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Comparison of Crystal Enteric/Nonfermenter System, API 20E System, and Vitek Automicrobic System for Identification of Gram-Negative Bacilli

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A comparative evaluation of the Crystal Enteric/Nonfermenter system (Crystal; Becton Dickinson, Cockeysville, Md.), API 20E (API; bioMérieux Vitek, Inc., Hazelwood, Mo.), and the Vitek GNI card (Vitek; bioMérieux Vitek) was performed with 512 clinical isolates of gram-negative bacilli, including 381 members of the family *Enterobacteriaceae* and 131 nonenteric bacilli. With supplemental testing, API, Crystal, and Vitek correctly identified to the genus and species level 505 (98.6%), 489 (95.5%), and 494 (96.5%) of the 512 isolates, respectively. Supplemental testing, as specified by the manufacturer, was required to identify 119 (23.2%), 18 (3.5%), and 5 (1.0%) of the isolates with the three systems, respectively. Of the 381 isolates from the family *Enterobacteriaceae*, API and Crystal correctly identified 90.3 and 91.6% by 18 to 24 h without supplemental testing, respectively, and Vitek identified 92.4 and 96.1% following 10 and 18 h of incubation, respectively. Of the 131 nonenteric organisms, API and Crystal correctly identified 28.2 and 93.9% by 18 to 24 h without supplemental testing, respectively, and Vitek identified 84.0% by 10 h and 93.9% by 18 h. Errors in identification with each system were infrequent and appeared to be randomly distributed among the genera evaluated. The three systems were comparable in accuracy when either a weighted clinical laboratory profile of organisms or a group of selected isolates in a stress test sample was evaluated ($P > 0.05$). There were no significant differences between the three systems in their ability to identify either the isolates in the weighted group or those in the stress test ($P > 0.05$). Significantly more isolates required supplemental testing with API than with Crystal or Vitek ($P < 0.05$). Crystal compared favorably with API and Vitek, which have established track records in clinical laboratories, and is acceptable for the identification of members of the *Enterobacteriaceae* and nonenteric bacilli in a clinical microbiology laboratory.

The clinical microbiology laboratory is an important contributor to the diagnosis of gram-negative bacterial infections in both ambulatory and hospital settings. The identification of gram-negative bacilli represents a significant supply and labor expense for many clinical microbiology laboratories. Therefore, it is essential that laboratories perform accurate and cost-effective identification of clinical isolates of gram-negative bacilli.

There are a number of conventional or commercially available systems to identify gram-negative bacilli. Except for reference testing, conventional macrotube biochemical tests for bacterial identification (4) have been virtually replaced by commercial systems, because the classical methods are too expensive, slow, and unwieldy for routine use in the clinical microbiology laboratory. Commercial systems range from visual interpretation of miniaturized biochemical panels with computerized taxonomic databases (1, 7, 12, 16, 21) to semiautomated or automated systems that can interpret and analyze results in a matter of hours (2, 3, 6, 11, 14, 15, 17, 20). The Crystal Enteric/Nonfermenter (E/NF) identification system (Becton Dickinson, Inc., Cockeysville, Md.) is a recently developed, miniaturized, 18-h, manual method that contains modified conventional and nonconventional biochemical and enzymatic tests (8, 10, 18). The system is designed to identify members of the family *Enterobacteriaceae* and also common isolates of clinically significant, glucose-nonfermenting gram-negative bacilli. This report presents the results of a comparative evaluation of the Crystal E/NF system, API 20E (bioMérieux Vitek, Inc., Hazelwood, Mo.), and the Vitek AutoMicrobic System (Vitek AMS; bioMérieux Vitek) for the identification of gram-negative bacilli using both a stress test and a weighted laboratory profile (9). In addition, in view of the increasing importance of labor costs, the results of timing studies of the three identification systems are summarized.

MATERIALS AND METHODS

Test organisms. A total of 512 gram-negative bacillus isolates, including 381 from the family *Enterobacteriaceae* and 131 nonenteric organisms, were tested. These organisms included 439 recent isolates from clinical specimens processed in the clinical microbiology laboratory at Hartford Hospital and 73 stock culture isolates from Hermann Hospital, Houston, Tex. The identification systems were evaluated by both a weighted laboratory profile and a stress test. The weighted profile included 412 recent clinical isolates from Hartford Hospital that reflected both the types and percentages of organisms isolated from clinical material during a 50-day time period. These 412 isolates included 19 *Acinetobacter* strains, 21 *Citrobacter* strains, 64 *Enterobacter* strains, 107 strains of *Escherichia coli*, 68 *Klebsiella* strains, 5 strains of *Morganella morganii*, 18 *Proteus* strains, 83 *Pseudomonas* strains, 3 *Salmonella* strains, 12 strains of *Serratia marcescens*, 9 strains of *Xanthomonas maltophilia*, and 1 strain each of *Flavobacterium meningosepticum*, *Providencia stuartii*, and *Vibrio parahaemolyticus*. The stress test, using many species not routinely isolated in most laboratories, included a subset of 173 recent clinical isolates from Hartford Hospital used in the weighted profile, 27 additional recent Hartford Hospital isolates, and 73 isolates from a stock culture collection accumulated from the Hermann Hospital clinical microbiology laboratory for a total of 273 test organisms. These 273 isolates included 17 *Acinetobacter* strains, 26 *Citrobacter* strains, 38 *Enterobacter* strains, 32 strains of *E. coli*, 26 *Klebsiella* strains, 15 strains of *M. morganii*, 20 *Proteus* strains, 16 *Providencia* strains, 28 *Pseudomonas* strains, 4 *Salmonella* strains, 23 strains of *S. marcescens*, 19 strains of *X. maltophilia*, 2 strains each of *Aeromonas hydrophilia* and *Hafnia*

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alvei, and 1 strain each of *Alcaligenes xylosoxidans*, *Bordetella bronchiseptica*, *Edwardsiella tarda*, *F. meningosepticum*, and *V. parahaemolyticus*.

Isolate preparation. All organisms were subcultured onto Trypticase soy agar plus 5% sheep blood (TSA II; Becton Dickinson Microbiology Systems) and MacConkey agar (MacConkey I; Becton Dickinson Microbiology Systems), incubated overnight at 35°C in an ambient atmosphere, inspected for purity, subcultured a second time onto blood agar, and incubated overnight at 35°C in an ambient atmosphere. Colonies with identical morphologies were then used to inoculate the various identification systems.

Isolate identification by API 20E. The API 20E strips (bioMérieux Vitek) were inoculated, incubated at 35°C for 18 to 48 h, and interpreted as recommended by the manufacturer. The API 20E system also requires the oxidase test result for organism identification. An acceptable identification required a biocode designation of excellent, very good, or acceptable as indicated in the current (1985 edition) API 20E Analytical Profile Index. A designation of good likelihood and low selectivity was acceptable if confirmed by the additional tests required by the manufacturer. The additional tests required to confirm identification to the genus or species level during the course of the study included adonitol, glucose, maltose, raffinose, trehalose, xylose, acetamide, cetrimide, DNase, esculin, flagellar stain, gelatin, growth at 42°C, Jordan's tartrate, lecithinase, malonate, methyl red, motility at 35°C, mucate, nitrate, polymyxin B, potassium cyanide, and triple sugar iron agar. A maximum of four additional biochemical tests was required to completely identify an isolate. Profile numbers that were not available in the index were entered into the API telephone identification system.

Isolate identification by Vitek AMS. The most current Vitek AMS gram-negative identification card was inoculated, incubated at 35°C in the reader-incubator module for 18 h, and automatically read hourly by the optical scanner according to manufacturer recommendations by using software version R06.5. The Vitek system also requires the oxidase test result for organism identification. Final results were available in 4 to 18 h. An acceptable identification required a likelihood equal to or greater than 90% as determined by the manufacturer's computer database. The additional tests needed to confirm organism identification to the genus or species level during the study included glucose, growth at 42°C, growth on MacConkey agar, nitrate, and triple sugar iron agar for gas production. A maximum of two additional tests was required to completely identify an isolate.

Isolate identification by Crystal E/NF. The Crystal E/NF panel (Becton Dickinson) contains the following 30 dried biochemical and enzymatic substrates: arabinose, mannose, sucrose, melibiose, rhamnose, sorbitol, mannitol, adonitol, galactose, inositol, *p*-nitrophenyl phosphate, *p*-nitrophenyl α-β-glucoside, *p*-nitrophenyl galactoside, proline nitroanalide, *p*-nitrophenyl bis-phosphate, *p*-nitrophenyl xyloside, *p*-nitrophenyl α-arabinoside, *p*-nitrophenyl phosphorylcholine, *p*-nitrophenyl-β-glucuronide, *p*-nitrophenyl-*N*-acetyl glucosaminide, gamma-L-glutamyl *p*-nitroanilide, esculin, *p*-nitro-DL-phenylalanine, urea, glycine, citrate, malonate, tetrazolium, arginine, and lysine. The Crystal system also requires oxidase and indole test results for organism identification. Crystal E/NF panels were rehydrated with one colony of organism suspended in the Crystal inoculum fluid by pouring all of the inoculum fluid into the target area of the panel and manually rotating the panel to fill all 30 wells according to manufacturer specifications. The lids, containing the 30 dehydrated substrates on the tips of plastic prongs, were then snapped into place, and the inoculated panels were incubated at 35°C for 18 h in an ambient atmosphere with 40 to 60% humidity. The colorimetric substrates require no additional reagents following incubation. Biochemical reactions were visually interpreted and manually converted into a 10-digit profile number that is the basis for identification of the organism, in conjunction with the oxidase and indole test results, by using the Crystal Electronic Codebook. The software provides access to interpretation of all profile numbers without telephone calls and includes a differentiation database to help identify organisms that cannot be differentiated on the basis of the profile and indole and oxidase tests. An acceptable identification required a likelihood equal to or greater than 90% as determined by the manufacturer's computer database. The additional tests needed to confirm organism identification to the genus or species level during the study included cellobiose, DNase, gelatin, growth at 42°C, motility at 35°C, nitrate, ornithine, triple sugar iron agar for H_2S production, and xylose. A maximum of two additional tests was required to completely identify an isolate.

Quality control. Quality control of each system was performed as recommended by the manufacturer. Each lot of API 20E strips was tested with *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumoniae* ATCC 13883, *Proteus vulgaris* ATCC 13315, and *Pseudomonas aeruginosa* ATCC 10145. The Vitek AMS gram-negative identification panels were quality controlled with the following organisms: *Acinetobacter baumannii* ATCC 19606, *B. bronchiseptica* ATCC 10580, *K. pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *P. aeruginosa* ATCC 27853, *Serratia odorifera* ATCC 33077, and *Shigella sonnei* ATCC 25931. The Crystal E/NF panel quality control consisted of testing with *K. pneumoniae* ATCC 33495.

Timing studies. Timing studies were performed each day of testing for each batch of isolates with each system. The studies were structured to measure only those activities integral to test performance and therefore included the test procedure from the time of inoculation to identification of the isolate. Time measurement was divided into three parts: inoculation of the panel, incubation, and identification determination. All materials for each system were assembled prior to starting the clock. Inoculation was timed from initiation of the panel inoculation procedure until the test panel was completely inoculated and ready for incubation. Incubation time was defined as the length of the incubation period before the identification of an isolate was available. For the interpretation and identification determination, the clock was started at the time that the panels were removed from the incubator and stopped when the last organism identification in the test batch was obtained. Supplemental test performance specified by each manufacturer was timed as needed to complete identifications.

Cost analysis. A cost analysis was performed for each of the three systems and included costs associated with quality control, supplemented testing, service contracts, and shipping.

Data analyses. A test isolate was considered to be correctly identified if all three systems yielded the same identification, with or without additional tests as specified by a particular system. Any strain that gave a discordant identification from that given by the other systems was retested on the system in question. If following retesting the discrepant result was corrected, the system identification was repeated once more, and the identification by the specific system was based on the best two of three responses.

Final organism identification by each system was differentiated on the basis of whether additional tests were required as specified by the system. An additional test was defined as any test that was not a specified part of each system. The identification results for the three systems were classified as (i) correct at the species level, in which an acceptable identification at the species level was obtained and was correct; (ii) correct at the genus level, in which an acceptable, correct identification was achieved only at the genus level; (iii) no identification, in which an acceptable level of identification was not achieved; or (iv) misidentification, in which an acceptable, incorrect identification was obtained.

Results from the stress test and weighted laboratory profile for each identification system were compared by chi-square testing, and P values were calculated to test the significance of the difference between the two sets of organisms for each system and the significance of the difference between the identification systems. The calculations were repeated following stratification of the isolates into two groups consisting of *Enterobacteriaceae* and non-*Enterobacteriaceae* isolates.

RESULTS

A total of 512 isolates, including 381 from the family *Enterobacteriaceae* and 131 nonenteric bacilli, were evaluated in each of the three systems (Table 1). Each system correctly identified at least 362 (95.0%) of the 381 isolates from the family *Enterobacteriaceae* to the species level and at least 127 (97.0%) of the 131 isolates of nonenteric bacilli to the species level. Eight (72.7%) of the 11 isolates that were correct only at the genus level with the Crystal system were *E. cloacae* isolates that the database identified as either *E. cloacae* or *Enterobacter sakazakii* without offering a means to differentiate between the two organisms. Five (71.4%) of the seven isolates correct only at the genus level with the Vitek system were *E. cloacae* isolates that the database reported as either *E. cloacae* or another *Enterobacter* sp. without specification of additional tests to differentiate between the species. System misidentifications at either the genus or species level are summarized in Table 2.

When results were compared after the completion of additional testing, there were significant differences between the Crystal and Vitek systems and API 20E ($P < 0.05$), with the API system correctly identifying to the species level significantly more isolates from the family *Enterobacteriaceae* than either the Crystal or the Vitek system. No significant differences ($P > 0.05$) were noted among the three systems in their ability to correctly identify the nonenteric bacilli to the species level. There was no significant difference ($P > 0.05$) in the accuracy of identification between the isolates of *Enterobacteriaceae* versus the non-*Enterobacteriaceae* group for any of the three systems.

Significantly more isolates required supplemental testing with the API 20E system than with the Crystal or the Vitek system ($P < 0.05$) to correctly identify either the *Enterobacteriaceae* isolates or the nonenteric bacilli. The members of the family *Enterobacteriaceae* that most frequently required supplemental testing with the API system included *Citrobacter diversus*, *E. cloacae*, and *K. pneumoniae*. A total of 15 (62.5%) of the 24 *C. diversus* isolates yielded a very good identification

TABLE 1. Identification of gram-negative bacilli by API 20E, the Crystal system, and Vitek AMS

Organism	Tested	No. (%) of isolates Identified as: Correct species[a]			Correct genus only			Incorrect genus or species			With no identification			Needing supplemental testing		
		API	Crystal	Vitek	API	Crystal	Vitek	API	Crystal	Vitek	API	Crystal	Vitek	API	Crystal	Vitek
Enterobacteriaceae																
Citrobacter amalonaticus	2	1	1	1	1	0	0	0	1	1	0	0	0	2	0	0
Citrobacter diversus	24	24	23	24	0	0	0	0	0	0	0	1	0	15	0	0
Citrobacter freundii	18	18	17	17	0	0	0	0	0	0	0	1	1	2	2	0
Edwardsiella tarda	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter aerogenes	28	28	27	28	0	1	0	0	0	0	0	0	0	0	0	0
Enterobacter agglomerans	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0
Enterobacter cloacae	38	35	29	32	1	8	5	2	0	0	0	1	1	4	3	0
Enterobacter gergoviae	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter taylorae	1	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0
Escherichia coli	107	107	107	104	0	0	2	0	0	0	0	0	1	1	2	0
Hafnia alvei	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella oxytoca	20	20	18	20	0	1	0	0	0	0	0	1	0	0	0	0
Klebsiella ozaenae	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Klebsiella pneumoniae	56	56	55	54	0	0	0	0	1	1	0	0	1	5	3	0
Morganella morganii	15	15	14	15	0	0	0	0	0	0	0	1	0	2	3	0
Proteus mirabilis	13	13	13	13	0	0	0	0	0	0	0	0	0	0	0	0
Proteus vulgaris	7	7	7	7	0	0	0	0	0	0	0	0	0	0	0	0
Providencia rettgeri	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0
Providencia stuartii	12	12	12	12	0	0	0	0	0	0	0	0	0	2	1	0
Salmonella sp.	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0
Serratia marcescens	23	23	23	23	0	0	0	0	0	0	0	0	0	1	0	0
Subtotal	381	377 (99.0)	362 (95.0)	366 (96.1)	2 (0.5)	11 (2.9)	7 (1.8)	2 (0.5)	3 (0.8)	2 (0.5)	0 (0)	5 (1.3)	6 (1.6)	35 (9.2)	14 (3.7)	0 (0)
Non-*Enterobacteriaceae*																
Acinetobacter baumannii	18	18	18	18	0	0	0	0	0	0	0	0	0	3	0	0
Acinetobacter lwoffii	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	2
Aeromonas hydrophila	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	2
Alcaligenes xylosoxidans	1	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0
Bordetella bronchiseptica	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Flavobacterium meningosepticum	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0
Pseudomonas aeruginosa	80	80	80	78	0	0	0	0	0	0	0	0	2	76	1	0
Pseudomonas cepacia[b]	3	2	2	3	0	0	0	0	0	0	1	1	0	0	1	0
Pseudomonas putida	2	1	2	1	1	0	0	0	0	0	0	0	1	2	2	1
Pseudomonas stutzeri	1	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0
Vibrio parahemolyticus	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Xanthomonas maltophilia[b]	19	19	19	19	0	0	0	0	0	0	0	0	0	1	0	0
Subtotal	131	128 (97.7)	127 (97.0)	128 (97.7)	1 (0.8)	0 (0)	0 (0)	0 (0)	1 (0.8)	0 (0)	2 (1.5)	3 (2.3)	3 (2.3)	84 (64.1)	4 (3.1)	5 (3.8)
Combined totals	512	505 (98.6)	489 (95.5)	494 (96.5)	3 (0.6)	11 (2.2)	7 (1.4)	2 (0.4)	4 (0.8)	2 (0.4)	2 (0.4)	8 (1.6)	9 (1.8)	119 (23.2)	18 (3.5)	5 (1.0)

[a] Includes results obtained with supplemental testing if needed.
[b] Correct name of organism at time of study.

TABLE 2. Incorrect identifications by the API 20E, Crystal E/NF, and Vitek systems

System	Identification	
	Incorrect	Correct
API 20E	*Citrobacter freundii*	*Enterobacter cloacae*
	Serratia liquefaciens	*Enterobacter cloacae*
Crystal E/NF	*Pseudomonas* sp.	*Bordetella bronchiseptica*
	Escherichia coli	*Citrobacter amalonaticus*
	Klebsiella pneumoniae	*Enterobacter agglomerans*
	Enterobacter aerogenes	*Klebsiella pneumoniae*
Vitek	*Escherichia coli*	*Citrobacter amalonaticus*
	Yersinia frederiksenii	*Klebsiella pneumoniae*

of *Citrobacter* species with API 20E and required additional testing to achieve species identification. Of the 38 *E. cloacae* isolates, 4 (10.5%) required supplemental testing, because API 20E for 1 gave a very good identification of *Enterobacter* sp. and with three other isolates yielded a designation of good likelihood but low selectivity that included *E. cloacae* versus either *Enterobacter asburiae*, *Serratia liquifaciens*, or *Citrobacter freundii*. Of the 56 *K. pneumoniae* isolates, an identification of good likelihood but low selectivity was given by API 20E for 5 (8.9%) isolates that included *K. pneumoniae* versus either *Enterobacter agglomerans*, *Enterobacter intermedius*, or *Klebsiella ozaenae*. The isolates of nonenteric bacilli that most frequently required supplemental testing with API 20E included *A. baumannii* and *P. aeruginosa*. Three (16.7%) of the 18 *A. baumannii* isolates yielded identifications of good likelihood but low selectivity that could not discriminate between *A. baumannii* and *Flavimonas oryzihabitans* without further testing. Of the 80 *P. aeruginosa* isolates, 76 (95.0%) required additional testing, including 53 isolates yielding an identification of good likelihood but low selectivity that included *P. aeruginosa* versus *Chromobacterium* sp. and required triple sugar iron agar to differentiate the organisms and 23 isolates that were identified as fluorescent *Pseudomonas* group requiring supplemental biochemicals for species identification. The Crystal system required supplemental testing significantly more often than the Vitek system ($P < 0.05$) to accurately identify isolates from the family *Enterobacteriaceae*. No significant difference in the frequency of supplemental testing was noted between the Crystal and Vitek systems ($P > 0.05$) for the isolates of nonenteric bacilli.

When the data were analyzed from the perspective of the 412 recent clinical isolates in the weighted laboratory profile and the 273 selected isolates in the stress test sample, there were no significant differences between the three systems in their ability to identify to the species level either the former group ($P > 0.05$) or the isolates in the latter category ($P > 0.05$). Supplemental testing was required for significantly more isolates with API 20E than with the Crystal or Vitek system ($P < 0.05$) to correctly identify either the weighted or stress test isolates. The Crystal system used supplemental tests significantly more often than the Vitek system ($P < 0.05$) to correctly identify isolates in the weighted laboratory profile, and there was no significant difference in the frequency of supplemental testing between the Crystal and Vitek systems ($P > 0.05$) for the stress test isolates.

Overall, 74.4% of all isolates were correctly identified at 18 to 24 h with API 20E, including 90.3% of the isolates from the family *Enterobacteriaceae* and 28.2% of the nonenteric isolates (Table 3). The Crystal system correctly identified 92.2% of all isolates at 18 h, including 91.6% of the isolates from the *Enterobacteriaceae* and 93.9% of the nonenteric bacilli. The cumulative percentages of identifications correctly completed by Vitek were 50.8% by 4 h, 90.2% by 10 h, and 95.5% by 18 h for all organisms. The cumulative percentages of *Enterobacteriaceae* and nonenteric identifications completed by Vitek were 59.3 and 26.0% by 4 h, 92.4 and 84.0% by 10 h, and 96.1 and 93.9% by 18 h, respectively.

DISCUSSION

A number of studies have previously reported the accuracies of gram-negative rod identification by API 20E and Vitek AMS. The reported accuracy of API 20E has ranged from 77.0 to 94.6% at 24 h (11, 13, 19, 23) and 95.2 to 97.5% following the performance of supplemental testing according to the manufacturer's recommendations (11, 13, 24). In the present study, API 20E correctly identified 74.4% of all isolates at 18 to 24 h, including 90.3% of the isolates of the family *Enterobacteriaceae* and 28.2% of the nonenteric bacilli at 24 h. Following additional testing, 99.0% of the members of the *Enterobacteriaceae* and 97.7% of the nonenteric bacilli were correctly identified to the species level. Other investigators found the Vitek AMS to have an accuracy of 84.5 to 97.6% at 24 h that improved to 92.8 to 98.2% following supplemental testing (13–15, 22). Similarly, in our study, the Vitek AMS correctly identified to the species level 95.5% of all isolates at 18 h, including 96.1% of the members of the *Enterobacteriaceae* and 93.9% of the nonenteric isolates. Performance of additional testing as specified by the manufacturer improved the accuracy of the Vitek system to 97.7% for the nonenteric bacilli. In comparison, the Crystal system correctly identified 92.2% of all isolates at 18 h, including 91.6% of the isolates from the *Enterobacteriaceae* and 93.9% of the nonenteric isolates. Following additional testing, 95.0% of the members of the *Enterobacteriaceae* and 97.0% of the isolates of nonenteric bacilli were correctly identified to the species level. These results are similar to those recently reported by Holmes et al. in which the Crystal E/NF system correctly identified 93% of the *Enterobacteriaceae* strains and 96% of the nonenteric isolates (5). When results for the three systems were compared after the completion of additional testing in the present study, there were significant differences between the Crystal and Vitek systems and API 20E ($P < 0.05$), with the API system correctly identifying to the species level significantly more isolates from the family *Enterobacteriaceae* than either the Crystal or the Vitek system. If pigment production were required for identification of an organism as *E. sakazakii*, as planned in the next Crystal software update by the manufacturer, there would be no significant differences in identification accuracy to the species level between the API 20E and the Crystal systems.

The Crystal E/NF panel was comparable in accuracy to API 20E and Vitek AMS when either a weighted clinical laboratory profile of organisms or a group of selected isolates in a stress test sample was evaluated. Following supplemental testing, the API, Vitek, and Crystal systems correctly identified to the species level 99.8, 95.6, and 95.9% of the 412 organisms in the weighted sample, respectively. The API, Vitek, and Crystal systems correctly identified 96.7, 93.8, and 91.6% of the 273 isolates in the stress test, respectively. There were no significant differences between the three systems in their ability to identify to the species level either the isolates in the weighted laboratory group ($P > 0.05$) or the isolates in the stress test category ($P > 0.05$). However, it should be noted that the evaluation did not include strains of *Shigella* spp. and *Yersinia* spp.

Although all three systems performed at an acceptable level,

TABLE 3. Incubation times required for API 20E, Crystal E/NF, and Vitek results correct to genus and species for all organisms tested

Organism	No. tested	No. (%) of isolates correctly identified by:					
		API at:		Crystal at 18 h	Vitek at:		
		18–24 h	48 h		4 h	5–10 h	11–18 h
Enterobacteriaceae							
Citrobacter amalonaticus	2	0	0	1	0	1	0
Citrobacter diversus	24	9	0	23	10	13	1
Citrobacter freundii	18	16	0	15	13	4	1
Edwardsiella tarda	1	1		1	0	1	
Enterobacter aerogenes	28	28		27	19	8	1
Enterobacter agglomerans	1	1		0	0	0	0
Enterobacter cloacae	38	32	0	27	24	7	1
Enterobacter gergoviae	4	4		4	2	1	1
Enterobacter taylorae	1	0	0	0	0	1	
Escherichia coli	107	106	0	105	98	5	1
Hafnia alvei	2	2		2	0	2	
Klebsiella oxytoca	20	20		18	12	8	
Klebsiella ozaenae	1	1		1	0	0	0
Klebsiella pneumoniae	56	51	0	52	32	19	3
Morganella morganii	15	13	0	11	1	12	2
Proteus mirabilis	13	13		13	6	7	
Proteus vulgaris	7	7		7	0	7	
Providencia rettgeri	4	4		4	0	4	
Providencia stuartii	12	10	0	11	0	8	4
Salmonella sp.	4	4		4	3	1	
Serratia marcescens	23	22	0	23	6	17	
Subtotal	381	344 (90.3)	0 (0)	349 (91.6)	226 (59.3)	126 (33.1)	14 (3.7)
Non-*Enterobacteriaceae*							
Acinetobacter baumannii	18	15	0	18	0	18	
Acinetobacter lwoffii	2	0	2	2	0	0	0
Aeromonas hydrophila	2	2		2	0	0	0
Alcaligenes xylosoxidans	1	0	0	0	0	0	1
Bordetella bronchiseptica	1	0	1	0	0	0	1
Flavobacterium meningosepticum	1	0	0	1	0	0	1
Pseudomonas aeruginosa	80	0	4	79	34	43	1
Pseudomonas cepacia[a]	3	1	1	1	0	1	2
Pseudomonas putida	2	0	0	0	0	0	0
Pseudomonas stutzeri	1	0	0	0	0	0	1
Vibrio parahemolyticus	1	1		1	0	1	
Xanthomonas maltophilia[a]	19	18		19	0	13	6
Subtotal	131	37 (28.2)	8 (6.1)	123 (93.9)	34 (26.0)	76 (58.0)	13 (9.9)
Combined totals	512	381 (74.4)	8 (1.6)	472 (92.2)	260 (50.8)	202 (39.5)	27 (5.3)

[a] Correct name of organism at time of study.

with at least 95% of the isolates correctly identified to the species level following manufacturer-specified supplemental testing, the extent of additional testing varied significantly between the systems. To achieve a high level of accuracy, significantly more isolates required supplemental testing with API 20E than with the Crystal or the Vitek system to correctly identify either the *Enterobacteriaceae* isolates or the nonenteric bacilli ($P < 0.05$). Supplemental testing was required for 9.2, 3.7, and 0% of the isolates of *Enterobacteriaceae* tested by the API, Crystal, and Vitek systems, respectively. Additional testing was needed for 64.1, 3.1, and 3.8% of the nonenteric isolates identified to the species level by the API, Crystal, and Vitek systems, respectively. A previous report indicated that API 20E required supplemental testing for 29% of the study isolates to achieve identification at the species level (24). In the present evaluation, 23.2% of the total isolates tested in API 20E required additional tests for identification, increasing the time to identification to 48 h or more. The Crystal system required supplemental testing to accurately identify 3.7% of the isolates from the family *Enterobacteriaceae* versus 0% for the Vitek system, and this difference was significant ($P < 0.05$). No significant difference in the frequency of supplemental testing was noted between the Crystal and Vitek systems ($P > 0.05$) for the isolates of nonenteric bacilli. Although supplemental testing and the identification delays associated with it occurred infrequently with the Vitek system, initial testing with the Vitek system can be delayed by 1 day in the clinical laboratory when working with patient specimens because the system requires an inoculum standardized to a 1.0 McFarland standard versus a single colony needed for the performance of either the API or the Crystal system.

The cost of identification per isolate was calculated for each of the three different systems on the basis of a current annual test volume of 7,200 isolates and related volume discounts (Table 4). The costs associated with quality control were also calculated. The labor and supply costs associated with supplemental testing needed to obtain an identification were tabulated. The cost of the service contract for the Vitek system was allocated equally among the 19,000 tests, including the 7,200 gram-negative rod identifications, performed annually with the

TABLE 4. Cost of identification per isolate

Item(s)	Cost ($) per isolate by:		
	API 20E	Crystal E/NF	Vitek AMS[a]
Strip, card, or panel	2.95	3.10	2.26
Saline tube	0.39	With panel	0.30
Transfer pipette	0.04		
Reagents	0.13		
Quality control	0.04	0.01	0.05
Supplemental supplies	0.15	0.04	0.01
Shipping or freight	0.05		0.08
Service contract			0.35
Labor ($18/h)	0.99	0.87	0.57
Total	4.74	4.02	3.62

[a] Excludes capital equipment expense.

Vitek system in our laboratory. The cost data do not include the capital equipment expense incurred with the Vitek system, because there are a number of methods, with variable cost impact, for allocation of this type of expense. It is important that cost calculations include all of the expenses associated with a test product, including the frequently ignored shipping and service contract costs.

The labor time studies were designed to measure only those activities essential for test performance, including the time interval from initiation to completion of the panel inoculation and the time interval from removal of the panel from the incubator to organism identification. Supplemental testing performance specified by the manufacturer was timed also. The test batch size ranged from 9 to 30 tests per system. An average of 1.5 min was needed to inoculate the API 20E system, with an additional 1.5 min needed the next day to identify an organism. The average total labor required to obtain an identification by API 20E was 3.0 min without additional testing and 3.3 min per isolate with supplemental testing. Inoculation of the Crystal panel required 1.4 min per isolate followed by an additional 1.4 min the next day to identify the organism. The total labor required to identify an isolate by the Crystal system was 2.8 min without additional testing and 2.9 min with the supplemental testing. For the Vitek system, 1.6 min was required to inoculate and load the cards, and an additional 0.2 min per isolate was needed to check the computer and remove the identification printouts. The total time required to identify an isolate was 1.8 min without supplemental testing and 1.9 min with additional testing.

In summary, API 20E, Crystal E/NF, and Vitek AMS were found to be reliable systems for the identification of members of the family *Enterobacteriaceae* and nonenteric gram-negative bacilli. Errors in identification with each system were infrequent and appeared to be randomly distributed among the genera evaluated. The accuracy of Crystal E/NF compared favorably with those of API 20E and Vitek AMS, which have established track records in clinical laboratories. The Crystal system provided accurate results for 92.2% of the isolates at 18 h and required minimal supplementary testing to correctly identify organisms to the species level. In addition, it was found to be simple and safe to use because of the one-step inoculation process and the closed system design, it conveniently accommodated existing laboratory work flow, and it was cost-effective. Therefore, the Crystal E/NF is an acceptable method for the identification of members of the family *Enterobacteriaceae* and nonenteric gram-negative bacilli and is a useful addition to clinical microbiology laboratories.

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