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Evaluation of a New Identification System, Crystal Enteric/Non-Fermenter, for Gram-Negative Bacilli

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A total of 505 fermentative and 201 nonfermentative gram-negative bacilli, identified by conventional methods, were tested by the Crystal Enteric/Non-Fermenter ID kit and by the API 20E or API 20NE identification system. The overall correct results for fermenters were 92.9% by the Crystal kit and 89.1% by the API 20E system. The false identifications (genus and species incorrect) accounted for 3.1 and 7.1% for the Crystal and API systems, respectively. For nonfermenters, figures for correct identifications by the two systems were comparable (Crystal, 75.9%; API 20NE, 75.3%) while the API 20NE system gave twice as many incorrect results (13.8%) as Crystal (6.3%); however, Crystal failed to precisely identify several species included in a "miscellaneous" group. The Crystal Enteric/Non-Fermenter system is an easy-to-use kit which compares favorably with other commercial systems.

Several automated and nonautomated systems have been developed and are commercially available for the identification of gram-negative rods (1, 6). Some of them are restricted to one single family or taxonomic group, most often members of the family *Enterobacteriaceae*, while others identify a broad range of gram-negative bacteria. The Crystal Enteric/Non-Fermenter ID kit (Crystal E/NF) (Becton Dickinson, Cockeysville, Md.) is a new nonautomated identification system based on 30 biochemical tests, assigned to the identification of members of the families *Enterobacteriaceae* and *Vibrionaceae*, nonfermenting gram-negative bacilli, and some members of the family *Pasteurellaceae.*

The aim of this study was to evaluate the accuracy of Crystal E/NF for the identification of different groups of gram-negative rods in comparison with a currently used nonautomated commercial system, API 20E and API 20NE (bio-Mérieux, Marcy l'Etoile, France), and with conventional methods.

MATERIALS AND METHODS

Isolates. In a multicenter study, a total of 706 clinical isolates of fermentative and nonfermentative gram-negative rods were tested (262 from Brussels and 228 from Leuven, Belgium, and 216 from Leiden, The Netherlands). Definitive identification of all cultures was done by conventional tests (2, 3, 5, 7, 8).

The majority of organisms were fresh clinical isolates. About 22% of the isolates, especially within the nonfermenter group, were stock strains, collected previously from clinical samples. The fresh clinical isolates were first identified by conventional methods and then, either immediately or after 1 to 16 weeks of storage on nutrient agar, subcultured on blood agar or MacConkey agar for simultaneous identification by the two commercial systems. Stock strains were likewise first subcultured on blood agar before being tested.

The 505 clinical isolates of fermentative bacteria, including 480 members of the family *Enterobacteriaceae*, 17 members of the family *Vibrionaceae*, and 8 *Pasteurella* spp., were identified in parallel with the API 20E and the Crystal E/NF systems. The 201 isolates of nonfermentative organisms were identified in parallel with the API 20NE and the Crystal E/NF systems. Some of these nonfermenters were identified by the Crystal system only as "miscellaneous." Of the 201 nonfermenters used in this study, 27 belonged to this category.

Identification systems. The Crystal E/NF panel is a new miniaturized system containing 30 dried biochemical and enzymatic substrates divided over three rows of 10 wells. The 30 enzymatic and biochemical substrates are arabinose, mannose, sucrose, melibiose, rhamnose, sorbitol, mannitol, adonitol, galactose, inositol, *p*-nitrophenyl-phosphate (p-n-p-phosphate), p-n-p-α-β-glucoside, ONPG (*o*-nitrophenyl-β-D-galactopyranoside), proline-*p*-nitroanilide, p-n-p-bis-phosphate, p-n-p-xyloside, p-n-p-α-arabinoside, p-n-p-phosphorylcholine, p-n-p-β-glucuronide, p-n-p-*N*-acetyl glucosaminide, γ-L-glutamyl *p*-nitroanilide, esculin, *p*-nitro-DL-phenylalanine, urea, glycine, citrate, malonate, tetrazolium, arginine, and lysine. The utilization and degradation of specific substrates are detected by various indicator systems: pH indicators and chromogenic reactions. The inoculum is prepared in a tube with an inoculum fluid provided in the kit by picking one well-isolated large colony from a blood agar or MacConkey agar plate with the tip of a sterile cotton swab. This bacterial suspension is transferred into the target area of the Crystal base, and all 30 wells are inoculated with one smooth, swirling movement. Afterwards, a lid with the 30 substrates is aligned with the base and snapped in place, which results in the rehydration of the dried substrates and initiation of the test reactions. The panels are incubated at 35°C for 18 h. Positive and negative results are read by using the Crystal light box according to the color chart. Each test result that is positive is given a value of 4, 2, or 1 corresponding to the row where the test is located. A value of 0 is given to any negative result. The numbers resulting from each positive reaction in each column are then added together so that a 10-digit profile number can be generated for the 30 tests. In addition, oxidase and indole tests are performed separately by conventional or spot tests. The profile number and the indole and oxidase test results are then entered on a personal computer in which the Crystal ID System Electronic Codebook has been installed.

The API 20E and API 20NE kits have been extensively described. The API strips were inoculated with suspensions prepared from nonselective media in 5 ml of 0.85% sterile saline approximating a 0.5 McFarland standard and incubated for 24 h (API 20E) and for 24 or 48 h (API 20NE). The addition of reagents and the interpretation of reactions were done according to the manufacturer's directions. The 20 biochemical reactions on the test strip were converted into an octal profile number and decoded by using the Analytical Profile Index (APILAB version 3.2.2). The API 20E has the following 11 tests in common with the Crystal system: ONPG, lysine, arginine, urease, phenylalanine, fermentation of glucose, mannitol, sorbitol, rhamnose, sucrose, and melibiose. The API 20NE strip has the following substrates in common with the Crystal E/NF panel: arginine, esculin, citrate, and urease. Arabinose, glucose, mannitol, and mannose are tested as an assimilation test (turbidity) in the API 20NE system and as an acidification test in the Crystal system.

Identification by both commercial systems was performed by one technologist in each laboratory.

Interpretation. For both systems, all first-choice identification results were accepted, even with a low degree of discrimination (less than 90% probability). The term "correct" used to describe an answer means that the identification was correct to the genus and species level. "Correct to genus only" means that the identification was correct to the genus level but incorrect to the species level. "Incorrect" indicates an incorrect genus or an unacceptable profile number. For the purposes of this study, all genus-level-only identifications as *Salmonella* species, *Shigella* species, or *Yersinia* species (except *Yersinia pseudotuberculosis*) were taken as correct. For the miscellaneous group of gram-negative bacilli, this

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TABLE 1. Comparison of Crystal E/NF and API 20E for identification of fermenting gram-negative rods

Family and species	No. of strains	No. of strains with identification:					
		Correct		Genus correct		Incorrect	
		API	Crystal	API	Crystal	API	Crystal
Enterobacteriaceae							
Escherichia coli	96	89	93	1		6	3
Shigella spp.	25	21	24			4	1
Klebsiella pneumoniae	39	37	33		3	2	3
Klebsiella oxytoca	23	19	21		1	4	1
Klebsiella ozaenae	3	3	2				1
Enterobacter cloacae	46	38	43	7	3	1	
Enterobacter aerogenes	10	9	7			1	3
Enterobacter agglomerans	2	1	1			1	1
Hafnia alvei	3	3	3				
Salmonella spp. (non-*typhi*)	32	31	32			1	
Citrobacter freundii	15	13	15			2	
Citrobacter diversus or *Citrobacter amalonaticus*	8	6	6		2	2	
Serratia marcescens	28	26	26	2	1		1
Serratia liquefaciens	2	2	1		1		
Serratia plymuthica	1	1			1		
Proteus mirabilis	49	47	44		4	2	1
Proteus vulgaris	20	19	19		1	1	
Proteus penneri	3	3	3				
Morganella morganii	27	26	27			1	
Providencia stuartii	10	7	10	3			
Providencia rettgeri	4	4	4				
Ewingella americana	1	1	1				
Cedecea lapagei	1	1	1				
Kluyvera spp.	3	3	3				
Rhanella aquatilis	2	2	2				
Enterobacter taylorae	1	1	1				
Enterobacter asburiae	1		1			1	
Moellerella wisconsensis	1	1	1				
Edwardsiella tarda	3	3	3				
Yersinia pseudotuberculosis	3	1	2			2	1
Yersinia enterocolitica group	18	16	18			2	
Vibrionaceae							
Aeromonas hydrophila or *Aeromonas caviae*	5	4	4	1	1		
Aeromonas sobria	2	2	2				
Vibrio fluvialis	1		1			1	
Vibrio mimicus	1		1	1			
Vibrio parahaemolyticus	1	1	1				
Vibrio alginolyticus	1		1	1			
Vibrio cholerae	6	3	6	1		2	
Pasteurellaceae							
Pasteurella multocida	7	6	6	1	1		
Pasteurella stomatis	1			1	1		
Total (%)	505	450 (89.1)	469 (92.9)	19 (3.8)	20 (4)	36 (7.1)	16 (3.1)

answer was accepted for the Crystal system while for the API 20NE system an answer to the genus or species level was taken into account.

Results were compared by chi-square analysis, and *P* values were calculated to test the significant differences between results obtained by the two systems.

RESULTS

Since fermenting and nonfermenting bacteria were identified by distinct API systems, results for the API 20E and API 20NE kits in comparison with those obtained with Crystal E/NF are given separately in Table 1 and Table 2, respectively. Moreover, a number of species belonging to the nonfermenting group are identified by Crystal E/NF as miscellaneous; therefore, their results are presented separately in Table 3, since neither an accurate identification nor a comparison with results obtained with other systems could be done. For the global evaluation, this miscellaneous group, comprising 27 strains, was discarded from the total of 706 strains examined.

Overall results for the 679 strains that could be compared were as follows. A total of 581 strains were correctly identified by the API system (85.6%), and 601 were correctly identified by Crystal E/NF (88.5%). The figures for correct identifications at the genus level were 38 strains (5.6%) and 51 strains (7.5%), respectively, and the figures for misidentifications (genus incorrect) were 60 strains (8.8%) and 27 strains (4%), respectively, for the API and Crystal systems. This difference is statistically significant ($\chi^2 = 14.7$, $P = 0.01$). In particular, the

TABLE 2. Comparison of Crystal E/NF and API 20NE for identification of nonfermenting gram-negative rods

Organism	No. of strains	No. of strains with identification:					
		Correct		Genus correct		Incorrect	
		API	Crystal	API	Crystal	API	Crystal
Acinetobacter lwoffii	6	2	6	2		2	
Acinetobacter baumanii	16	14	16	1		1	
Acinetobacter haemolyticus	4	4			4		
Acinetobacter junii	7	1		5	7	1	
Acinetobacter johnsonii	3	1		1	3	1	
Flavobacterium odoratum	7	4	6		1	3	
Pseudomonas diminuta	5		4		1	5	
Flavobacterium meningosepticum	7	4	7	2		1	
Flavobacterium indologenes	5	3	3	1	1	1	1
Flavobacterium breve	1		1			1	
Agrobacterium radiobacter	6	6	5				1
Pseudomonas aeruginosa	45	41	36		5	4	4
Pseudomonas putida	11	6	7	3	4	2	
Pseudomonas fluorescens	13	10	9	3	2		2
Pseudomonas stutzeri	8	7	7	1	1		
Sphingobacterium multivorum	2	2	2				
Sphingomonas paucimobilis	1	1	1				
Flavimonas oryzihabitans	2	1	1			1	1
Chryseomonas luteola	3	3	2				1
Xanthomonas maltophilia	17	16	16			1	1
Pseudomonas cepacia	3	3	1		2		
Pseudomonas vesicularis	2	2	2				
Total (%)	174	131 (75.3)	132 (75.9)	19 (10.9)	31 (17.8)	24 (13.8)	11 (6.3)

number of misidentifications was more than twice as high for the API system as it was for Crystal E/NF. There was no difference between the two systems when fermenting and nonfermenting bacteria were considered separately, but the reliability of both systems was much lower for identification of nonfermenters. Indeed, excluding the miscellaneous group, they identified only 75% of the nonfermenters correctly.

Misidentifications were generally scattered over many genera and species, but in several cases the small number of strains tested did not allow assessment of reliability for the identification of a given species. However, for the nonfermenter group, the identification score by the Crystal system for *Pseudomonas aeruginosa* (36 of 45 strains) was particularly low, as were those by the API 20NE system for *Pseudomonas diminuta* (0 of 5) and some *Flavobacterium* species (7 of 13).

One strain, clearly identified by conventional methods as *Pasteurella multocida*, was misidentified by both commercial systems as *Pasteurella haemolytica.*

Nine strains were not identified satisfactorily by either commercial system, namely, one strain of each of the following species: *Escherichia coli*, *Enterobacter cloacae*, a *Shigella* sp., *Proteus mirabilis*, *Y. pseudotuberculosis*, *P. diminuta*, *Pseudomonas stutzeri*, *Flavimonas oryzihabitans*, and *Flavobacterium indologenes.*

Low discrimination scores occurred for 87 strains with the Crystal system with 55 (63.2%) accurate results and for 143 strains with the API system with 96 (67.1%) accurate results.

DISCUSSION

In a recent evaluation of the Crystal E/NF system, 93% of 266 members of the family *Enterobacteriaceae* and 88% of 26

TABLE 3. Identification by Crystal E/NF and API 20NE of nonfermentative gram-negative rods belonging to the miscellaneous group

Organism	No. of strains	No. of strains with identification:					
		Correct		Genus correct		Incorrect	
		API	Crystal	API	Crystal	API	Crystal
Alcaligenes xylosoxidans subsp. *denitrificans*	5	2	5	3			
Alcaligenes xylosoxidans subsp. *xylosoxidans*	6	6	4				2
Alcaligenes faecalis	3	3	3				
Pseudomonas pseudoalcaligenes	2		2			2	
Comamonas testosteroni	1	1	1				
Comamonas terrigena	2		2	2			
Comamonas acidovorans	2	2	2				
Pseudomonas pickettii	2	1	2			1	
Ochrobactrum anthropi	4	4	4				
Total (%)	27	19 (70.4)[a]	25 (92.6)[a]	5 (18.5)		3 (11.1)	2 (7.4)

[a] Identification to the genus and species level by API 20NE, identification as miscellaneous group by Crystal E/NF.

oxidase-positive fermentative gram-negative rods were correctly identified (4). These figures are in agreement with our overall results of 92.9% correct identifications among 505 fermentative isolates. For nonfermenters, Holmes et al. (4) reported 100% correct identifications in comparison with the 75% obtained in our study, excluding the miscellaneous group. This difference may be due to the smaller number of isolates, belonging to only 10 taxa, investigated in the former study. Furthermore, only five *P. aeruginosa* strains have been tested.

Our study was slightly biased by the fact that species identification by conventional methods was already known. Consequently, the choice to use API 20E for members of the family *Enterobacteriaceae* and API 20NE for nonfermenters was easy. In laboratory practice, it is not always possible to distinguish a fermenter from a nonfermenter unless previous screening tests have been done.

Overall performance of Crystal E/NF is good for fermentative bacteria, but identification levels for some genera lack precision. This is particularly true for the *Yersinia enterocolitica* group, in which a number of related species may have different significances in clinical and food bacteriology. Although even within the species *Y. enterocolitica*, biotype 1^A has no clinical significance, the first step in identification for the *Yersinia* group should nevertheless be to differentiate between pathogenic and environmental species. In comparison, the API 20E system allows the identification of some related species, but two of four such strains failed to be correctly identified in our study. Furthermore, *Yersinia bercovieri*, which is rather common; *Yersinia mollaretii*; and *Yersinia rohdei* are not included in the API database.

Within the *Shigella* group, *Shigella dysenteriae* was identified to the species level only by the Crystal system.

Some lack of accuracy with the API system is found insofar as it gathers two species under one single code: *Citrobacter diversus* and *Citrobacter amalonaticus* on the one hand and *Aeromonas hydrophila* and *Aeromonas caviae* on the other hand.

Sixteen of 17 strains of the family *Vibrionaceae* were correctly identified by Crystal versus 10 correctly identified by API. Of particular concern was the fact that half of the *Vibrio cholerae* strains were misidentified by API.

It has generally been stated that identification of nonfermenters by commercial systems is usually less reliable than that of other gram-negative bacteria. In this respect, both systems were able to identify approximately the same proportion of those species included in their databases. A major drawback of the Crystal E/NF system is its failure to correctly identify 20% of strains of *P. aeruginosa*, which is by far the most clinically relevant nonfermenter. However, more than half of the isolates that are misidentified can easily be recognized by colony morphology, pigment, and odor.

Only two *Acinetobacter* species are recognized by Crystal E/NF: *Acinetobacter baumanii*, including all glucose oxidizers, and *Acinetobacter lwoffii*, including the non-glucose oxidizers. This leads to many misidentifications at the species level, particularly for the non-glucose oxidizers, since *Acinetobacter junii*, *Acinetobacter johnsonii*, and nonsaccharolytic strains of *Acinetobacter haemolyticus* together represent a considerably larger number of isolates than does *A. lwoffii*. The API system recognizes the six named *Acinetobacter* species. However, only 22 of the 36 strains tested (61%) were correctly identified at the species level and for 5 (13.8%) the genus *Acinetobacter* was not recognized at all, whereas Crystal E/NF correctly identified all 36 strains at the genus level. Considering the present database of the Crystal E/NF system, it would probably be more advisable to limit the identification of *Acinetobacter* strains to the genus level, allowing the user to perform additional tests for species determination when necessary.

Crystal E/NF includes several nonfermenters in a miscellaneous group. Species belonging to this group are usually correctly identified as miscellaneous, but failure to accurately identify important and relatively common nosocomial pathogens such as *Alcaligenes xylosoxidans* may be a serious drawback in an identification system for nonfermenters. One wonders why the Crystal system does not attempt to identify, at least at the genus level, some medically relevant isolates such as *Alcaligenes*, *Comamonas*, and *Ochrobactrum* spp. In this respect, four strains belonging to the genus *Comamonas* displayed the same or very similar profile numbers: 1000100100 for three strains and 1000100110 for another one. These numbers were not found for any other strains and may support the possibility of identifying those strains at the genus level. The identification score achieved by the API 20NE system for species included in the Crystal E/NF miscellaneous group is neither worse nor better than the score for the other nonfermenters. However, the overall percentage of incorrect identifications for nonfermenters was substantially higher for API (13.8%) than it was for Crystal E/NF (6.3%).

An advantage of the Crystal system over the API system is that it includes in one panel both fermenters and nonfermenters, whereas for API the decision to use API 20E or API 20NE depends on results of preliminary tests like glucose fermentation and oxidase reproduction. In addition, in some instances the API 20NE system requires a reading at 48 h, when a code at 24 h indicates "not acceptable before 48 h." Sometimes the answer was correct at 24 h but changed and was incorrect after 48 h.

The Crystal E/NF system is easy to handle, requiring a one-step inoculation and addition of neither paraffin oil before incubation nor reagents before reading. The inoculated panels are tightly closed and safe to handle. The time needed to perform the full procedure for the API and Crystal systems was measured in two laboratories, and mean times of 3.7 and 3.5 min for Crystal E/NF and 4.8 and 5.0 min for the API system were obtained. A drawback might be the larger amount of waste produced by the Crystal system.

In conclusion, Crystal E/NF is a valuable single identification system for fermenting and nonfermenting gram-negative bacilli and compares favorably with other commercial sets like API 20E and API 20NE. Its main advantage may be the safe and very simple handling procedure.

As for other systems, identification of nonfermenters is less reliable, and some relevant species should be included in the database.

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