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Kit systems for identifying Gram negative aerobic bacilli: report of the Welsh Standing Specialist Advisory Working Group in Microbiology

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SUMMARY Under the auspices of the Welsh Standing Specialist Advisory Working Group in Microbiology (WMG) 10 clinical microbiology laboratories in Wales undertook a collaborative study to assess 10 commercial kits for the identification of aerobic Gram negative bacilli. In excess of 1000 such strains were examined in parallel with each kit system. Accuracy, reproducibility of accuracy, and reproducibility alone were assessed, together with the cost effectiveness of the kits used. A ranking order of kit performance based on the above variables was drawn up.

Since the foundation of bacteriology as a science in the second half of the nineteenth century, the use of various substrate reactions as an aid to identification has been well exploited. This is particularly true of *Enterobacteriaceae* and other associated Gram negative aerobic bacilli. Antigenic analysis apart, biochemical reactions remain the primary means by which these organisms are identified.

The past decade has witnessed the introduction from commercial sources of several miniaturised kit identification systems designed to replace traditional methods of substrate testing. Prompted by these developments, the Standing Specialist Advisory Working Group in Microbiology of the Welsh Scientific Advisory Committee (WMG) decided to undertake a collaborative study to assess the value of the 10 kits, which were then available in the United Kingdom, for identifying Gram negative aerobic bacilli (Table 1). Of the kits listed, the API RapiD 20E, Minitek Enterobacteriaceae III, and Micro ID kits were designed to produce a result after four hours' incubation at 37°C: these three kits, together with the Titertek Enterobac, were not intended for the identification of oxidase positive, afermentative, Gram negative aerobic rods.

Previously published reports have included a study of six commercial systems by Smith,[1] five systems by Nord *et al*,[2] comparisons between two commercial systems and conventional media by Barry *et al*,[3] and comparisons between a single commercial kit and traditional substrates by Holmes *et al*,[4] and a comprehensive review article by D'Amato *et al*.[5] We could find no reference to the kind of large scale collaborative and comparative study that we propose.

Material and methods

A detailed protocol was agreed by the WMG and the kit manufacturers to: evaluate the accuracy and reproducibility of the systems; attempt to assess the cost effectiveness of the systems.

The Neath laboratory, having been designated as the source laboratory, collected 860 isolates (representing 48 taxa) of aerobic Gram negative bacilli from within West Glamorgan and other parts of Wales (Table 2). Strains were identified using the API 20E system, subcultured on to Dorset egg medium, and held at room temperature until required. A preprinted index card was used to record the source of the strain, the profile score, and the identification: these cards were subsequently used to record the results from participating laboratories and formed the manual data base used in compiling a series of progress reports considered by the Working Group throughout the study.

In addition to the clinical series, a collection of control strains were assembled from the National Collection of Type Cultures (NCTC), the American Type Culture Collection (ATCC), and the Microbiology Quality Assessment Scheme (MQAS), supplemented with local isolates of *Salmonella* spp and *Yersinia* spp. The identities of *Yersinia* spp were confirmed by the respective PHLS reference laboratories. The control

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Table 1 *Kit systems used*

Source	*Product name*	*Abbreviation*
API Laboratory Products	API 20E	API 20E
API Laboratory Products	API RapiD 20E	API RAP
Becton Dickinson	MINITEK Enterobacteriaceae II	ENT II
Becton Dickinson	MINITEK Enterobacteriaceae III	ENT III
Roche Products	OXI/FERM ENTEROTUBE II	OXI ENT
LIP Services	MICROBACT 24E	MIC 24E
Seward Laboratory	SENSITITRE ENTERIC IDENTIFICATION PLATE	SEN EIP
General Diagnostics	MICRO ID	MIC ID
Flow Laboratories	TITERTEK ENTEROBAC	TTK EBC
Cathra International	REPLIDEX	REPDEX

Although all systems are designed to identify members of the family *Enterobacteriaceae*, other related extra taxa, which include oxidase positive afermenting organisms, are not handled by four of the systems: the API RapiD 20E, MINITEK Enterobacteriaceae III, MICRO ID, and the TITERTEK ENTEROBAC. Because of this constraint, it was necessary to account for this in reporting results.

series was derived from the 63 strains representing 40 taxa (Table 3).

It was agreed that the source laboratory should use the API 20E kit, although the remaining participating laboratories were randomly assigned a particular kit by means of a draw. Apart from the considerable experience with the API 20E kit by the source laboratory, experience with the other kits was minimal or nil. The agreed protocol, however, contained provision for full "on site" training to be given by the manufacturers and for their representatives to ensure close liaison with the respective laboratories throughout the study. At weekly intervals 24 cultures were selected from the clinical series together with one control culture. After subculture on MacConkey agar to check purity and viability a single colony was suspended in 5 ml of sterile distilled water. This suspension was used to inoculate an API 20E strip, a pair of glucose oxidation/fermentation media, 10 nutrient agar slopes, and a segment of a MacConkey agar plate as a further purity and viability check. Preprinted, self adhesive, serially numbered labels were used to identify each culture, and the same numbered label affixed to the index card. After incubation of all cultures at 37°C for 24 hours the MacConkey plate was inspected for purity and viability. If satisfactory the API 20E strip was dosed with the appropriate reagents and the results read, together with the reaction of the pair of oxidation/fermentation media. The profile score obtained was compared with the scores

Table 2 *Clinical isolates: taxa and numbers tested*

Taxon	*No*	*Taxon*	*No*
Achromobacter xylosoxidans	1	*Plesiomonas shigelloides*	2
Acinetobacter aniltratus	23	*Proteus mirabilis*	58
Acinetobacter iwoffii	4	*Proteus morganii*	40
Aeromonas hydrophila	2	*Proteus vulgaris*	14
Alcaligenes spp	11	*Providencia alcalifaciens*	9
CDC Group IV-E	1	*Providencia rettgeri*	3
Citrobacter spp	2	*Providencia stuartii*	36
Citrobacter diversus	15	*Pseudomonas* spp	1
Citrobacter freundii	42	*Pseudomonas aeruginosa*	18
Enterobacter cloacae	52	*Pseudomonas fluorescens*	7
Enterobacter agglomerans	9	*Pseudomonas cepacia*	2
Enterobacter aerogenes	10	*Pseudomonas maltophilia*	5
Enterobacter gergoviae	1	*Pseudomonas stutzeri*	2
Enterobacter sakazakii	3	*Salmonella* spp	25
Edwardsiella tarda	2	*Salmonella typhi*	2
Escherichia coli	209	*Serratia liquefaciens*	9
Escherichia coli lysine negative, ornithine negative	20	*Serratia marescens*	28
Escherichia coli Alkalescens-dispar	24	*Shigella* spp	1
Escherichia coli hydrogen sulphide positive	1	*Shigella boydii*	5
Hafnia alvei	22	*Shigella dysenteriae*	1
Klebsiella pneumoniae	91	*Shigella flexneri*	1
Klebsiella oxytoca	20	*Shigella sonnei*	6
Klebsiella ozaenae	2	*Yersinia enterocolitica*	11
Pasteurella aerogenes	1	*Yersinia pseudotuberculosis*	6
		Total clinical isolates	860

Table 3 *Control strains and sources used*

Organism	*Source*	*Organism*	*Source*
Acinetobacter calcoaceticus	NCTC 7844	*Proteus rettgeri*	NCTC 7475
Aeromonas hydrophila	NCTC 8049	*Proteus vulgaris*	ATCC 6380
Alcaligenes faecalis	NCTC 8764	*Proteus vulgaris*	NCTC 10020
Alcaligenes odorans	NCTC 10416	*Providencia alcalifaciens*	NCTC 10286
*Citrobacter ballerupensis**	NCTC 6021	*Providencia stuartii*	NCTC 10318
Citrobacter freundii	MQAS 673	*Pseudomonas aeruginosa*	NCTC 10701
Citrobacter freundii	NCTC 9750	*Pseudomonas aeruginosa*	MQAS 627
Edwardsiella tarda	NCTC 1036	*Pseudomonas aeruginosa*	ATCC 14207
Enterobacter aerogenes	NCTC 10006	*Pseudomonas cepacia*	NCTC 10661
Enterobacter agglomerans	MQAS 632	*Pseudomonas fluorescens*	MQAS 650
Enterobacter cloacae	ATCC 23355	*Salmonella arizona*	NCTC 8297
Enterobacter cloacae	MQAS 644	*Salmonella bovis morbificans*	MQAS 661
Escherichia alkalescens†	NCTC 1601	*Salmonella enteritidis*	MQAS 649
Escherichia coli	NCTC 86	*Salmonella typhi*	West Glam 6
Escherichia coli	ATCC 12228	*Salmonella typhi*	West Glam 23
Escherichia coli	MQAS 649	*Salmonella typhimurium*	MQAS 632
Escherichia dispar†	NCTC 7721	*Salmonella typhimurium*	ATCC 14028
Hafnia alvei	NCTC 5678	*Serratia marescens*	ATCC 8100
Klebsiella aerogenes	NCTC 5005	*Shigella boydii serotype 1*	NCTC 9327
Klebsiella aerogenes	NCTC 8172	*Shigella dysenteriae serotype 4*	NCTC 9759
Klebsiella edwardsii (var) atlantae	NCTC 10896	*Shigella flexneri serotype 4b*	MQAS 662
Klebsiella ozaenae	NCTC 5050	*Shigella sonnei*	MQAS 669
Klebsiella pneumoniae	ATCC 23357	*Shigella sonnei*	MQAS 764
Klebsiella pneumoniae	MQAS 644	*Shigella sonnei*	MQAS 719
Klebsiella pneumoniae	NCTC 9633	*Vibrio* sp (NAG)	MQAS 698
Klebsiella rhinoscleromatis	NCTC 50465	*Yersinia enterocolitica*	West Glam 40
Plesiomonas shigelloides	NCTC 10360	*Yersinia enterocolitica*	MQAS 776
Proteus mirabilis	MQAS 669	*Yersinia enterocolitica*	West Glam 65
Proteus mirabilis	MQAS 680	*Yersinia enterocolitica*	West Glam 90
Proteus mirabilis	NCTC 7827	*Yersinia fredericksensii*	West Glam 98
Proteus morganii	NCTC 235	*Yersinia fredericksensii*	West Glam 104
		Yersinia pseudotuberculosis	West Glam 25

*Now classified as a strain of *Citrobacer freundii* (NCTC, personal communication).
†Now regarded as strains of *Escherichia coli.*

contained in the manufacturers' profile index. Any additional tests indicated in the index were undertaken and the final identification, together with the profile score recorded on the relevant index card. In accordance with the manufacturers' instructions, strains showing no utilisation of glucose and less than two other positive reactions were returned to the incubator for a further 24 hours before reagents were added. The nutrient agar slopes were inspected for evidence of visible growth, the caps tightened, and sets of 25 cultures, together with a results sheet were suitably packed and despatched by post to participating laboratories. Returned results, comprising profile score and identification, were entered on the respective index cards. This data was subsequently transcribed for computer input and so stored for future detailed analysis.

The identities of all cultures used in the study were not known by the participating laboratories until the study had been completed.

Results

It was agreed by the WMG that the results should be analysed in three ways: how well a kit performed in correctly identifying a strain; the reproducibility of accuracy achieved; the reproducibility achieved, irrespective of accuracy.

Results of both clinical and control series were analysed and presented at both genus and species levels of identification. To compensate for those kits which were not intended to identify the oxidase positive afermenters—that is, the API RapiD 20E, Minitek Enterobacteriaceae III, and Titertek Enterobac, the appropriate figures were corrected so as to exclude such organisms.

Table 4 shows identification performance achieved with the control strains and Table 5 kit performance with the 860 clinical strains.

Reproducibility of accuracy together with reproducibility alone were determined from results obtained by the issue on three separate occasions during the study of 60 of the control strains. The identity and origin of these strains were unknown to the participants. Results were recorded (in percentage terms) of the number of times reproducibility of accuracy was achieved (Table 6). Similarly, Table 7 shows the reproducibility, irrespective of accuracy.

The participating laboratories completed a questionnaire designed to provide information on the tim-

Table 4 *Accuracy of performance: control series*

Kit	*Percentage*	
	Genus	*Species*
API 20E	96·3	95·0
API RapiD 20E	89·3	81·3
MINITEK Enterobacteriaceae II	85·5	78·2
MINITEK Enterobacteriaceae III	66·0	54·3
OXI/FERM ENTEROTUBE II	85·9	76·4
MICROBACT 24E	81·4	73·3
REPLIDEX	64·7	57·4
SENSITITRE EIP (AP60)	94·1	85·9
MICRO ID	79·3	68·4
TITERTEK ENTEROBAC	88·8	82·0

ing of setting up and reading tests, the cost of kits, shelf life, user problems encountered, problems of nomenclature, and microbiological safety.

Discussion

Examination of the results provided evidence for the ranking of the 10 kits examined in terms of percentage accuracy at both levels of identification. With the control series the best performing kits at genus and species levels were the API 20E and the Sensititre EIP kit, with the Replidex and Minitek Enterbacteriaceae III kits performing poorly, and the remaining kits occupying the middle ground. A similar order of performance in terms of reproducibility of accuracy and reproducibility alone was observed with the control strains.

With the clinical series the identification achieved by the source laboratory using the API 20E kit was used as a reference point. Our experience with the control series supports this standpoint and accords with the views expressed by Hayek and Willis[6] regarding the API 20E kit. In only nine instances (four strains at genus level and five at species level)

Table 5 *Accuracy of performance: clinical series*

Kit	*Percentage*	
	Genus	*Species*
API 20E	99·5	99·3
API RapiD 20E	87·0	84·9
MINITEK Enterobacteriaceae II	93·1	84·5
MINITEK Enterobacteriaceae III	80·0	63·0
OXI/FERM ENTEROTUBE II	81·4	76·7
MICROBACT 24E	81·5	70·6
REPLIDEX	68·0	56·5
SENSITITRE EIP (AP60)	91·6	78·4
MICRO ID	88·1	77·0
TITERTEK ENTEROBAC	85·4	85·0

Table 6 *Reproducibility of accuracy: control series*

Kit	*Percentage*	
	Genus	*Species*
API 20E	96·6	90·0
API RapiD 20E	80·0	72·7
MINITEK Enterobacteriaceae II	76·6	61·6
MINITEK Enterobacteriaceae III	41·8	27·3
OXI/FERM ENTEROTUBE II	71·6	55·0
MICROBACT 24E	65·0	51·6
REPLIDEX	45·0	36·6
SENSITITRE EIP (AP60)	83·3	71·6
MICRO ID	41·5	33·9
TITERTEK ENTEROBAC	79·2	58·5

Table 7 *Reproducibility, irrespective of accuracy: control series*

Kit	*Percentage*	
	Genus	*Species*
API 20E	98·3	93·3
API RapiD 20E	85·5	81·8
MINITEK Enterobacteriaceae II	86·0	73·3
MINITEK Enterobacteriaceae III	53·7	35·2
OXI/FERM ENTEROTUBE II	76·6	63·0
MICROBACT 24E	66·6	56·6
REPLIDEX	48·3	43·3
SENSITITRE EIP (AP60)	86·6	78·3
MICRO ID	43·4	37·3
TITERTEK ENTEROBAC	79·2	64·1

Table 8 *Summary of accuracy of performance*

Kit	*Percentage*			
	Control		*Clinical*	
	Genus	*Species*	*Genus*	*Species*
API 20E	96·3	95·0	99·5	99·3
API RapiD 20E	89·3	81·3	87·0	84·9
MINITEK Enterobacteriaceae II	85·5	78·2	93·1	84·5
MINITEK Enterobacteriaceae III	66·0	54·3	80·0	63·0
OXI/FERM ENTEROTUBE II	85·9	76·4	81·4	76·7
MICROBACT 24E	81·4	73·3	81·5	70·6
REPLIDEX	64·7	57·4	68·0	56·5
SENSITITRE EIP (AP60)	94·1	85·9	91·6	78·4
MICRO ID	79·3	68·4	88·1	77·0
TITERTEK ENTEROBAC	88·8	82·0	85·4	85·0

Table 9 *Identification failures in control series*

Kit	Percentage		
	Pathogens*	Non-pathogens†	Total‡
API 20E	0·0	0·0	0·0
API RapiD 20E	6·6	3·4	4·5
MINITEK Enterobacteriaceae II	6·6	6·2	6·3
MINITEK Enterobacteriaceae III	28·6	16·6	20·4
OXI/FERM ENTEROTUBE II	17·1	1·4	6·8
MICROBACT 24E	19·7	2·8	8·6
REPLIDEX	51·3	5·6	21·3
SENSITITRE EIP (AP60)	3·9	3·4	3·6
MICRO ID	18·4	9·0	12·2
TITERTEK ENTEROBAC	6·6	2·8	4·1

*Pathogens incorrectly identified (n = 79).
†Non-pathogens identified as pathogens (n = 142).
‡Total failures of identification (n = 221).

was the identification obtained by the source laboratory, with the 860 clinical isolates being totally at variance with the identification obtained by the other participants. These strains were recorded as out of agreement, thus giving the API 20E kit less than 100 per cent accuracy of identification (Table 5). Examination of results from the clinical series provides evidence of a similar ranking to that obtained with the control series. From a summary of results of performance accuracy of both clinical and control series, however, it seems that a marginal increase in performance was achieved by all kits in respect of the clinical series (Table 8). This increase may relate to the larger number of strains examined in the clinical series, or the fact that roughly 25% of these comprised strains of *Escherichia coli*, or both factors: additionally, stock cultures (comprising most of the control series) are known to have impaired enzyme activity, resulting in aberrant biochemical activity in substrates. Of particular concern to the WMG were the failures of identification in the control series, and these were considered from two aspects.

Firstly, that strains with clinical or epidemiological importance were not correctly identified due to misidentification or non-identification. Genera regarded of critical importance in this context were *Edwardsiella, Plesiomonas, Salmonella* or *Arizona, Shigella, Yersinia*, and *Vibrio*. Secondly, that organisms were not correctly identified as non-pathogens either by non-identification or misidentifying a non-pathogen—such as *E coli* incorrectly identified as *Shigella flexneri*.

Table 9 shows the identification failures in the control series and specific examples included the following: *S flexneri* misidentified as *E coli* on one occasion and as a *Yersinia* on another occasion by the same kit, *S dysenteriæ* misidentified by three of the kits as *Acinetobacter iwoffii, Proteus morganii*, and a *Salmonella* sp, respectively. Examples of non-pathogens identified as pathogens included *Klebsiella pneumoniae* misidentified as *Yersinia pseudotuberculosis* on one occasion and as a *Salmonella* sp on the second occasion by the same kit. *Serratia marcescens* was misidentified as a *Salmonella* sp and subsequently again misidentified as *Yersinia pseudotuberculosis* by another kit.

In evaluating the activity analysis questionnaires, it was evident that a valid objective assessment of timings was impractical. Unit costs at the time of the study (excluding available discount) ranged from 16 pence for the Replidex kit to 196 pence for the Micro ID kit, with an average unit cost of 120 pence. Most kits could be stored at between 2°C and 8°C, but for the Titertek Enterobac storage at −20°C was recommended. Shelf life varied from two months (Replidex kit) to two years (Sensititre EIP); most kits had an average shelf life of one year.

Microbiological safety was assessed with particular reference to aerosol and droplet dispersal, with the exception of the inbuilt inoculation wire of the Oxi/Ferm Enterotube II, and inoculation of the kits was by means of a pipette. Using the accepted level of care, no evidence of environmental contamination could be detected with any of the kits. Autoclaving or incineration, or both, provided safe after use disposal of all kits examined.

User problems reported were largely confined to initial difficulties in interpretation of colour changes with the carbohydrates, decarboxylases, and the arginine dihydrolase, but these diminished with increasing experience. The Replidex kit, however, caused persistent problems of interpretation due to colour diffusion around adjacent discs.

Problems of nomenclature were few, apart from the fact that none of the data bases took account of the Cowan[7] classification of the Klebsiellas, and some data bases classified *Salmonella* of subgenus III as *Arizona* sp.

The WMG believe it to be axiomatic that potential users of identification kits should require accuracy and that reproducibility is of paramount importance, with cost effectiveness a secondary consideration. The WMG also believed that the ideal kit should speciate not only the *Enterobacteriaceae* but should also identify the associated Gram negative oxidase positive afermenting rods. The results of this collaborative study suggest that only two kits approached this ideal, the API 20E and Sensititre EIP. Other workers, however, may not agree with this ideal and will be prepared to sacrifice some degree of accuracy and reproducibility for other perceived benefits. For example, some may be satisfied with genus level identification, particularly if the answer is available in four hours. Others may be content to reserve the use of these kits only for the identification of the *Enterobacteriaceae* and use more conventional methods when dealing with the oxidase positive afermenting aerobic Gram negative rods.

The reasons for the less than ideal performance of some kits examined in this study was not part of our remit, but it is clear that expansion of data bases could be helpful in improving performance, as could a review of the test substrates used.

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References

[1] Smith PB. *Performance of six bacterial identification systems.* Atlanta: US Department of Health, Education and Welfare, 1975.

[2] Nord C-E, Linberg AA, Dahlback A. Evaluation of five test kits—API, Auxotab, PathoTec, and R/B for identification of Enterobacteriaceae. *Med Microbiol Immunol* 1974;**159**:211.

[3] Barry AL, Badal RE. Rapid identification of Enterobacteriaceae with the Micro-ID system versus API 20E and conventional media. *J Clin Microbiol* 1979;**10**:293–8.

[4] Holmes B, Willcox RR, Lapage SP. Identification of Enterobacteriaceae by the API 20E system. *J Clin Pathol* 1978; **31**:22–30.

[5] D'Amato RF, Holmes B, Buttone EJ. The systems approach to diagnostic microbiology. *CRC Crit Rev* 1981;**9**:1–44.

[6] Hayek LJ, Willis GW. Identification of the Enterobacteriaceae: a comparison of the Enterotube II with the API 20E. *J Clin Pathol* 1984;**37**:344–7.

[7] Cowan ST, Steel KG. *Manual for the Identification of Medical Bacteria.* 2nd ed. London: Cambridge University Press, 1974.

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