Identification of Gram-negative non-fermenters and oxidase-positive fermenters by the Oxi/Ferm Tube

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SUMMARY Since the recent introduction of the Roche Oxi/Ferm Tube to the UK two identification schemes have been developed by the manufacturer for use with the kit. We evaluated the success of these two schemes in identifying 222 predominantly culture collection strains belonging to 45 taxa of non-fermenters and nine taxa of oxidase-positive fermenters. The strains were chosen to represent all the taxa included in the two identification schemes developed by the manufacturer and we have therefore been able to assess the overall success of identification by the two schemes. Since, however, our choice of strains does not reflect their incidence in clinical material, our identification rates are not necessarily those that might be obtained in a routine clinical laboratory. The most advanced identification scheme so far developed for the Oxi/Ferm Tube (CCIS System 1977-1432) allowed 62% of the 222 strains to be correctly identified although a disturbing feature was that more of the strains that were not correctly identified were incorrectly identified (24%) rather than not identified (14%); these figures represent an improvement over the earlier identification scheme (CCIS System 1976-621-74346) for which the corresponding figures were 56%, 32%, and 12%. CCIS System 1977-1432 seems likely to give a better performance in a routine clinical laboratory than in this study since for those taxa which, we would judge from the material sent to us for identification, are most commonly seen in a routine laboratory (*Acinetobacter calcoaceticus*, *A. lwoffii*, *Pseudomonas aeruginosa*, *P. fluorescens*, *P. maltophilia*, *P. pseudocalcaligenes*, and *P. putida*) 89% were correctly identified, none remained unidentified, and 11% were incorrectly identified. Thirty strains, each of a different taxon, were tested in triplicate to assess the reproducibility of reactions in the Oxi/Ferm Tube.

The Oxi/Ferm Tube, a diagnostic kit designed for the identification of aerobic, Gram-negative, non-fermentative bacteria and of oxidase-positive fermentative bacteria, was initially introduced into the USA by the manufacturer of the Enterotube, a kit designed on similar principles for the identification of enterobacteria. Evaluations have been published (Freundlich *et al.*, 1977; Isenberg and Sampson-Scherer, 1977; Kilbourn, 1977; Oberhofer *et al.*, 1977a, 1977b; Nadler *et al.*, 1978; Smith *et al.*, 1978; Timm *et al.*, 1978). The Oxi/Ferm Tube has been evaluated in parallel with the API 20E system, a kit designed primarily for enterobacteria, and modified for the identification of non-fermentative bacteria by the addition of six supplementary tests

(Dowda, 1977; Nord *et al.*, 1977; Rinehart and Oberhofer, 1978; Snyder *et al.*, 1978). Other workers have evaluated the Oxi/Ferm Tube in parallel with the API 20E system and one or more other systems (Bannister *et al.*, 1978; Otto *et al.*, 1978). The evaluations of the various authors suggested that the Oxi/Ferm Tube was a useful product, the proportion of strains correctly identified ranging from 84% (Nadler *et al.*, 1978) to 98% (Rinehart and Oberhofer, 1978). Misidentification rates were correspondingly low, for example, 4.5% (Dowda, 1977).

Isenberg and Sampson-Scherer (1977), McHale *et al.* (1978), Nadler *et al.* (1978), Smith *et al.* (1978), and Snyder *et al.* (1978) all found the reproducibility of the Oxi/Ferm Tube tests to be good in the main, although Smith *et al.* (1978) reported a poor reproducibility for the citrate test of the kit. Freundlich

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Table Identification of 242 strains by two different systems

Taxon	Strains examined	Total no. of strains	CCIS System 1976-621-74346 No. of strains:			CCIS System 1977-1432 No. of strains:		
			Correctly identified	Not identified	Incorrectly identified	Correctly identified	Not identified	Incorrectly identified
<i>Achromobacter</i> sp biotype 1	A9112, C349, C675, C7259	4	1	0	3	3	0	1
<i>Achromobacter</i> sp biotype 2	D8708, D9035, D9053	3	0	0	3	0	1	2
<i>Achromobacter xylosoxidans</i>	NCTC 10807*, NCTC 10808, NCTC 10809, CL356/75, CL434/75	5	3	1	1	4	0	1
<i>Acinetobacter calcoaceticus</i>	NCTC 7250, NCTC 7363, NCTC 7364, NCTC 7412, NCTC 7844*	5	5	0	0	5	0	0
<i>Acinetobacter lwoffii</i>	NCTC 5866*, NCTC 5867, NCTC 7976, E632, E871	5	5	0	0	5	0	0
<i>Aeromonas hydrophila</i>	NCTC 7810, NCTC 7812, NCTC 8049*, E232, E326	5	3	2	0	5	0	0
<i>Alcaligenes odorans</i>	NCTC 655, NCTC 10388, NCTC 10416, CL98/73, CL158/73	5	3	0	2	4	0	1
<i>Alcaligenes denitrificans</i>	NCTC 8582	1	0	0	1	1	0	0
<i>Alcaligenes faecalis</i>	NCTC 415*, NCTC 8764, NCIB 9387, NCIB 9986, CL675/76	5	2	2	1	4	1	0
<i>Bordetella bronchiseptica</i>	NCTC 452*, NCTC 454, NCTC 455, NCTC 456, NCTC 458	5	5	0	0	5	0	0
<i>Flavobacterium meningosepticum</i>	NCTC 10016*, NCTC 10585, NCTC 10586	3	3	0	0	3	0	0
<i>Flavobacterium</i> group IIb	NCTC 10795, NCTC 10796, NCTC 10797	3	3	0	0	3	0	0
<i>Flavobacterium</i> group IIc	NCTC 10798, NCTC 10799, NCTC 10800, CL112/67, CL172/69	5	4	0	1	1	3	1
<i>Flavobacterium</i> group IIj	B6306*, B9541	2	0	0	2	0	0	2
<i>Pseudomonas paucimobilis</i>	NCTC 11030*, NCTC 11031, NCTC 11032, CL173/70, CL135/72	5	1	4	0	5	0	0
<i>Flavobacterium</i> group IIk-2	NCTC 11033*, NCTC 11034, B9042, D8510, D8864	5	2	1	2	2	1	2
<i>Alcaligenes</i> group IVe	C9573, D2312, D7460, D8817	4	3	0	1	3	1	0
<i>Pseudomonas</i> group Va-1	B19	1	0	0	1	1	0	0
<i>Pseudomonas</i> group Va-2	A5310, A8661	2	0	0	2	1	0	1
<i>Pseudomonas pickettii</i>	230, 279, 286, 288*, 298	5	0	1	4	2	1	2
<i>Pseudomonas</i> group Ve-1	A7701*, B237, B1117, B5264	4	4	0	0	3	1	0
<i>Pseudomonas</i> group Ve-2	B993*, B2524, B5066, B5142	4	4	0	0	3	0	1
<i>Moraxella</i> group M-4	B9265, D7836, D8054, KC 1290	4	0	0	4	0	0	4
<i>Flavobacterium odoratum</i>	NCTC 11036*, CL607/74, CL103/75, CL588/75, CL164/76	5	5	0	0	0	4	1
<i>Moraxella bovis</i>	NCTC 8561*, NCTC 9425	2	2	0	0	1	1	0
<i>Moraxella lacunata</i>	NCTC 7985, NCTC 7986	2	2	0	0	2	0	0
<i>Moraxella liquefaciens</i>	NCTC 7911, NCTC 10358	2	1	0	1	1	1	0
<i>Moraxella nonliquefaciens</i>	NCTC 7784, NCTC 10464	2	2	0	0	2	0	0
<i>Moraxella osloensis</i>	NCTC 10465, CL161/70	2	2	0	0	2	0	0
<i>Moraxella phenylpyruvica</i>	NCTC 10526, CL157/67, CL113/69, CL173/69, CL456/73	5	2	0	3	1	2	2
<i>Pasteurella haemolytica</i>	NCTC 9712, NCTC 10369, NCTC 10370, NCTC 10609, NCTC 10631	5	3	0	2	4	0	1
<i>Pasteurella multocida</i>	NCTC 3195*, NCTC 5868, NCTC 8080, NCTC 8282, NCTC 10382	5	3	0	2	3	0	2
<i>Pasteurella ureae</i>	NCTC 10219, NCTC 10220, NCTC 10221, NCTC 10222, CL576/76	5	0	0	5	5	0	0
<i>Plesiomonas shigelloides</i>	NCTC 10360*, NCTC 10363, NCTC 10364, E387, CL582/77	5	4	1	0	2	3	0
<i>Pseudomonas aeruginosa</i>	NCTC 1999, NCTC 2000, NCTC 6750, NCTC 7244, NCTC 10332*	5	5	0	0	5	0	0
<i>Pseudomonas acidovorans</i>	NCTC 9991, NCTC 10683*, ATCC 17406, CIP 60.78, CIP 64.36	5	0	3	2	2	1	2
<i>Pseudomonas alcaligenes</i>	NCTC 10367*	1	1	0	0	1	0	0
<i>Pseudomonas cepacia</i>	NCTC 10661, NCTC 10734, NCTC 10743*, NCTC 10744, CL37/70	5	1	2	2	4	0	1
<i>Pseudomonas diminuta</i>	NCTC 8545*, AB236, AB328, AB359	5	4	0	1	5	0	0
<i>Pseudomonas fluorescens</i>	NCTC 3756, NCTC 10038*, NCTC 10392, CL170/68, CL174/68	5	2	0	3	2	0	3
<i>Pseudomonas mallei</i>	NCTC 3708, NCTC 3709, NCTC 10229, NCTC 10230, NCTC 10245	5	0	1	4	0	1	4
<i>Pseudomonas maltophilia</i>	NCTC 10257*, NCTC 10258, NCTC 10259, NCTC 10498, NCTC 10499	5	4	1	0	4	0	1
<i>Pseudomonas pseudoalcaligenes</i>	NCTC 8769, NCTC 10860*, ATCC 17442, ATCC 17443, NCIB 9390	5	5	0	0	5	0	0
<i>Pseudomonas pseudomallei</i>	NCTC 1688, NCTC 4845, NCTC 4846, NCTC 6700, NCTC 7383	5	3	1	1	1	1	3

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Taxon	Strains examined	Total no. of strains	CCIS System 1976-621-74346 No. of strains:			CCIS System 1977-1432 No. of strains:		
			Correctly identified	Not identified	Incorrectly identified	Correctly identified	Not identified	Incorrectly identified
<i>Pseudomonas putida</i>	NCTC 10936*, CL237/73, CL238/73, CL239/73, CL240/73	5	5	0	0	5	0	0
<i>Pseudomonas putrefaciens</i>	NCTC 10735*, NCTC 10737, NCTC 10738, NCTC 10762, NCTC 10763	5	2	3	0	2	3	0
<i>Pseudomonas stutzeri</i>	NCTC 10450, NCTC 10473, NCTC 10475*, CL142/73, CL147/73	5	3	1	1	0	0	5
<i>Pseudomonas mendocina</i>	NCTC 10897, NCTC 10898, NCTC 10899, CH34, CH35	5	0	0	5	0	0	5
<i>Pseudomonas testosteroni</i>	NCTC 10698*, ATCC 17407, NCIB 8893, NCIB 9682, NCIB 9683	5	4	0	1	4	0	1
<i>Pseudomonas vesicularis</i>	NCTC 10900*, AB 102, AB 794, AB 995, AB 1014	5	0	1	4	1	2	2
<i>Vibrio alginolyticus</i>	NCTC 10675	1	0	1	0	0	1	0
<i>Vibrio cholerae</i>	NCTC 5395, NCTC 7254, NCTC 7270, NCTC 8021, NCTC 8457	5	4	0	1	4	0	1
<i>Vibrio parahaemolyticus</i>	NCTC 10441, NCTC 10884, NCTC 10885, NCTC 10886, NCTC 10903	5	0	1	4	2	2	1
<i>Chromobacterium violaceum</i>	NCTC 8683, NCTC 8684, NCTC 8685, NCTC 9370, NCTC 9757	5	5	0	0	5	0	0
	Totals:	222	125 (56%)	27 (12%)	70 (32%)	138 (62%)	31 (14%)	53 (24%)
<i>Bordetella parapertussis</i>	NCTC 5952, NCTC 7385, NCTC 8250, NCTC 10520, NCTC 10521	5	0	0	5	0	0	5
<i>Brucella</i> spp	NCTC 10084, NCTC 10094, NCTC 10095, NCTC 10096, NCTC 10316	5	0	0	5	0	3	2
<i>Chromobacterium lividum</i>	NCTC 7150, NCTC 7917, NCTC 9796, NCTC 10646, E779	5	0	0	5	0	1	4
' <i>Pseudomonas thomasi</i> '	NCTC 10893, NCTC 10894, NCTC 10895, CL21/70, CL283/70	5	0	0	5	1†	0	4

Explanation of Table: Taxa are listed in the order in which they appear in the Reaction Chart in CCIS System 1976-621-74346. *Chromobacterium violaceum* was not initially included in this scheme but was added in a January 1977 addendum. Taxa are listed in the same order and by the same nomenclature in the Reaction Chart in CCIS System 1977-1432 except that *Alcaligenes denitrificans* and *A. odorans* are no longer considered separately but are both included within *A. faecalis*. In the above Table we have substituted more recent names for taxa where applicable: *Acinetobacter calcoaceticus* instead of *A. anitratus*, *Pseudomonas paucimobilis* instead of group IIk, type 1, and *Flavobacterium odoratum* instead of group M-4f. In both CCIS Systems *Flavobacterium meningosepticum* and group IIb are included together in a single taxon *Flavobacterium*; *Moraxella bovis*, *M. lacunata*, *M. liquefaciens*, *M. nonliquefaciens*, and *M. osloensis* are included together in a single taxon *Moraxella* species; *Pseudomonas pickettii* is included in group Va-2 and *Pseudomonas mendocina* in *P. stutzeri*. *Bordetella parapertussis*, *Brucella* spp, *Chromobacterium lividum*, and '*Pseudomonas thomasi*' (which is probably synonymous with *P. pickettii*) are not included in either CCIS System, and these taxa are therefore not included in the calculation of identification rates; they were included in order to assess their likelihood of being misidentified if examined in the Oxi/Ferm Tube.

Explanation of strain numbers: ATCC = American Type Culture Collection, Rockville, Maryland, USA; CIP = Collection de l'Institut Pasteur, Paris, France; NCIB = National Collection of Industrial Bacteria, Aberdeen, UK; NCTC = National Collection of Type Cultures, Colindale, London, UK; strains prefixed by the letters A, B, C, D, or KC are from the Center for Disease Control, Atlanta, Georgia, USA; strains prefixed by the letters CL or E are stock cultures of the NCTC Computer Identification Laboratory; *Pseudomonas diminuta* and *P. vesicularis* strains prefixed by the letters AB are from the Statens Serum Institut, Copenhagen, Denmark; *Pseudomonas mendocina* strains prefixed by the letters CH are from Hoffman-La Roche Inc, Nutley, New Jersey, USA; *Pseudomonas pickettii* strains are from the University of California, Los Angeles, California, USA.

*Strain tested in Oxi/Ferm Tube in triplicate for assessment of reproducibility.

†Identified as group Va-2.

et al. (1977) also studied test reproducibility of this kit but did not publish their results.

Material and methods

ORGANISMS

Two hundred and forty-two strains were used to assess the success of the Oxi/Ferm Tube system in identification (Table), 222 of which represented all the 45 non-fermentative and nine oxidase-positive fermentative taxa included in the two identification systems so far produced by the manufacturer, one in 1976 and one in 1977. These strains comprised 188 reference strains (142 of which were from culture

collections and 46 from reference experts) and 34 field strains referred to the National Collection of Type Cultures (NCTC) for computer-assisted identification; if type or neotype strains had been designated for a particular taxon then these were included.

The remaining 20 strains (17 culture collection and three field strains) represented four additional non-fermentative taxa not included in the two Oxi/Ferm Tube identification systems. These strains were, therefore, not used in the calculation of identification rates but were included in order to see whether they were likely to be misidentified.

Of the 242 strains, all the non-fermenters had been

previously examined in at least the 68 characteristics described by Holmes *et al.* (1975) and their identity confirmed or determined by the computer identification method of Lapage *et al.* (1973) with the use of an unpublished probability matrix devised in the NCTC for non-fermenters. All the fermenters had been previously examined for 50 characteristics and their identity confirmed or determined by the computer identification method of Lapage *et al.* (1973) and probability matrix of Bascomb *et al.* (1973).

TESTS AND METHODS

All 242 strains were examined in the Roche Oxi/Ferm Tube, which consists of a plastic tube within which are compartments of test media in agar slopes, and contains the following nine tests: acid from dextrose under anaerobic conditions; production of arginine dihydrolase, nitrogen gas, hydrogen sulphide, and indole; acid from xylose and from dextrose under aerobic conditions; production of urease; and citrate utilisation. The oxidase test, performed conventionally, is used to supplement these nine tests so that the basic identification system consists of 10 tests.

In accordance with the manufacturer's instructions, the Oxi/Ferm Tubes were each inoculated with well isolated colonies from 24-hour cultures grown on nutrient agar. The media are inoculated by withdrawing a pre-sterilised inoculating wire (which is part of and inside the kit) through the compartments. The manufacturer states that the Oxi/Ferm Tube must be inoculated with a heavy inoculum, but when the inoculating wire is drawn through the Oxi/Ferm Tube it passes initially through an aperture of a diameter equal to that of the wire. As a result, no matter how large an inoculum is placed on the end of the wire, most bacterial growth is scraped off on passage through the aperture. With some of the more fastidious organisms, in particular, the expected positive reactions often could not be obtained with the Oxi/Ferm Tube, and no method was found to increase the inoculum size sufficiently to give the expected reactions.

The Oxi/Ferm Tubes were incubated at 37°C (as recommended by the manufacturer) for most strains, but with some species which prefer lower growth temperatures (for example, *P. fluorescens* and *P. putida*) incubation at 30°C was necessary for the expected reactions to be obtained. Strains of *Chromobacterium lividum* were incubated at room temperature (18–22°C), the highest temperature at which they grow.

The reactions obtained with the Oxi/Ferm Tube are recorded by comparison with a colour chart after 48 hours' incubation, except for the urease test,

which is recorded at both 24 and 48 hours since 'rapid urea hydrolysis' is one of the additional tests that may be necessary for identification. Indole is the only test requiring the addition of a reagent, introduced by a needle and syringe through the plastic film covering the H₂S compartment. Although the manufacturer recommends that the reagent should be allowed to reach the agar surface, we found the test easier to interpret by introducing sufficient reagent to form a droplet on the underside of the plastic film that covers the test compartment. By rotating the tube from side to side, the development of a pink colour in the reagent droplet was easily discerned even when the reaction was only weakly positive.

IDENTIFICATION OF STRAINS

The reactions of the nine Oxi/Ferm Tube tests are recorded as a three-digit number based on octal numbers. The oxidase test result is used to form a fourth digit. This four-digit number is looked up in a register entitled *Computer Coding and Identification System for < Oxi/Ferm Tube > Roche* (CCIS System), of which two versions were produced during the course of this evaluation, the first 1976-621-74346 together with January 1977 addendum, and the second 1977-1432 (referred to in the following text as the 1976 and 1977 systems respectively). Most strains could not be adequately differentiated on the results of the basic 10 Oxi/Ferm Tube tests and so it was necessary to carry out the additional conventional tests suggested in the register. The results of these additional tests were often available when the register was consulted either from reading of the Oxi/Ferm Tube itself (eg, rapid urea hydrolysis, alkaline reaction in xylose medium) or from a purity plate (eg, pigment, wrinkled colonies). In some cases, final identification was delayed until the results of further tests were available (eg, anaerobic growth, growth at 42°C).

If the four-digit number derived from the reactions of a strain was listed in the identification register, together with the results of any additional tests required, an identification was considered 'correct' when the indicated taxon agreed with the identity of the strain and as 'incorrect' when they disagreed. Strains were considered 'not identified' when their four-digit number was not listed in the identification register, or when the number was listed but the results of the additional conventional tests prevented either correct identification or an incorrect identification.

For details, the reader is referred to the manufacturer's instructions, which give full details of the kit and additional conventional tests, how they are to be inoculated and incubated, and how interpreted.

REPRODUCIBILITY OF TESTS

Thirty strains (Table), each representing a different taxon, were each tested three times in Oxi/Ferm Tubes so that each test (excluding the oxidase test, which is not performed in the kit) on each of the 30 strains was carried out three times. When the results were being recorded, all three Oxi/Ferm Tubes for each strain were compared so that the differences in test results were due to actual differences in the colours of the reactions and not to differences in interpretation.

A convenient way of expressing test reproducibility is the probability of erroneous test results (Sneath and Johnson, 1972). In the present study, each test was repeated three times, and for simplicity in presentation of the results it is assumed that if the three results were consistent (+++ or ---) then all three results were correct, and if the results were inconsistent (eg, ++- or --+), the single minority result was an error. For the results of three replicate tests presented in this way an estimate of the probability of an erroneous test result, which is not biased by these assumptions, is given by C ,

$$C = \frac{1}{3} (1 - \sqrt{1 - 4E/N})$$

where E is the number of errors observed, N is the total number of test results, and C is termed the corrected error rate. This formula is obtained from formulae (4) and (14) of Sneath and Johnson (1972).

Results

IDENTIFICATION OF STRAINS

As can be seen from the Table, the 1977 system yielded a 6% improvement in identification rate over the 1976 system. The proportion of strains remaining not identified differed by only 2% between the two systems; however, use of the 1977 system decreased the rate of incorrect identification by 8%. Although the overall identification rate for the 1977 system was only 62%, the identification rate varied for individual taxa (Table) and was satisfactory for strains of *Acinetobacter calcoaceticus*, *A. lwoffii*, *Aeromonas hydrophila*, *Bordetella bronchiseptica*, *Flavobacterium meningosepticum*, *Flavobacterium* group IIb, *Pseudomonas paucimobilis* (group IIk, type 1; Holmes *et al.*, 1977a), *Pasteurella ureae*, *Pseudomonas aeruginosa*, *P. diminuta*, *P. pseudoalcaligenes*, *P. putida*, and *Chromobacterium violaceum*, which include most of the non-fermentative species most frequently isolated from routine clinical material. For some taxa, the improvement in identification rate between the two systems was dramatic; for example, all the strains of *Pasteurella ureae* were incorrectly identified

on the 1976 system but correctly identified on the 1977 system. For other taxa, the performance in identification was worse with the 1977 system; for example, all five strains of *Flavobacterium odoratum* (group M-4f; Holmes *et al.*, 1977b) identified correctly on the 1976 system but four remained not identified and one was incorrectly identified on the 1977 system.

The Table shows that the strains of taxa not included in the identification systems were more likely to be incorrectly identified than to remain not identified.

REPRODUCIBILITY OF THE TESTS

The 30 strains were examined in triplicate in nine tests, giving a total of 810 test results. In 14 of the triplicates the results were inconsistent, that is, there were 14 errors and hence the corrected error rate was 1.8% with a 95% confidence interval 0.9%-2.7%.

Discussion

Despite its name, the *Computer Coding and Identification System for < Oxi/Ferm Tube > Roche* is a register of patterns of results, and no use is made of probabilistic or any other method of computer-assisted identification, as is also the case with the Encise System originally used with the Enterotube, but unlike the more recently devised system for Enterotube and that for the API 20E, both of which are computer-assisted, a strategy that increases the identification rate (Holmes *et al.*, 1977c). Not unexpectedly, therefore, the identification rate of 62% with 24% incorrectly identified found for the Oxi/Ferm Tube using the 1977 system is similar to that obtained with the Enterotube using the Encise System (70% correctly identified, 29% incorrectly identified; Holmes *et al.*, 1977c). The low identification rate found for the Oxi/Ferm Tube is due partly to the small number of tests employed (10 basic and a maximum of nine additional in the 1977 system) and partly to the lack of computer-assisted identification. Identification rates of 85-90% have been achieved in the NCTC for non-fermentative bacteria using a probability matrix based on 68 tests and computer assistance. Completion of the matrix may improve performance and enable us to publish it.

Difficulties in evaluating and comparing both different test kits and the results obtained with kits by various authors have been discussed by Holmes *et al.* (1978). In our evaluation of the Oxi/Ferm Tube, we examined more taxa than the routine laboratory is likely to encounter, and as the strains chosen do not reflect their distribution in routine clinical

material, the identification rate we obtained cannot be applied directly to clinical practice. Our results indicate that the Oxi/Ferm Tube 1977 system might be expected to give a better performance in the clinical laboratory than the identification rate of 62% achieved in our study, as identification was better with many of the commoner taxa likely to be found in clinical material. This is borne out by the identification rates of 84% to 98% found by various authors who evaluated the kit in clinical laboratories, or in special studies using smaller numbers of the commoner taxa (not exceeding 33 and often less than 20 compared to the 54 used in our study). Smith *et al.* (1978) tested the Oxi/Ferm Tube with cultures of rarely encountered organisms and obtained identification rates of around 50%. Such evaluations must be treated with caution because to check identifications obtained from kits, the identity of the strains must be known on independent criteria, and this is not always done, or the criteria used, which must be stringent, are not stated satisfactorily. Conventional tests and schemes for the identification of non-fermenters have been developed only in recent years and are not yet widely used, at least to our knowledge in the UK, so the established identities in evaluation in clinical laboratories must be carefully inspected.

For comparison of kit identifications, the particular identification systems (manufacturer's number, date, etc) used must be known, and precise comparison can be made only between evaluations using the same system; less accurate comparisons may, however, still have some validity, for example, the 6% gain in identification of the 1977 over the 1976 system may still allow some comparison if one laboratory used the earlier and the other the later system.

Details in the publication may be inadequate to ascertain the use of the same system: for example, Oberhofer *et al.* (1977b) used for the Oxi/Ferm Tube an 'original' and then a 'revised' manual. These may or may not correspond respectively to our 1976 system (CCIS 1976-621-74346) and 1977 system (1977-1432), as Oberhofer *et al.* (1977b) stated rather ambiguously: 'Eleven strains of *Alcaligenes odorans* were correctly identified using the original code, whereas no code was provided in the revised manual'. In CCIS System 1977-1432, *A. odorans* was included in *A. faecalis*.

The differentiation of the alkali-producing pseudomonads is particularly difficult, and although in the 1976 system the register contained identification to species level, in the 1977 version these pseudomonads were grouped together with the misleading title '*Pseudomonas* species', misleading since *P. aeruginosa*, for example, was a separate taxon, and

if a strain of this species identified as '*Pseudomonas* species' then it should be considered an incorrect identification. Oberhofer *et al.* (1977b) also noted this combination of species and did not consider '*Pseudomonas* species' sufficiently precise to constitute an entirely accurate identification, which illustrates the confusion in kit comparison when one kit identifies the species and another a genus or a group of species. Oberhofer *et al.* (1977b) also appeared in their evaluation to have considered '*Pseudomonas* species' at genus level identification to include all pseudomonads not just the alkali-producers. With such pitfalls, it seems simplest to consider a strain to be correctly identified when its known identity corresponds to a taxon defined by the manufacturer as containing the species to which that strain belongs, irrespective of whether the identification is to species, genus, or some other level.

The probability of erroneous test results for the Oxi/Ferm Tube of 1.8% is near the typical range of 2%-4% for the average probability of errors for conventional biochemical tests within a single laboratory certainly to be expected with the potential central quality control of kits. Isenberg and Sampson-Scherer (1977) found the Oxi/Ferm Tube gave results with a better level of reproducibility than we found; from their results we estimated a probability of erroneous test results of 0.7% (cf our value of 1.8%). From the results of McHale *et al.* (1978) we estimate a probability of errors of 4% for the citrate and urea tests and 2.5% or less for other tests. Other reports on test reproducibility do not define how 'reproducibility' was calculated and so estimates of the probabilities of errors cannot be obtained from the results quoted. The probability of errors between laboratories for conventional tests is typically 6%-10% on average (Holmes *et al.*, 1977c). Provided that interpretation schemes for kit tests are accurate and sophisticated, it should be possible to obtain a level of reproducibility between laboratories using the same kit within the range obtained in a single laboratory using conventional tests. The Oxi/Ferm Tube tests were fairly easy to read but the colour chart provided by the manufacturer was not considered to be very realistic, and the chart could be improved to aid in the interpretation of weak or doubtful reactions.

The Oxi/Ferm Tube was developed from the Enterotube and therefore possesses the advantages and disadvantages of the latter kit; both are speedily inoculated, and even a Bunsen burner is not necessary. However, the manufacturer recommends that the indole reagent should be added to the indole test compartment by means of a syringe and needle. This could prove dangerous should the needle

come into contact with pathogens in the compartment and the needle subsequently accidentally puncture the skin of the user. If the indole compartment cannot be modified so as to obviate the use of a needle for addition of the reagent, then the manufacturer might also consider recommending that the reagent be introduced as a droplet just under the transparent film covering the compartment and reading the result there instead of allowing the reagent to reach the agar surface of the medium.

The manufacturer recommends inoculation of the Oxi/Ferm Tube with a heavy suspension yet, because of the design of the tube (see Material and methods), the inoculum cannot be increased beyond a certain amount, and may be inadequate to give the expected results with certain fastidious organisms. Possibly widening the aperture through which the inoculum is drawn might enable adequate inoculum to be used. It should be recommended that the tubes are incubated, not at 37°C for all organisms, but at the optimum growth temperature of the strain being tested, since some of the strains we tested, eg, *Pseudomonas fluorescens*, failed to give the expected reactions unless incubated at 30°C. The user should also be instructed to examine the urea compartment of the tube at 24 hours in case rapid urea hydrolysis proves to be one of the necessary additional tests. Finally, since the 20 strains of the four taxa not included in the calculation of identification rates tended to be incorrectly identified rather than remain not identified, the manufacturer might at least take account of these taxa in the compilation of future data bases, even if they are not directly included in the identification scheme.

Our results suggest that, to improve the identification rate, the number of tests may need to be increased, or the number of taxa reduced, retaining only those taxa most commonly found in clinical material, and placing emphasis on those additional tests that prevent strains belonging to taxa not included in the identification scheme from being incorrectly identified into included taxa.

Although the results were available, we have not compared for each strain the test results obtained in the Oxi/Ferm Tube with the test results obtained in the corresponding conventional tests. We consider that such a comparison has little scientific merit since, in considering different identification systems, it is not the comparability of individual test results that is important but the equivalence of the final identifications.

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