The Enterotube System in the Identification of *Enterobacteriaceae* and *Yersinia*

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SUMMARY

A trial of the Enterotube system for the identification of *Enterobacteriaceae* and a comparison with the methods at present in use in the Tygerberg Hospital Microbiology Laboratory, were carried out. One hunded cultures belonging to the family *Enterobacteriaceae* including *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Proteus morgani, Proteus rettgeri, Providencia, Edwardsiella, Shigella, Klebsiella, Enterobacter, Serratis, Salmonella, Citrobacter* and *Arizona* were tested with conventional procedures and also with the Enterotube. The conventional procedures, designed to identify the organisms within 24 hours after isolation, were used. Three of the cultures examined were not identified by the Enterotube technique when their identity was established by routine conventional methods. These were non-urease-producing non-motile organisms which may have been members of the tribe *Klebsiellae*. Good agreement was therefore observed in 97% of the cultures when the Enterotube was used compared with the conventional technique. Three cultures of *Yersinia enterocolitica* and two of *Yersinia pseudotuberculosis* were identified by the Enterotube was used, compared with the conventional levels.

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MATERIALS AND METHODS

The original Enterotube system incorporates 9 biochemical tests and comprises a moulded semicircular plastic tube divided into 8 compartments, each of which contains a slope of one of the following test media:

Citrate agar for detection of citrate utilisation; this would, if negative, exclude *Escherichia, Shigella* and *Edwardsiella.*

Lysine agar to detect lysine decarboxylase produced early by *Hafnia* and *Serratia* and possibly late by *Klebsiella* and *Enterobacter aerogenes.*

Lactose agar for detection of acid production, absent in *Shigella* (except *S. sonnei*), *Proteus* sp., *Providencia, Salmonella,* and *Edwardsiella.*

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Dulcitol agar for detection of acid production, absent in *Proteus* sp., most *Enterobacters* sp., *Hafnia* and *Serratia, Arizona* and *Edwardsiella.*

Urea agar for detection of urea cleavage, absent in *Escherichia, Shigella, Providencia, Enterobacter aerogenes, Hafnia, Salmonella, Arizona* and *Edwardsiella.*

Phenylalanine agar for detection of phenylalanine deaminase by production of a green colouration after addition of ferric chloride solution. This reaction is positive only in the *Proteus* and *Providencia* groups.

Hydrogen sulphide and indole agar for detection of hydrogen sulphide and indole, the latter being detected after the addition of Kovac's reagent to the compartment. Hydrogen sulphide is produced by some *Proteus* strains and by *Salmonella, Citrobacter, Arizona* and *Edwardsiella* while indole is produced by *Escherichia,* some *Proteus* strains, *Providencia* and *Edwardsiella.*

Dextrose agar showing acid production in all the *Enterobacteriaceae* and also in *Yersinia.* It is claimed that the Enterotube provides a 'ready to use' system for the screening and differentiation of *Enterobacteriaceae.* It avoids the preparation and sterilisation of media and permits the simultaneous inoculation of the medium in the 8 compartments with little risk of contamination.

A redesigned Enterotube is now available. In this tube, the lactose has been removed from the lysine decarboxylase compartment, a test for ornithine decarboxylase has been added to one compartment, and the tests for phenylalanine deaminase and dulcitol fermentation have been combined in another compartment. An iron salt has been added to this compartment, thus eliminating the need to add ferric chloride to the phenylalanine agar. The compartments containing tests for dextrose fermentation and the 2 carboxylase tests have been covered with sterile wax, allowing for detection of gas from dextrose and improving the carboxylase reactions. In our opinion these changes greatly enhance the value of the Enterotube system, especially in the separation of *Shigella* strains, *S. typhi* and *P. rettgeri,* and also the more positive identification of *Klebsiella pneumoniae.*

Only the original Enterotube is available in South Africa at present. This was tested by us and compared with the conventional methods in use in our laboratory. These include the following biochemical reactions: lactose, dextrose, sucrose, mannite, arabinose fermentation, with or without gas production, indole, hydrogen sulphide and ornithine decarboxylase production, urea cleavage and gluconate conversion. In addition, presence or absence of motility is recorded by a Craigie tube.

TABLE II. ENTEROTUBE REACTIONS OF *YERSINIA*

	Biochemical reactions							
Organism	Dextrose	H_2S	Phenyl alanine	Urea	Dulcitol	Lactose	Lysine	Citrate
Yersinia	+	−	−	+	−	−	−	−

One hundred strains of *Enterobacteriaceae* were tested by the original Enterotube and by our conventional methods. These organisms are shown in Table I.

TABLE I. ORGANISMS TESTED BY ENTEROTUBE

	No.
Escherichia coli	22
Shigella **sp.**	7
Edwardsiella tarda	1
Citrobacter freundii	2
Salmonella **sp.**	9
Arizona hinshawii	3
Klebsiella pneumoniae	26
Enterobacter aerogenes	6
Serratia marcescens	8
Providencia **sp.**	4
Proteus mirabilis	3
P. vulgaris	3
P. morgani	3
P. rettgeri	3

RESULTS

Three of the organisms tested were not identified by the Enterotube technique. They were non-motile organisms which did not produce urease and which were possibly *Klebsiella* or non-motile *Enterobacter*. They were, however, ornithine decarboxylase-negative and fermented arabinose in the conventional tests and should therefore be classified as *Klebsiella*.

Although not normally classified as *Enterobacteriaceae*, in view of their isolation from cases of gastro-enteritis, 5 strains of *Yersinia*, 3 of *Y. enterocolitica*, and 2 of *Y. pseudotuberculosis* were examined by the Enterotube technique. These strains were well-differentiated from the *Enterobacteriaceae*, and their Enterotube reactions are shown in Table II. *Y. enterocolitica* was not differentiated from *Y. pseudotuberculosis*.

CONCLUSIONS

Although our comparisons were limited because only 100 Enterotubes were available, we are of the opinion that the Enterotube system compares favourably with the conventional system in use in our laboratory. One of the great advantages of the Enterotube system is that all tubes are inoculated in sequence, and rapidly, from a single isolated colony. Most of the cultures examined were correctly identified, the only serious difficulties being with members of the *Klebsiella-Enterobacter* group. Thirty-two strains of these organisms were tested and 3 strains (approximately 10%) were possibly incorrectly identified. The redesigned Enterotube with an ornithine decarboxylase compartment should eliminate such problems. We are in agreement with Martin *et al.*[1] in this regard, and also support their recommendations that lactose be removed from the lysine decarboxylase compartment (as has been done) and that arabinose be added in place of dulcitol.

In our opinion the Enterotube system, in particular the redesigned Enterotube, should give a valuable diagnostic aid in small laboratories and also in field laboratories. Superficially it would appear to be rather expensive, but when the number and variety of tests is considered, it is doubtful whether the media could be produced at a lower cost in laboratories which are not equipped and staffed for large-scale media production.

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REFERENCE

1. Martin, W. J., Wu, P. K. W. and Washington, J. A. (1971): Appl. Microbiol., **22**, 96.