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Evaluation of Two BBL Crystal Systems for Identification of Some Clinically Important Gram-Negative Bacteria

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The BBL Crystal system (Becton Dickinson Microbiology Systems, Cockeysville, Md.) is a miniaturized bacterial identification method employing modified conventional and chromogenic substrates. Two products are currently available, the Rapid Stool/Enteric ID Kit and the Enteric/Nonfermenter ID Kit, each comprising thirty tests. We report an evaluation of both systems (using database version 1.1 for both) in the identification of 51 gram-negative taxa likely to be encountered commonly in the clinical laboratory. In all, 266 strains were tested in the Enteric/Nonfermenter ID Kit, and these represented 36 taxa of the family *Enterobacteriaceae* (188 strains), 5 oxidase-positive fermentative taxa (26 strains), and 10 nonfermentative taxa (52 strains). The majority of these same strains (203 of 266) were also tested in the Rapid Stool/Enteric ID Kit. The Enteric/Nonfermenter ID Kit performed as follows: *Enterobacteriaceae*, 93% correct, 6% not identified, and 1% incorrect; oxidase-positive fermenters, 88, 12, and 0%, respectively; and nonfermenters, 100% correct, although several only to the genus or group level. The Rapid Stool/Enteric ID Kit gave the following results: *Enterobacteriaceae*, 91% correct, 7% not identified, and 2% incorrect; oxidase-positive fermenters, 80, 13, and 7%, respectively (but results were based on only 15 strains); and nonfermenters, 100% correct (but results were based on only 11 strains). We found the systems extremely easy and rapid to use, and for the Enteric/Nonfermenter ID Kit an identification rate of 100% in 40 of 51 taxa was achieved, with corresponding figures of 29 of 39 taxa for the Rapid Stool/Enteric ID Kit.

The BBL Crystal system (Becton Dickinson Microbiology Systems, Cockeysville, Md.) has been introduced only comparatively recently. There are two products currently available: the Rapid Stool/Enteric ID Kit (RS/E kit) and the Enteric/Nonfermenter ID Kit (E/NF kit). Each is based on modified conventional and chromogenic substrates (30 total) contained within a novel plate. Each kit comprises a plastic base containing reaction wells to which, following inoculation, is clipped a lid with dehydrated substrates on the tips of plastic prongs. Results of reactions are read after 3 h for the RS/E kit and after 18 to 20 h for the E/NF kit. The test results are interpreted visually and recorded manually. The results are converted to a 10-digit profile number which is then entered into the appropriate database held on a microcomputer. The database for the RS/E kit comprises 59 taxa, and that for the E/NF kit comprises 105 taxa. Two calculations, a biotype validity and a confidence value, are made and are displayed on screen.

There do not yet appear to have been any evaluations of either system published. We therefore report here an evaluation of both the BBL RS/E and E/NF kits, using database version 1.1 for both, to test their abilities to identify 51 gram-negative taxa likely to be encountered commonly in the clinical laboratory.

MATERIALS AND METHODS

Bacterial strains. About five strains of each taxon were chosen to represent 51 gram-negative taxa (Table 1) considered to be encountered commonly in the clinical laboratory; in all, 266 strains were tested. The 51 taxa comprised 36 of members of the family *Enterobacteriaceae* (188 strains), 10 of nonfermenters (52 strains), and 5 of oxidase-positive fermenters (26 strains). When available, the type strain for each taxon was included, together with other reference strains maintained in the National Collection of Type Cultures and field strains.

Biochemical tests. All strains had been previously examined by up to 66 conventional biochemical tests; the identities of the field strains had been determined, and those of the reference strains had been confirmed, by processing the results through the appropriate probability matrices of Holmes et al. (3, 4).

Whole-cell protein electrophoresis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Whole-cell protein patterns were obtained for the strains representing the majority of taxa by the methods described by Costas (1). These patterns were compared visually for each taxon as a further confirmation of identity, to ensure within-taxon homogeneity, and as a method of detecting contaminated cultures. The protein patterns were compared prior to the testing of the strains in the BBL Crystal systems so that isolates with protein patterns atypical of the taxon could be excluded from this study. In some cases, however, this was not possible because certain taxa are known to be genomically heterogeneous, and variability in protein patterns was therefore expected.

Inoculation of BBL Crystal panels. Stock cultures were subcultured several times to increase their levels of metabolic activity. Inocula were prepared from overnight growth on blood agar plates (incorporating 5% [vol/vol] horse blood). A sterile cotton swab was used to carefully collect one well-isolated large colony or several small colonies. The growth was suspended, for both kits, in the Crystal Enteric/Stool ID Inoculum Fluid Tubes provided with the kits.

Although the instructions supplied with the E/NF kit do not include a standardization of inoculum densities, the manufacturer states that for the RS/E kit the turbidity should be at least equivalent to a McFarland 0.5 standard but should not exceed a McFarland 1.0 standard. In this study, a turbidimeter (API

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TABLE 1. Identification of gram-negative bacteria by the Crystal identification system

Taxon	No. of strains	Identification[a] by:					
		RS/E kit (3 h)			E/NF kit (18 to 20 h)		
		CI	NI	II	CI	NI	II
Enterobacteriaceae							
Citrobacter amalonaticus	6	6 [1]	0	0	6	0	0
Citrobacter diversus	5	5	0	0	5 [1]	0	0
Citrobacter freundii	5	5	0	0	5	0	0
Edwardsiella tarda	5	5	0	0	5	0	0
Enterobacter aerogenes	5	5	0	0	5 [1]	0	0
Enterobacter agglomerans	5				4 [1]	1	0
Enterobacter cloacae	6	6	0	0	6	0	0
Enterobacter sakazakii	6				6	0	0
Escherichia coli	5	5	0	0	5	0	0
Hafnia alvei	5	4	1	0	4 [1]	1	0
Klebsiella oxytoca	5	5	0	0	5	0	0
Klebsiella ozaenae	5	5 [1]	0	0	5 [2]	0	0
Klebsiella pneumoniae	7	7 [1]	0	0	6	0	1
Klebsiella rhinoscleromatis	5	4 [2]	1	0	5	0	0
Morganella morganii	5	5 [1]	0	0	5	0	0
Proteus mirabilis	5	5	0	0	5 [1]	0	0
Proteus penneri	5	5 [4]	0	0	5 [2]	0	0
Proteus vulgaris	5	5	0	0	5	0	0
Providencia alcalifaciens	6	6	0	0	6	0	0
Providencia rettgeri	5	3	2	0	5	0	0
Providencia stuartii	5	5	0	0	4	1	0
Salmonella arizonae	5	5 [3] (3)	0	0	5 [1]	0	0
Salmonella choleraesuis	5	4 (4)	1	0	4 (2)	1	0
Salmonella gallinarum	6	0	4	2 [1]	1 [1]	4	1 [1]
Salmonella paratyphi A, B, and C	5	5 [1] (3)	0	0	5 (5)	0	0
Salmonella typhi	5	5 (4)	0	0	4 (3)	1	0
Salmonella typhimurium	5	5 (5)	0	0	3 (3)	2	0
Salmonella sp.	5	4 (4)	0	1	5 (5)	0	0
Serratia liquefaciens	5	4 [3]	0	1	5 [3]	0	0
Serratia marcescens	5	5	0	0	5	0	0
Shigella boydii	5	5 [1] (5)	0	0	5 (5)	0	0
Shigella dysenteriae	6	6 [2] (5)	0	0	6 (4)	0	0
Shigella flexneri	5	5 [2] (5)	0	0	5 (5)	0	0
Shigella sonnei	5	2 [1]	3	0	5 [1]	0	0
Yersinia enterocolitica	5	5 [2]	0	0	5	0	0
Yersinia pseudotuberculosis	5	5 [1]	0	0	5	0	0
Total		161 (91%)	12 (7%)	4 (2%)	175 (93%)	11 (6%)	2 (1%)
Oxidase-positive fermenters							
Aeromonas hydrophila	5	4 [1]	0	1	4	1	0
Pasteurella haemolytica	5				5 [2]	0	0
Pasteurella multocida	6				6	0	0
Vibrio cholerae	5	3 [2]	2	0	4	1	0
Vibrio parahaemolyticus	5	5 [5]	0	0	4	1	0
Total		12 (80%)	2 (13%)	1 (7%)	23 (88%)	3 (12%)	0 (0%)
Nonfermenters							
Acinetobacter baumanii	5				5	0	0
Acinetobacter lwoffii	5				5	0	0
Alcaligenes faecalis	6				6 (6)	0	0
Flavobacterium meningosepticum	5				5	0	0
Moraxella lacunata	5				5 [1] (5)	0	0
Moraxella osloensis	5				5 (5)	0	0
Pseudomonas aeruginosa	5	5	0	0	5	0	0
Pseudomonas fluorescens	5				5 [3]	0	0
Pseudomonas putida	5				5 [5]	0	0
Xanthomonas maltophilia	6	6	0	0	6	0	0
Total		11 (100%)	0 (0%)	0 (0%)	52 (100%)	0 (0%)	0 (0%)
Grand total		184 (91%)	14 (7%)	5 (2%)	250 (94%)	14 (5%)	2 (<1%)

[a] CI, number of strains correctly identified; NI, number of strains not identified; II, number of strains incorrectly identified (see Materials and Methods). Numbers in brackets indicate identification to two or more groups, one of which is correct. Numbers in parentheses indicate identification to genus or group level.

ATB 1550), precalibrated with McFarland standards, was employed to give readings in the appropriate range for both kits. The inoculum, which was always used immediately after preparation, was poured into the reservoir of the kit base. The base was then carefully manipulated so that the inoculum ran along a convoluted channel, filling the test wells as it did so; excess inoculum was rolled back to the reservoir. The kit lid was then clipped to the base; this causes the prongs bearing the substrates to enter the inoculated reaction wells while a sponge absorbs excess inoculum remaining in the reservoir. The kits were always incubated at 35 to 37°C, even for organisms preferring lower growth temperatures. The results of the reactions were read after 3 h for the RS/E kit and after 18 to 20 h for the E/NF kit.

In addition, the indole and oxidase tests were performed separately, as directed by the manufacturer, using BBL *p*-dimethylaminocinnamaldehyde (DMACA) Indole Reagent and BBL Oxidase Reagent.

Interpretation of results. The panels were read visually with the aid of a light box supplied by the manufacturer. Results were compared with the appropriate color reaction chart supplied with the kits and recorded as + or −. The results were recorded on a pad and grouped into triplets from which a 10-digit profile number was derived (2). This profile number was then entered into a microcomputer on which the Crystal ID System Electronic Codebook had been installed. Results for the indole and oxidase tests were also entered.

Identifications with the Crystal system are reported together with a display, for information purposes, of biotype validity and confidence values (identifications are reported when set threshold levels for these values are exceeded). When a strain is not reported as definitively identified, the use of additional screens permits the user to determine additional tests with which to further the identification. In this study, we have used the term correct identification when the correct species, genus, or other taxonomic group (i) is reported, (ii) concurs with the identification produced by using conventional phenotypic tests, and, when possible, (iii) is supported by protein profile data. Incorrect identification is recorded when the identification given does not agree with that of the phenotypic and/or protein profile. When no identification is reported, this is indicated.

For both *Salmonella* and *Shigella* spp. the manufacturer recommends that the identification be confirmed serologically; if a strain of either genus was incorrectly identified as belonging a particular taxon within the appropriate genus, it was considered not identified. Although the serological examination was not carried out, it was assumed that it would have refuted the incorrect identification.

RESULTS

The numbers of strains correctly identified, incorrectly identified, and not identified with each of the two kits and for each taxon are given in Table 1. With the E/NF kit, 100% of the strains were correctly identified in 40 of 51 taxa examined; in 9 of the remaining taxa only one strain was not identified correctly, and in only 2 taxa (*Salmonella gallinarum* and *Salmonella typhimurium*) were two or more strains not identified. Among the 16 strains not correctly identified, 14 were not identified and only 2 were incorrectly identified (Table 1). With the RS/E kit, 100% of the strains were correctly identified in 29 of 39 taxa examined; in 6 of the remaining taxa only one strain was not identified correctly, while in 4 taxa two or more strains were not identified. Among the 19 strains not correctly identified, 14 were not identified and only 5 were incorrectly identified (Table 1). Of the total number of strains not correctly identified, seven were not identified in both kits, but five of these were strains of *S. gallinarum*.

In the E/NF kit, 93% of *Enterobacteriaceae* strains were correctly identified, while 88% of the oxidase-positive fermenters and 100% of the nonfermenters (although with several only to genus or group level) were correctly identified. In the RS/E kit the corresponding results were 91%, 80% (although based on only 15 strains), and 100% (although based on only 11 strains).

DISCUSSION

Probability matrices for the identification of atypical or rare gram-negative rods have been published (3, 4); one for fermenters (110 taxa and 66 conventional tests) gave a correct identification rate of 89.2%, with 10.8% not identified and none incorrectly identified, and one for nonfermenters (66 taxa and 83 conventional tests) gave corresponding identification rates of 91.5, 8.5, and 0%. A successful identification system should thus yield, for organisms which may include atypical or rare isolates, a correct identification rate of about 90% and should have a low misidentification rate. As can be seen from Table 1, both the E/NF and RS/E kits performed very satisfactorily. The correct identification rates compare favorably with those obtained in a similar previous evaluation of the API 20E system (API 20E, 88% [5]; Crystal E/NF, 94%).

In any laboratory, it is important that strains which cannot be correctly identified should remain unidentified rather than be misidentified. With the E/NF kit, the misidentification rate was <1%, while with the RS/E kit, the rate was only 2%. The misidentification rates compare favorably with those obtained in a similar previous evaluation of the API 20E system (API 20E, 2% [5], Crystal E/NF, <1%).

It should be remembered that good identification relies on sound classification. Certain taxa, such as *Pseudomonas fluorescens*, are known to be genomically heterogeneous and are therefore also likely to be phenotypically heterogeneous. For such taxa, a high rate of correct identification may not be possible whichever system is used. In the present study, three of five strains of *P. fluorescens* (E/NF kit) were not identified to the species level; this taxon was one of the likely taxa suggested, but additional tests were necessary for complete identification.

The present study included strains of *S. gallinarum*, which was the only taxon whose members were, in the main, not identified. It is likely that these strains have a distinct pattern of reactions in these kits, which is not included in the entry for *Salmonella* sp. in the present databases. Since the five strains here had a distinct pattern, this would indicate that *S. gallinarum* could be added to the present database without difficulty. The number of tests in the system (30) is larger than those in several other comparable systems, and, in theory, these tests should permit discrimination among additional taxa. The E/NF kit includes a number of nonfermenters, and although the identification rate was high, many strains can be identified only as being in the broad group "miscellaneous gram-negative bacilli" and are then referred to further conventional tests for species identification. A separate kit, with more relevant tests, might usefully be developed for these organisms.

The Crystal system is very convenient to use and is supplied complete with all consumables required except for cotton swabs. Samples are easily prepared, and only a small volume of test material is required. The use of a turbidimeter permits standardization of inoculum density, while the innovative design of the panel ensures standardization of inoculum volume delivered to each test well. The design of the panel also allows for a rapid inoculation of all wells, none of which

require oil overlay or addition of reagents following incubation. It is important that all kits are always incubated at 35 to 37°C, even for organisms preferring lower growth temperatures, since the database entries for such organisms were created from data obtained at the higher temperature range. We tested some strains of *P. fluorescens* at 30°C, but they could not be identified (data not shown).

The Crystal system presented no obvious safety hazards, requiring only a small volume of inoculum (2.2 ml). However, since the sample is vortexed (in a sealed tube) in order to prepare a uniform suspension, care is necessary to avoid spillage and the creation of aerosols. Following inoculation (which does not require the use of a pipette), the system is clipped together and thereby sealed, making it safe even when mishandled.

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