwards and Ewing (1). The same interpretation presented in an earlier publication (11) should be applied here, i.e., the number of organisms tested in this study does not approach the numbers which led to the published percentages of Edwards and Ewing. Thus, some differences in reaction frequencies must be expected in comparing results. Generally speaking, *Escherichia coli*, *Shigella sonnei*, *Salmonella enteritidis*, and *Citrobacter* reacted as one would predict on citrate and rhamnose. The reactions of the genus *Proteus* must be modified by considering that incubations in excess of 18 h are required for eliciting responses to citrate. Since the basic r/b system must be read after 18 h, the Cit/Rham tube responses cannot be evaluated in terms of citrate production without further incubation. It is important to mention that the citrate reaction has no bearing on the identification of members of this tribe when the r/b system is used. The reactions of the genus *Klebsiella* on citrate and rhamnose are very close to the percentages cited by Edwards and Ewing. The major difference is observed in rhamnose fermentation. In the Cit/Rham tube, 78.3% of 194 *Enterobacter cloacae* fermented rhamnose,

TABLE 1. Reactions of clinical *Enterobacteriaceae* with the citrate-rhamnose tube of the r/b system

Organism	No. tested	Percent positive			
		Citrate		Rhamnose	
		r/b	Ewing <sup>a</sup>	r/b	Ewing
<i>Escherichia coli</i> .....	545	1.3	0.2	80.5	82.3
<i>Shigella sonnei</i> .....	5	0	0	0	16.6
<i>Salmonella enteritidis</i> .....	17	94.1	88.7	88.2	94.0
<i>Citrobacter freundii</i> .....	90	91.1	90.4	91.1	99.4
<i>C. diversus</i> .....	30	100.0	99.1	100.0	99.1
<i>Proteus mirabilis</i> .....	651	16.1 <sup>b</sup>	58.7	0.4	1.5
<i>P. vulgaris</i> .....	24	8.3	10.5	4.1	9.4
<i>P.morganii</i> .....	92	1.0	0	1.0	0
<i>P. rettgeri</i> .....	8	50.0	95.6	25.0	67.9
<i>Providencia stuartii</i> .....	4	75.0	95.6	0	0
<i>P. alcalifaciens</i> .....	7	57.1	97.9	0	0
<i>Klebsiella pneumoniae</i> .....	572	95.9	97.7	95.1	99.3
<i>Enterobacter aerogenes</i> .....	117	96.5	93.7	96.6	98.7
<i>E. cloacae</i> .....	194	89.2	99.5	78.3	92.0
<i>E. hafniae</i> .....	17	17.6	(58) <sup>c</sup>	82.3	93.0
<i>E. liquefaciens</i> .....	47	91.4	91.2	6.3	0
<i>Serratia marcescens</i> .....	103	84.4	98.6	2.9	0
<i>E. agglomerans</i> .....	31	80.6	61.8	80.6	84.0

<sup>a</sup> Edward and Ewing (1).

<sup>b</sup> When incubated in excess of 18 h, percentages comparable to those of Edwards and Ewing (1) were obtained with conventional and r/b methods.

<sup>c</sup> Percentage obtained after 3 or more days.

whereas in the larger series reported by Ewing, 92% positive fermentations was reported. The comparatively large number of *E. cloacae* included here might lead one to expect closer correspondence. However, the positive reactions recorded in Edwards and Ewing may be extended to 48 h. In our study, readings were made in 24 h, in keeping with the basic r/b system. At any rate, the 13.7% difference is not so great as to endanger recognition of *Enterobacter cloacae* on the basis of the single characteristic. In Table 2, the reactions of *Enterobacter*, *Serratia*, and the non-hydrogen sulfide-producing *Salmonella* are listed for the fourth tube (Soranas). This tube is intended to allow the recognition and speciation of *Enterobacter liquefaciens*, *E. aerogenes*, and *E. hafniae*, as well as the *Salmonella* and *Serratia* species. Unfortunately, percentages for the elaboration of deoxyribonuclease (DNase) by most of these organisms are not, to our knowledge, published. However, the percentages of *Serratia* that elaborated this enzyme in this study are similar to those published by Ewing et al. (2). Interestingly enough, the organism listed as *Enterobacter liquefaciens* is now proposed to belong to the genus *Serratia* (2). During the first 2 days, only 7.3% of *E. (S.) liquefaciens* elaborated DNase in our studies as compared with 88.3% of 109 reported by Ewing et al. in the same amount of time. Once more, this apparent discrepancy is brought about by the established convention of reading the r/b system in 24 h or less, a convention that ought not be sacrificed for the sake of one or two biochemical reactions of one or two species. The tube in question, i.e., Cit/Rham or Soranas, can be kept for an additional 24 h when there is a question of identification in the laboratory. The remaining tests shown in Table 2 gave results similar to those of Ewing et al.

One hundred samples were selected randomly from this series, coded, and submitted to the Clinical Bacteriology Section at CDC. No dif-

ferences in identification were encountered in the two laboratories when the total r/b system was used.

We challenged the ability of the r/b system to identify medically significant representatives of the *Enterobacteriaceae* by using bacteria with one or more atypical, biochemically significant reactions. The identity of these bacteria was determined by an extended number of biochemical and physiological tests that are not generally used in clinical laboratories and are not present in the rapid test systems. The 118 "pedigreed" bacteria, submitted as unknowns from the Enterobacteriology Section of CDC, were evaluated with the r/b and conventional systems by the Clinical Bacteriology Section at CDC. At LIJ-HMC, the r/b system constituted the initial approach. If the identity of the bacterium could not be established with these initial reactions, additional tests were performed in accordance with the guides represented in Fig. 1 through 3 and as follows. (Abbreviations: PAD, phenylalanine deaminase; LAC, lactose; LYS, lysine decarboxylase; ORN, ornithine decarboxylase; IND, indole; MOT, motility; CIT, citrate; RHA, rhamnose; RAF, raffinose; SOR, sorbitol; ARA, arabinose.)

For differentiating rare strains of *Escherichia* and *Enterobacter aerogenes*, if r/b reactions are PAD-, H<sub>2</sub>S-, LAC+, GAS+, LYS+, ORN+, IND-, MOT+, CIT-, RHA+, DNase-, RAF+, SOR+, and ARA+, perform methyl red (MR) and Voges-Proskauer (VP) tests. Positive MR and negative VP indicates *Escherichia coli*; negative MR and positive VP indicates *Enterobacter aerogenes*.

For differentiating rare strains of *Klebsiella* and *Escherichia*, if r/b reactions are PAD-, H<sub>2</sub>S-, LAC+, GAS+, LYS+, ORN-, IND+, MOT-, CIT-, and varying with RHA, perform tests in malonate and KCN broth. Positive and negative reactions in both broths indicate *Klebsiella* and *E. coli*, respectively.

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TABLE 2. Reactions of appropriate clinical *Enterobacteriaceae* with the Soranas expander of the r/b system

Organism	No. tested	Percent positive							
		DNase		Raffinose		Sorbitol		Arabinose	
		r/b	Ewing <sup>a</sup>	r/b	Ewing	r/b	Ewing	r/b	Ewing
<i>Enterobacter liquefaciens</i> .....	41	7.3	88.3	90.2	86.8	100	97	95.1	92.6
<i>E. aerogenes</i> .....	98	0		93.8	96	100	100	100	100
<i>E. hafniae</i> .....	27	0		0	0	14.7	0	96.2	96
<i>Serratia</i> .....	101	99	96.7	0	1.7	93	98.3	0	0
H <sub>2</sub> S-negative <i>Salmonella</i> .....	4	0		0	3.0	100	94.1	100	89.2

<sup>a</sup> Edwards and Ewing (1).



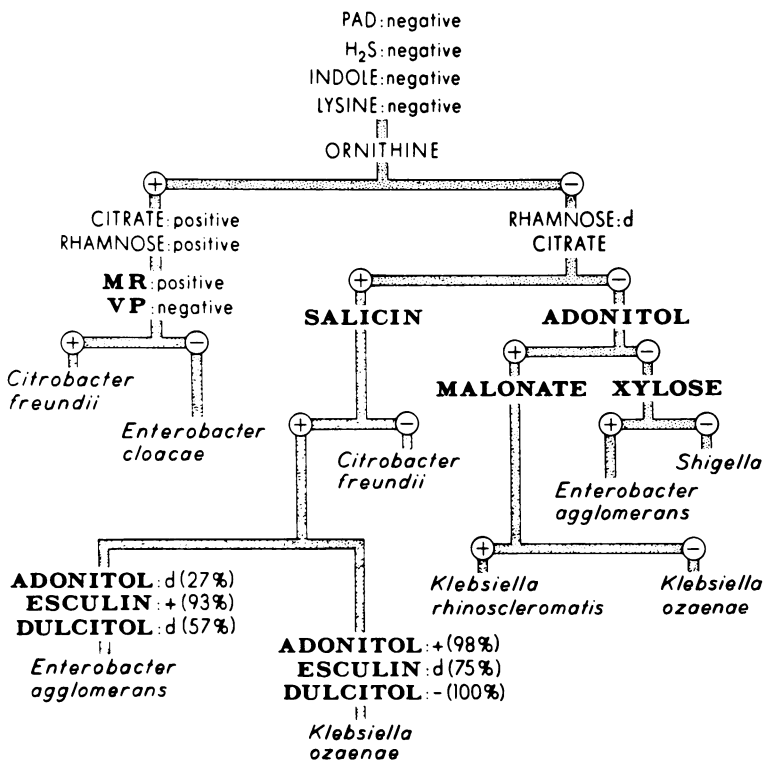


FIG. 2. Guide to additional reactions useful in the classification of anomalously reacting and rarely encountered Enterobacteriaceae incompletely identifiable with the r/b system. Additional test substrates or reactions are in boldface type. Symbols: +, positive; -, negative; d, variable.

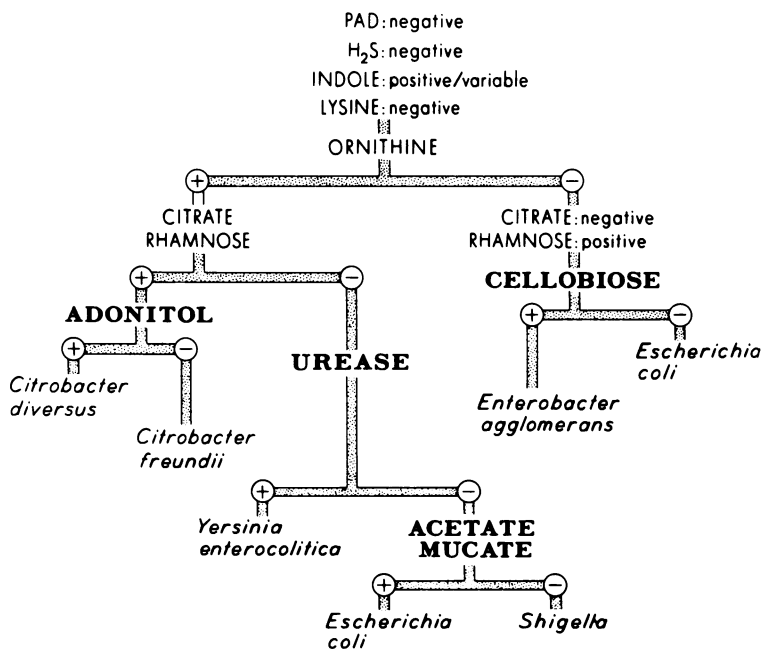


FIG. 3. Guide to reactions required in addition to the r/b system for the identification of anomalously reacting and rarely encountered Enterobacteriaceae and *Yersinia enterocolitica*. Additional test substrates are in boldface type. Symbols: +, positive; -, negative.

presence of cyanide, all of which argues against identifying this organism as *E. coli*. The LIJ-HMC laboratory misidentified three indole-negative *E. coli*; one was reported as an *Enterobacter aerogenes* because it failed to produce indole. The second misidentified *E. coli* was reported as a *Salmonella*; although the citrate was negative, sorbitol was positive, which led to serological screening. The results were positive with *Salmonella* polyvalent and group C<sub>1</sub> antisera. The third *E. coli* in the indole-negative group displayed only one positive reaction in the first three r/b tubes, namely, gas from glucose. Since it was nonmotile, citrate negative, and indole negative, acetate, mucate, methyl red, acetyl methyl carbinol, and adonitol were inoculated. With the exception of the methyl red reaction, these tests were all negative, which made the identification of *Shigella*

the most likely despite the failure of commercial *Shigella* antisera to confirm this conclusion. These failures of correct identification must be ascribed to the laboratory rather than the r/b system, since the r/b reactions were correct and the errors in judgment were based on reactions beyond the capabilities of the r/b system. The failure to identify *Edwardsiella tarda* was also due to an error not in the r/b system. In this instance, mannitol was fermented, which led to the identification of this bacterium as *E. coli*, obviously as the result of conventional reagent failure. The *Proteus vulgaris* cultures reacted typically and presented no problem. All of the *K. pneumoniae* cultures were indole positive. The misidentification of one of these by the LIJ-HMC laboratory was the result of a recording error: the LDC-positive reaction was listed as ODC positive, and the organism was identified as *Citrobacter diversus*. Both clinical laboratories failed in the recognition of the other four klebsiellae with all approaches (Table 3). The consistent reactions which led to these conclusions raise serious questions about the true incidence of *K. rhinoschleromatis* and *K. ozaenae* and the means of assessing aberrantly reacting members of these species in any laboratory.

The four *Enterobacter aerogenes* cultures did not ferment lactose. Both laboratories misidentified the same strain as *E. cloacae* with the r/b system since this bacterium failed to elicit a positive LDC; the CDC conventional approach recognized this organism properly on the basis of its arginine dihydrolase reaction.

*E. cloacae* members of the pedigreed group of organisms presented quite a challenge. Five of the six were lactose negative. One produced no gas in fermenting glucose, was nonmotile, and did not utilize citrate. LIJ-HMC identified one culture as *E. liquefaciens* on the basis of its consistent inability to ferment rhamnose. In two

TABLE 3. Summary of identifications of unusual bacteria

Bacterium	No. tested	No. properly identified	
		LIJ-HMC	CDC
<i>Escherichia coli</i> .....	26	22	25
<i>Edwardsiella tarda</i> .....	1	0	1
<i>Shigella</i> .....	7	7	7
<i>Proteus vulgaris</i> .....	1	1	1
<i>Klebsiella pneumoniae</i> ..	10	9	10
<i>K. rhinoschleromatis</i> ..	2	0	0
<i>K. ozaenae</i> .....	2	0	0
<i>Enterobacter aerogenes</i> ..	4	3	3
<i>E. cloacae</i> .....	6	4	4
<i>E. liquefaciens</i> .....	6	4	4
<i>E. hafniae</i> .....	5	4	4
<i>E. agglomerans</i> .....	10	7	6
<i>Serratia marcescens</i> .....	4	4	4
<i>Citrobacter diversus</i> .....	10	9	9
<i>C. freundii</i> .....	9	7	8
<i>Salmonella</i> .....	15	14	13
Total correct (%)	118	92	99
	(100)	(78.0)	(83.9)

TABLE 4. Organisms not identified by r/b and conventional methods in both laboratories

Enteric laboratories (CDC)	Identification of:		Aberrant reactions <sup>a</sup>
	Clinical microbiology laboratory		
	LIJ-HMC	CDC	
<i>Escherichia coli</i> .....	<i>Enterobacter liquefaciens</i>	<i>E. liquefaciens</i>	CIT + ; KCN +
<i>Klebsiella rhinoschleromatis</i> ..	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	CIT + ; LYS + ;
<i>K. rhinoschleromatis</i> .....	<i>Escherichia coli</i>	<i>E. coli</i>	LYS + ; ORN + , MOT +
<i>K. ozaenae</i> .....	<i>Enterobacter hafniae</i>	<i>E. hafniae</i>	MOT + ; ORN +
<i>K. ozaenae</i> .....	<i>Serratia</i>	<i>E. liquefaciens</i>	MOT + ; ORN +
<i>Enterobacter cloacae</i> .....	<i>Enterobacter aerogenes</i>	<i>E. aerogenes</i>	LYS +

<sup>a</sup> Abbreviations: CIT, citrate; LYS, lysine decarboxylase; ORN, ornithine decarboxylase; MOT, motility.

instances, LDC gave false-positive reactions in the r/b system, leading to the designation of one of these bacteria as *E. aerogenes*. The second of these LDC-positive organisms did not ferment rhamnose and was reported as *E. liquefaciens*. A fourth *E. cloacae* was identified as *E. aerogenes* by both laboratories when they used the r/b system and encountered LDC-positive reactions. The conventional approach at CDC led to the correct identity. This particular culture is the biochemically inactive bacterium mentioned above. The *E. cloacae* listed in Table 8 which produced LDC detectable even with the conventional media at CDC undoubtedly belonged to the small number of positives (0.5%) reported by Edwards and Ewing (1). It was identified as *E. aerogenes*.

The six pedigreed *E. liquefaciens* included: one culture which did not produce gas from glucose but was indole positive, one representative which was citrate negative, four which elaborated DNase, three which were raffinose negative, and two that did not ferment arabinose. LIJ-HMC identified two members of this species as *Serratia*, one as the result of a Soranase tube error with an organism which was DNase positive and rhamnose negative but which also yielded a false-negative arabinose. The second organism was missed because it displayed negative raffinose, rhamnose, and arabinose fermentations, a combination very suggestive of *Serratia*. CDC incorrectly identified two other *E. liquefaciens*; one as *E. aerogenes* on the basis of rhamnose, raffinose, and arabinose fermentations and lack of DNase, and the other as a *Serratia* with a weakly positive DNase, positive sorbitol fermentation, and absence of fermentation of arabinose and raffinose. With the conventional approach in this laboratory, both organisms were recognized as *E. liquefaciens*.

The selected *Enterobacter hafniae* represented five variants, one of which was nonmotile and lacked the ability to ferment arabinose. Another member of the species fermented sorbitol. It is this latter bacterium which was identified by LIJ-HMC as an *Escherichia coli* because it was able to ferment sorbitol. Although the conventional methods at CDC yielded the proper designation for all five *E. hafniae*, a culture distinct from the one missed by LIJ-HMC and mentioned above was diagnosed as *E. aerogenes*.

The ten pedigreed members of *Enterobacter agglomerans* gave various reactions: two produced phenylalanine deaminase, none elaborated H<sub>2</sub>S, seven fermented glucose with the production of gas, two fermented lactose, none

produced LDC, one produced ODC, three produced indole, eight were motile, nine elaborated citratase, and three fermented rhamnose. One was designated *Escherichia coli* by LIJ-HMC, but unfortunately was lost during freezer storage. Using the r/b system, CDC designated an ODC-positive culture as *Enterobacter cloacae* on the basis of esculin hydrolysis and failure to ferment adonitol; thus, their designation was based on tests other than those in the r/b evaluation. A second member of the species was reported as *E. aerogenes* primarily because this bacterium elicited weakly positive reactions with the r/b LDC and ODC. Both laboratories identified the same *E. agglomerans* as *Citrobacter freundii* because it failed to hydrolyze esculin. Another bacterium was designated by both laboratories as *E. hafniae* on the basis of a positive ODC. The conventional system at CDC led to the correct identity of *E. agglomerans*.

The four *Serratia* species submitted were correctly recognized in both laboratories. The ten *Citrobacter diversus* cultures all belonged to the aerogenic biogroup; three were nonmotile, and two lacked citratase. The r/b system in both laboratories designated one representative as *Escherichia coli* because it was citratase negative. At LIJ-HMC, this misidentification was strengthened further by the inability of the bacterium to grow in KCN broth. It is of interest that another *C. diversus* reacted similarly but gave a positive citratase reaction at LIJ-HMC when tested further. All of the *C. diversus* except the one missed jointly fermented adonitol, a confirmatory test to distinguish this species from H<sub>2</sub>S-negative *C. freundii*.

Nine pedigreed *C. freundii* were evaluated. All were H<sub>2</sub>S negative, two produced no gas from glucose, two fermented lactose, two did not elaborate ODC, eight were indole positive, one was nonmotile, and one did not utilize citrate. One of these bacteria was identified as *C. diversus* at LIJ-HMC because it fermented adonitol. Another was designated as *E. agglomerans* by virtue of its lacking ODC and its ability to ferment cellobiose. However, at CDC the r/b system demonstrated ODC for this organism, which makes this failure ascribable to the system. CDC r/b results identified one member of this series as *E. agglomerans* since it lacked ODC as well. This particular organism was recognized at LIJ-HMC as *C. freundii* by virtue of its inability to hydrolyze esculin and its inability to ferment cellobiose and adonitol, which emphasizes that tests in addition to the usual conventional approaches and the commercial systems are required in the diagnosis of

these rarely encountered *Enterobacteriaceae*.

The 15 salmonellae in this special group of bacteria displayed the following characteristics: all were aerogenic, 11 were H<sub>2</sub>S negative, 5 fermented lactose, all produced LDC and ODC, none produced indole, 1 was nonmotile, 2 did not grow in citrate, 1 did not ferment rhamnose, 1 produced DNase or fermented raffinose, and 14 fermented sorbitol and arabinose. For identification, all of these bacteria required tests not included in the r/b system when their identity as salmonellae became suspect. Usually dulcitol fermentation, malonate intolerance, and serological confirmation were used at LIJ-HMC. The *Salmonella* which did not ferment sorbitol in the Soranase expander was called *E. hafniae* since it was arabinose and rhamnose positive, and it produced citratase, ODC, and LDC. The organism also fermented lactose, which should have been a clue to an anomalously reacting bacterium, a clue missed in the laboratory. The r/b system approach at CDC led to the designation of two salmonellae as *E. aerogenes*, both on the basis of lactose fermentation, although neither bacterium fermented raffinose and one produced a slight amount of H<sub>2</sub>S. Unfortunately, in this instance the laboratory at CDC did not use the Soranase expander although the failure to ferment raffinose may have increased the suspicion and led to further examinations. With the conventional system at CDC, these bacteria were recognized as salmonellae, obviating the requirement to consider tests beyond the r/b expanders.

It seems only proper to consider these results from several aspects. Table 4 shows that six of these bacteria were well outside any system, based on biochemical and physiological reactions; the presentation of the results emphasizes that lack of proper functioning of tests beyond the r/b system was responsible for failure to identify these unique and challenging organisms in five instances. The judgment exercised in the interpretation of results at both laboratories can be questioned somewhat in four instances. Instances of r/b system mistakes ascribable to failures of single reactions occurred seven times; this did not necessarily influence the proper identification of an organism.

## DISCUSSION

The results of this study must be examined and analyzed from several viewpoints. The r/b system enhanced by the two expander tubes performed well in identifying medically significant members of the *Enterobacteriaceae*. The methodology involved is simple, as is the read-

ing of reactions and reaching a correct conclusion, as long as the user follows precisely the instructions provided by the manufacturer. This study confirms earlier reports (6, 12) that the system functions efficiently in identifying the bacteria usually encountered in the clinical situation. Few difficulties were encountered with the more than 2,000 enteric bacteria recovered from clinical specimens and included in this study. Random samples, sent from the clinical laboratory to CDC, confirmed this impression.

This approach of using commercially prepared identification test systems has much to recommend it. Its most obvious advantage is the potential improvement in clinical microbiological proficiency in all areas of the country. Such systems enable all laboratories staffed by adequately trained personnel to perform a service often unavailable to the clinician, to obtain reproducible results and with dispatch, and to provide health authorities with a much more accurate measure of the incidence of primary infectious diseases. The potential of such commercially prepared systems cannot be fully appreciated if the systems are evaluated only with microorganisms encountered routinely in the clinical situation. In addition, the capability of any system or other approach can only be established by defining the limits of performance of the system. Comparisons of a properly manufactured, quality-controlled product can only be made with reagents and media of equal or better quality, stability, and reproducibility, preferably in universal use and fully described. This problem became obvious during this study because entirely different conventional methods were used in the two laboratories to assess the identical biochemical and physiological reactions. Recognition of this shortcoming did not come to the fore during the testing of bacteria isolated from clinical specimens; rather, it became obvious during the challenge provided the total r/b system by the bacteria pedigreed in the Enteric Laboratory at CDC.

The exact identity of a bacterium involved in an infectious disease process is of little importance to the clinician who must urgently help his patient. An intimation of genus identity and reliable guidance to antimicrobial therapy satisfy the most pressing needs. This can be and is accomplished with the means available in today's good clinical microbiology laboratories. Further tests to focus on information of admitted significance to the local and greater communities are imperative, but not with the urgency found in the clinical setting. The challenge with the pedigreed bacteria indicates that the r/b



system with its expanders can easily accommodate both of these requirements at almost the same level of accuracy as other laboratory procedures. This efficiency of the r/b system must be viewed against the frequency with which bacteria of the type used to challenge the system are encountered in actual clinical specimens. Although exact information is not available, it is estimated that bacteria with these reaction patterns are encountered in only 1 out of 5,000 to 10,000 isolates. When the r/b system is operated by knowledgeable technical personnel, its performance is equal to that of any biochemical-physiological diagnostic approach.

Of course, any quality-controlled commercial system has the advantage over the laboratory-prepared systems of enabling laboratories to compare results more readily and more reliably. A significant conclusion arising from this investigation is that microorganisms do not respond identically on substrates of different composition that contain different indicators even if the major test substrate is the same. Discrepant results were observed between the two laboratories in tests beyond the r/b screen. Such observations underline the need to agree beforehand on the so-called conventional procedures against which the performance of an untested innovative method will be compared. In the absence of such agreement, exchange of such substrates between participants would be advisable. The identical material used in each laboratory ought to be tested by all participants in order to determine the full extent of variations. Conversely, commercial preparations such as the r/b system and similar devices have the potential benefit of providing many laboratories with standard materials to use in assessing bacterial reactions. In fact, our data suggest that this must be a mandate of the industry.

This study indicates that the present r/b system is an efficient one for recognizing medically significant members of *Enterobacteriaceae*. Representatives of the family encountered routinely can be categorized with the re-

actions contained in the four-tube system. Exceptional, rarely isolated members of the family can be diagnosed with only a few additional tests. The use of the system and the interpretation of the results are clear cut and easily learned. However, neither this nor any other device can be substituted for adequate training and experience in clinical microbiology.

#### LITERATURE CITED

1. Edwards, P. R., and W. E. Ewing. 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.
2. Ewing, W. H., B. R. Davis, and M. A. Fife. 1972. Biochemical characterization of *Serratia liquefaciens* and *Serratia rubidaea*. In DHEW Publication no. (HSM) 73-8209, Washington, D.C.
3. Goldin, M. 1972. A comparison of multiple-test systems for the presumptive identification of *Enterobacteriaceae*. Amer. J. Med. Technol. **38**:288-291.
4. 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.
5. Isenberg, H. D., and J. I. Berkman. 1966. Recent practices in clinical bacteriology. Progr. Clin. Pathol. **1**:237-317.
6. Isenberg, H. D., and B. G. Painter. 1971. Comparison of conventional methods, the R/B system, and modified R/B system as guides to the major divisions of *Enterobacteriaceae*. Appl. Microbiol. **22**:1126-1134.
7. 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.
8. Martin, W. J., P. K. W. Yu, and J. A. Washington II. 1971. Evaluation of the Enterotube system for identification of members of the family *Enterobacteriaceae*. Appl. Microbiol. **22**:96-99.
9. Morton, H. E., and M. A. J. Monaco. 1971. Comparison of Enterotubes and routine media for the identification of enteric bacteria. Amer. J. Clin. Pathol. **56**:64-66.
10. O'Donnell, E. D., F. J. Kaufman, E. D. Longo, and P. D. Ellner. 1970. Evaluation of the r/b system for the identification of *Enterobacteriaceae*. Amer. J. Clin. Pathol. **53**:145-148.
11. Painter, B. G., and H. D. Isenberg. 1973. Clinical laboratory experience with the improved Enterotube. Appl. Microbiol. **25**:896-899.
12. Smith, P. B., D. L. Rhoden, K. M. Tomfohrde, C. R. Dunn, A. Balows, and G. H. Hermann. 1971. R/B enteric differential system for identification of *Enterobacteriaceae*. Appl. Microbiol. **21**:1036-1039.