A comparison of two commercial methods for the identification of the Enterobacteriaceae—API 20E and the Enterotube—with conventional methods

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SYNOPSIS The API 20E, the Enterotube, and routine methods of Cowan and Steel were used in parallel to identify 245 members of the Enterobacteriaceae.

The API 20E and conventional methods gave the same identification in all but 2 (0.8%) of the 245 organisms tested. The Enterotube correctly identified 85% of these organisms on first testing. On re-testing those organisms incorrectly identified the Enterotube results agreed with the conventional ones in a further 20 (8%). There was no change in the identification obtained by the API or conventional methods.

Further conventional sugar tests were necessary before final identification was available by the API system in 7 (3%) against 106 (47%) of the 226 organisms correctly identified by the Enterotube. The Enterotube relied on serological testing alone to distinguish between alternatives in 17 (7%) isolates.

Other advantages and disadvantages of these systems are discussed.

During the last few years there has been increasing interest in the role of Gram-negative bacilli in hospital infection, while the rapid and accurate identification of these organisms is becoming more important because of the increasing frequency of severe infections with these species.

Recently, several rapid multitest systems have appeared to facilitate the identification of the Enterobacteriaceae. We have compared two such methods now available in the UK with the conventional methods used in this laboratory and examined their accuracy, reliability, and convenience.

Methods and Material

The API 20E system consists of a plastic card containing 20 cupules of dried reagents. These are inoculated with a bacterial suspension, made by emulsifying one colony of the organism to be tested in 4.5 ml of distilled water, anaerobiosis, where appropriate, being achieved by overlaying with sterile liquid paraffin. The inoculated cards are incubated overnight in a plastic container with a

small amount of water to maintain humidity. Further reagents must be added after incubation to the Voges-Proskauer, tryptophan deaminase, and indole compartments. Results may be recorded and interpreted in the conventional way or, if preferred, they may be converted to a numerical profile which is very simply located in a table provided by the manufacturers.

The Enterotube tested was the improved Enterotube introduced in the UK in 1973 to replace the earlier original version. It consists of an ingenious plastic cartridge with eight separate compartments containing agar slopes with incorporated reagents. Eleven parameters can be tested as three of the compartments contain complex media designed to produce combined results (glucose/gas, indole/H₂S, and dulcitol/phenylalanine deaminase). This device is inoculated by touching a single colony with a straight wire, which is then pulled through the length of the cartridge, inoculating all eight compartments at once. Anaerobiosis is achieved by breaking the wire so that a section is retained in the appropriate compartments. The Enterotube is incubated overnight, and results are recorded and interpreted in the usual way, or by means of a numerical profile and register.

The conventional methods of identification used were those described by Cowan and Steel (1965) and Cowan (1974), using material supplied by Oxoid Ltd, Difco, or Southern Group Laboratories.

Two hundred and forty five cultures were tested by all three methods, and 36 of these were later re-tested. The organisms were all clinical isolates, mostly arising during the three-month period of the trial, although, as the number of Salmonella, Shigella, and Serratia isolates during this period was low, a small number of freeze-dried cultures previously isolated in our laboratory was included. All organisms were tested within 48 hours of isolation or, in the case of freeze-dried cultures, of reconstitution.

Results

All three methods gave broadly the same result in

209 (85%) of the 245 cultures on first testing (table I). It will be seen that the Enterotube does not identify beyond genus level in the *Citrobacter*, *Serratia*, or *Klebsiella* groups. The identification of one organism as *Serratia liquefaciens* by the API and *Enterobacter liquefaciens* by the other methods is accepted as provisionally recommended by Edwards and Ewing (1972). The remaining 36 cultures in which identification did not agree at first testing were then re-tested using the same methods but materials of different batches. Table II gives details of a further 20 cultures (8% of the total) which were given the same identification. The changes in the reactions producing the new identifications are listed. Most of the relevant changes occurred in the Enterotube results, though there were a few changes in the sugar reactions given by the API system, but these did not alter the identification achieved. Table III gives details of the 16 cultures (7% of the total) in which

No. Tested	Identification		
	Conventional	API	Enterotube
60	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
15	<i>Enterobacter cloacae</i>	<i>Enterobacter cloacae</i>	<i>Enterobacter cloacae</i>
21	<i>Citrobacter freundii</i>	<i>Citrobacter freundii</i>	<i>Citrobacter</i>
6	<i>Enterobacter liquefaciens</i>	<i>Serratia liquefaciens</i>	} <i>Serratia</i>
5	<i>Serratia marcescens</i>	<i>Serratia marcescens</i>	
1	<i>Enterobacter liquefaciens</i>	<i>Serratia liquefaciens</i>	<i>Enterobacter liquefaciens</i>
33	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	} <i>Klebsiella</i>
6	<i>Klebsiella oxytoca</i>	<i>Klebsiella pneumoniae oxytoca</i>	
12	<i>Proteus mirabilis</i>	<i>Proteus mirabilis</i>	<i>Proteus mirabilis</i>
8	<i>Proteus morganii</i>	<i>Proteus morganii</i>	<i>Proteus morganii</i>
5	<i>Proteus rettgeri</i>	<i>Proteus rettgeri</i>	<i>Proteus rettgeri</i>
4	<i>Proteus vulgaris</i>	<i>Proteus vulgaris</i>	<i>Proteus vulgaris</i>
9	<i>Providencia B</i>	<i>Providencia stuartii</i>	<i>Providencia stuartii</i>
13	<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>
7	<i>Shigella</i>	<i>Shigella</i>	<i>Shigella</i>
4	<i>Hafnia alvei</i>	<i>Enterobacter hafniae</i>	<i>Enterobacter hafniae</i>

Table I Cultures given the same or equivalent names by all three methods

No. Tested	API and Conventional Results on First and Second Testing	Enterotube		Change in Enterotube Results on Second Testing to produce 'Correct' Identification
		First Testing	Second Testing	
1	<i>Enterobacter cloacae</i>	<i>Citrobacter</i>	<i>Enterobacter cloacae</i>	VP +
2	<i>Enterobacter cloacae</i>	<i>Citrobacter</i>	<i>Enterobacter cloacae</i>	VP +, Gas +, Ornithine +
1	<i>Klebsiella oxytoca</i>	<i>Citrobacter</i>	<i>Klebsiella</i>	Gas -, lysine +, urea +, dulcitol +
1	<i>Klebsiella oxytoca</i>	<i>Citrobacter</i>	<i>Klebsiella</i>	Lysine +
4	<i>Klebsiella pneumoniae</i>	<i>Citrobacter</i>	<i>Klebsiella</i>	Lysine +
1	<i>Klebsiella pneumoniae</i>	<i>Citrobacter</i>	<i>Klebsiella</i>	Lysine +, gas -
1	<i>Klebsiella pneumoniae</i>	<i>Enterobacter cloacae</i>	<i>Klebsiella</i>	Lysine +, citrate -
2	<i>Klebsiella pneumoniae</i>	<i>Enterobacter cloacae</i>	<i>Klebsiella</i>	Lysine +
1	<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	<i>Klebsiella</i>	Ornithine decarboxylase -, urea -
1	<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	<i>Klebsiella</i>	Ornithine decarboxylase -, gas -
1	<i>Serratia marcescens</i>	<i>Enterobacter liquefaciens</i>	<i>Serratia</i>	Lysine +, gas -
1	<i>Escherichia coli</i>	Not listed	<i>Escherichia coli</i>	Gas, lysine, ornithine, indole, lactose, and dulcitol all +
1	<i>Serratia liquefaciens</i>	<i>Enterobacter cloacae</i>	<i>Enterobacter liquefaciens</i>	Lysine +, lactose +, gas +
1	<i>Serratia liquefaciens</i>	Not listed	<i>Enterobacter liquefaciens</i>	Citrate +
1	<i>Enterobacter hafniae</i>	<i>Enterobacter liquefaciens</i>	<i>Enterobacter hafniae</i>	Gas +

Table II Results of 20 cultures in which discrepancies in identification were resolved on re-testing

No. Tested	API Identification		Enterotube	
	API	Conventional	First Testing	Second Testing
4	<i>Enterobacter agglomerans</i>	<i>Erwinia</i> / <i>Enterobacter agglomerans</i>	<i>Citrobacter</i>	<i>Citrobacter</i>
2	<i>Enterobacter agglomerans</i>	<i>Erwinia</i> / <i>Enterobacter agglomerans</i>	<i>Enterobacter cloacae</i>	<i>Enterobacter cloacae</i>
1	<i>Enterobacter agglomerans</i>	<i>Erwinia</i> / <i>Enterobacter agglomerans</i>	<i>Enterobacter aerogenes</i>	<i>Klebsiella</i>
1	<i>Enterobacter agglomerans</i>	<i>Erwinia</i> / <i>Enterobacter agglomerans</i>	<i>Enterobacter aerogenes</i>	Not listed ¹
1	<i>Enterobacter hafniae</i>	<i>Enterobacter hafniae</i>	<i>Enterobacter cloacae</i>	Not listed
1	<i>Enterobacter hafniae</i>	<i>Enterobacter hafniae</i>	Not listed	Not listed
1	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter cloacae</i>	<i>Citrobacter</i>
1	<i>Klebsiella ozaenae</i>	<i>Klebsiella ozaenae</i>	<i>Citrobacter</i>	<i>Citrobacter</i>
1	<i>Escherichia coli</i>	<i>Escherichia coli</i>	Not listed	Not listed
1	<i>Proteus morganii</i>	<i>Proteus morganii</i>	<i>Proteus vulgaris</i>	<i>Proteus vulgaris</i>
2	<i>Proteus vulgaris</i>	<i>Proteus rettgeri</i>	<i>Proteus rettgeri</i>	<i>Proteus rettgeri</i>

Table III Details of identification given to 16 organisms which on re-testing failed to agree

¹Where the identification is given as 'not listed' it was not possible to name the organisms using the tables provided by the manufacturer.

discrepancies in the identification remain after re-testing.

Results from both systems are normally available after 18 hours' incubation. Extra conventional tests involving further overnight incubation were necessary before final identification was available from the API system in seven of the 238 (3%) cultures which were correctly identified. These were all single sugar fermentation reactions. A total of 244 extra tests had to be performed on 106 of the 226 (47%) of those cultures correctly identified by the Enterotube method. The Enterotube method relied on serological testing alone to distinguish between alternatives on 17 (7%) isolates identified, and in conjunction with other tests on 24 (10%) occasions. The API system did not require any serological differentiation between alternatives. Both systems recommended the use of serological testing to confirm the biochemical identification of *Salmonella* and *Shigella* species.

Discussion

While we feel that the finer points of the taxonomy of the Enterobacteriaceae will long remain fertile ground for discussion, it seems a pity that there still seems no universally agreed nomenclature within the group, thus rendering much inter-laboratory comparison of results of little value. One of the causes of this apparent confusion must be that the Enterotube and API methods are based on the system of Edwards and Ewing, whereas most medical microbiologists in the UK use that of Cowan and Steel. Unfortunately, these systems do not quite match, the position being especially confusing with the *Klebsiellae*. Where there has been any change between the 1965 and 1974 editions of Cowan and Steel the names shown in the earlier edition are used in this study. We have tried as nearly as possible to

equate organisms whose similarity is not doubted and yet whose names may differ (for example, *Serratia liquefaciens* and *Enterobacter liquefaciens*, or *Erwinia herbicola* and *Enterobacter agglomerans*). Despite this, these two commercial methods fail to reach the same identification in 38 out of 245 (15%) instances on first testing. There were no changes in the identification produced by the API system on re-testing, but changes in the Enterotube identification reduced the discrepancy rate to 7%. This improved success rate was then due only to the obvious discrepancy between methods used concurrently which had led to the re-testing. If the Enterotube had been used alone, the mistakes would have gone unnoticed.

We feel that any device which can correctly identify only 85% of a fairly random collection of organisms has considerable scope for improvement in design and quality control.

A number of workers have examined the correlation between individual tests carried out by conventional methods and by these commercial methods. Nord *et al* (1974) found that the API tests had an overall 99% agreement with conventional methods, while in the Enterotube system 9 out of 10 tests had a correlation of 98%, but the phenylalanine deaminase had only a 90% accuracy. We did not carry out a direct comparison of individual parameters, but it is interesting to note in table II how a high proportion of the changed identifications were due to changes in the decarboxylase tests given by the Enterotube, which may have been due to batch variation. We found that the API system gave 100% reproducible results, though two cultures identified as *Proteus rettgeri* by Enterotube and conventional methods were consistently identified as *Proteus vulgaris* by the API.

One of the problems associated with any method of identification which involves testing many

parameters is the number of variations in the reactions given by any one recognized species. Among the 60 different *Escherichia coli* isolates identified, the API system recognized 26 different biochemical variants, while the Enterotube, testing fewer parameters, recognized 20. To allow for such variations and to be able to give correct weight to the key characteristics requires great experience, which may not always be available in a routine laboratory. This difficulty seems to be avoided if one of these systems is used, as the manufacturers provide a comprehensive and continuously up-dated register of the biochemical profiles.

Both systems were successful in identifying organisms of the *Salmonella* or *Shigella* genera, where early and accurate identification may be of the greatest importance, though the Enterotube identified the *Shigella* after overnight incubation as one of several possible organisms, for example, *Citrobacter/Shigella/Salmonella/Enterobacter liquefaciens*, final identification being made by serological testing only. The API method gave correct biochemical identification after overnight incubation, serological testing being used as confirmatory evidence only.

As a test to exclude or confirm the presence of these organisms, we consider that both systems might provide a useful alternative to conventional

methods. We do, however, feel that such screening could well be done more economically with a small number of conventional sugars, though as a method of rapidly and accurately identifying unusual organisms or isolates of epidemiological importance we consider the API system to be without parallel.

In these safety-conscious days we consider that the recommendations of the manufacturers of the Enterotube to use a syringe and needle to add Kovac's reagent to the indole compartment is to be deprecated owing to the risk of the inevitable accidents in the presence of dangerous pathogens. We should also add that the mixture of concentrated hydrochloric acid and amyl alcohol softens and distorts the polypropylene syringes now in use.

References

- Cowan, S. T. (1974). *Manual for the Identification of Medical Bacteria*, 2nd edition. Cambridge University Press, London.
- Cowan, S. T. and Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*, Cambridge University Press, London.
- Edwards, P. R. and Ewing, W. H. (1972). *Identification of Enterobacteriaceae*, 3rd edition. Burgess, Minneapolis.
- Nord, C. E., Lindberg, A. A., and Dahlbäck, A. (1974). Evaluation of five test-kits,—A P I., Auxotab, Enterotube, PathoTec, and R/B—for identification of Enterobacteriaceae, *Med. Microbiol. Immunol. (Berlin)*, **159**, 211-220.