An evaluation of the Modified R/B Enteric Differential System for the identification of the Enterobacteriaceae

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SUMMARY Two hundred and twenty-two organisms were identified by conventional methods and by the Modified R/B Enteric Differential System. Two hundred and eighteen (98.2%) of these organisms were correctly identified by the R/B system. The results of individual tests were also compared. The convenience and general ease of handling of the R/B system are discussed.

In recent years a number of commercial systems for the identification of the Enterobacteriaceae have appeared on the market. Two of these, the API system and the Enterotube, have been available in the UK for some years and have been widely reviewed (Morton and Monaco, 1971; Smith *et al.*, 1972; Washington, 1972; Leers and Arthurs, 1973; Brooks *et al.*, 1974; Nord *et al.*, 1974; Smith, 1975; Hayek and Willis, 1976). We have recently tested the R/B system, which has been available in various forms in the USA for some time and may be marketed in the UK.

Material and methods

Two hundred and twenty-two clinical isolates were examined over a three-month period in our routine laboratory. They were all tested within one week of isolation, except for a few cultures of *Shigella*, *Serratia*, and the single culture of *Salmonella typhi* which were taken from a stock culture collection. The organisms were tested in parallel by conventional methods and by the R/B system.

The conventional methods used were those described by Cowan (1974) and Edwards and Ewing (1972). Decarboxylase media were supplied by Difco Laboratories. H₂S production was tested for in Kligler triple sugar iron agar, locally prepared from Oxoid dehydrated medium. All other media were obtained from Southern Group Laboratories.

The Modified R/B Enteric Differential System consists of four constricted Beckford tubes. Three of these contain different media above and below the

constrictions, allowing up to six different parameters to be read from one tube. The general principle of at least some of these tubes seems to be similar to that of Kligler's triple sugar medium, where the capacity of an organism to perform different reactions in the same medium under different conditions of oxygen tension is exploited. Tube 1 gives reactions for phenylalanine deaminase, lactose and glucose fermentation, and H₂S production above the constriction, with lysine decarboxylase below. Tube 2 gives indole production, motility, and ornithine decarboxylase; tube 3 Simmon's citrate utilisation above the constriction, with rhamnose fermentation below; and tube 4 DNase production, raffinose, and sorbitol fermentation above the constriction, with arabinose fermentation below. Gas production is read by displacement of the agar in any tube. Indole production, tested by saturating a swab in a modified Kovac's reagent and touching this on the surface of the growth medium, is indicated by the development of a red colour.

Inoculation of the R/B system is extremely simple and takes only a few seconds for all four tubes. This is achieved with a 10 cm long wire of heavier gauge than standard, with the terminal 1 cm folded back on itself. The centre of a single colony is touched with this wire, which is then stabbed to the bottom and streaked over the surface of each tube in turn without re-flaming or re-charging the wire. The screw caps are replaced loosely before incubation for 18-24 hours at 35-37°C.

Results are read using a colour chart of reactions supplied by the manufacturers. In many cases the organism can be identified by direct reference to these charts, though in some instances we found it

necessary to refer to the conventional type of percentage reaction table which was also supplied. This chart is based on data supplied by the Centre for Disease Control, Atlanta, Georgia, USA.

Results

Two hundred and eighteen of the 222 (98.2%) organisms examined were correctly identified by the R/B system. These are listed in Table 1.

Table 1 Organisms tested and given the same identification by conventional and R/B systems

Organism	No. tested
<i>Proteus mirabilis</i>	16
<i>Proteus vulgaris</i>	7
<i>Proteus rettgeri</i>	5
<i>Proteus morganii</i>	4
<i>Providencia stuartii</i>	7
<i>Providencia alkaliifaciens</i>	3
<i>Klebsiella pneumoniae</i>	34
<i>Klebsiella pneumoniae oxytoca</i>	3
<i>Enterobacter hafniae</i>	8
<i>Enterobacter aerogenes</i>	4
<i>Enterobacter cloacae</i>	21
<i>Enterobacter agglomerans</i>	7
<i>Enterobacter liquefaciens</i>	4
<i>Serratia marcescens</i>	9
<i>Citrobacter freundii</i>	8
<i>Citrobacter diversus</i>	4
<i>Salmonella</i> spp.	26
<i>Shigella</i>	7
<i>Escherichia coli</i>	41

Four organisms were incorrectly identified by the R/B system. These are shown in Table 2 together with the reasons for the incorrect identification.

In most instances no further tests other than those included in the R/B set of four tubes were needed, though a urease test was required to differentiate *Proteus rettgeri* from *Providencia*. Dulcitol and malonate tests are recommended to differentiate *Salmonellae* from *Arizona* in cases where the result of lactose fermentation is obscured, and an acetate test is recommended to differentiate some strains of *Shigella* from atypical *Escherichia coli*. In practice, we found it quicker to use slide agglutination with polyvalent and specific antisera in the latter two

Table 2 Organisms incorrectly identified by R/B

Identification by conventional tests	Identification by R/B	Reasons for incorrect identification by R/B system
<i>Proteus morganii</i>	<i>Escherichia coli</i>	False negative PPA
<i>Escherichia coli</i>	Unidentifiable	Only glucose and lactose fermented (shown to be cysteine-dependent strain)
<i>Citrobacter freundii</i>	<i>Enterobacter aerogenes</i>	False positive lysine
<i>Salmonella typhi</i>	<i>Salmonella</i> spp.	False positive gas production

instances, though widespread sharing of antigens between *E. coli* and *Shigella* could occasionally lead to difficulties.

Individual parameters were tested for agreement, and a rather higher discrepancy rate was apparent, though this very rarely seemed to make any difference to the final identification. Table 3 shows the breakdown of individual test results.

Table 3 Correlation of individual tests performed by conventional and R/B systems

Test	No. of agreements	Differences		R/B unreadable	% Agreement of readable tests
		R/B+	R/B-		
PPA	215	1	6	—	96.8
Lactose	156	6	21	39	85.2
H ₂ S	218	4	0	—	98.2
Glucose	222	0	0	—	100
Lysine	211	3	8	—	95.0
Ornithine	218	2	2	—	98.2
Indole	215	1	6	—	96.8
Motility	195	3	24	—	87.8
Citrate	196	24	2	—	88.3 (95)
Rhamnose	206	1	15	—	92.8
DNAse	217	1	4	—	97.7
Raffinose	187	6	22	7	87.0
Sorbitol	178	1	25	18	87.3
Gas	203	11	8	—	91.4

Discussion

Our results have shown the accuracy of individual tests performed by the R/B system when compared with conventional tests to vary from 100% to only 85% correct. Glucose fermentation was 100% correctly assessed by the R/B tubes, but this reaction was not always readable in tube 1, where it was sometimes obscured by the black colouration of a positive H₂S test. In this event growth in the decarboxylase tubes could be taken to indicate glucose fermentation as glucose is included in these media.

The manufacturers state in their literature that the appearance of any visible growth on the citrate slope should be interpreted as positive, but so many organisms that were citrate negative by conventional methods gave a positive reaction when read in this way that we finally recorded as positive only those organisms which produced a blue colour change in the medium as well. With this correction there was a final agreement in 95% of instances.

The R/B system is a fascinating new approach to the identification of the Enterobacteriaceae, but in the early stages of our trial we found that, due to the combination of several reactions in each tube, interpretation of the results was not easy, though with experience we were later able to identify an organism very rapidly on examining the four tubes. Because of this initial difficulty of interpretation we

do not feel that the R/B system is as useful for the occasional 'difficult' organism as some other commercial systems, but that it is more suited to routine identifications performed every day. It seems particularly suited for the identification of very large numbers of organisms, as this is one of the most rapid methods of setting up a set of biochemical tests that we have tried.

We found considerable difficulty in reading the raffinose and sorbitol reactions in the presence of some other positive reactions. DNase production is indicated by a strong reddish-purple colouration which obscures raffinose fermentation. Sorbitol fermentation tends to be obscured by a red colouration which appears in this tube when organisms belonging to the *Proteus*, *Providencia*, *Salmonella* Arizona, and *Citrobacter* groups are being tested, though this is of little significance as the use of this tube is not required for the identification of these organisms. In practice the inability of the R/B system to perform certain tests on some organisms is of little importance in identification as the combination of tests in each tube has been so designed with this in mind.

One test, not included in the R/B system yet frequently used in many conventional schemes of identification, is that for urease production. The manufacturers of the R/B system have relied heavily on the phenylalanine deaminase test, which produces parallel results to urease production for most organisms except *Providencia*, *Citrobacter*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*. We only found it necessary to perform urease tests to differentiate *Proteus rettgeri* from *Providencia* but due to the rapidity of this reaction there was no significant delay in reaching an identification.

If we critically examine those instances where an organism was wrongly identified by the R/B system it will be noted that one of these organisms, an *E. coli*, was cysteine dependent and succeeded in fermenting only glucose and lactose. This in itself would have led to further investigation, as identification by the R/B system cannot be made on the results of two positive tests only. The *Proteus morganii* inexplicably failed to give a positive PPA test on the R/B system, even on repeat testing, while the *Citrobacter freundii* gave a false positive lysine

decarboxylase result. A potentially very serious disagreement was the apparent production of gas by *Salmonella typhi*, which in inexperienced hands could lead to serious error. Shayegani *et al.* (1975) also found false positive gas production from glucose in three of the nine *Salmonella typhi* strains examined. In our case this did not lead us to discard a dangerous pathogen, as the provisional identification was *Salmonella* spp., final identification being by slide agglutination.

References

- Brooks, Karen A., Jens, Martha, and Sodeman, T. M. (1974). A clinical evaluation of the API Microtube System for identification of Enterobacteriaceae. *Amer. J. med. Technol.*, **40**, 55-61.
- Cowan, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edition. Cambridge University Press, London.
- Edwards, P. R. and Ewing, W. H. (1972). *Identification of Enterobacteriaceae*, 3rd edition. Burgess, Minneapolis.
- Hayek, L. J. and Willis, G. W. (1976). A comparison of two commercial methods for the identification of the Enterobacteriaceae—API 20E and the Enterotube—with conventional methods. *J. clin. Path.*, **29**, 158-161.
- Leers, W-D. and Arthurs, G. K. (1973). Routine identification of Enterobacteriaceae with the 'new improved Enterotube'. *Canad. J. publ. Hlth.*, **64**, 584-587.
- Morton, H. E. and Monaco, M. A. J. (1971). Comparison of Enterotubes and routine media for the identification of enteric bacteria. *Amer. J. clin. Path.*, **56**, 64-66.
- Nord, C.-E., Lindberg, A. A. and Dahlbäck, Ann (1974). Evaluation of five test-kits—API, AuxoTab, Enterotube, PathoTec and R/B for identification of Enterobacteriaceae. *Med. Microbiol. Immunol.*, **159**, 211-220.
- Shayegani, M., Hubbard, M. E., Hiscott, T. and McGlynn, D. (1975). Evaluation of the R/B and Minitex Systems for identification of Enterobacteriaceae. *J. clin. Microbiol.*, **1**, 504-508.
- Smith, K. E. (1975). An investigation into three rapid multiple-test systems for identification of Enterobacteriaceae and their possible application in a clinical pathology laboratory. *Lab. Med.*, **6**, (7), 25-27.
- Smith, P. B., Tomfohrde, K. M., Rhoden, D. L. and Balows, A. (1972). API system: A multitube micro-method for identification of Enterobacteriaceae. *Appl. Microbiol.*, **24**, 449-452.
- Washington, J. A. (1972). An assessment of new techniques in the identification of Enterobacteriaceae. *Infectious Diseases Symposium, Washington May 3/5*.