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2003

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O'Hara, Caroline M.; Sowers, Evangeline G.; Bopp, Cheryl A.; Duda, Sarah B.; and Strockbine, Nancy A., "Accuracy of Six Commercially Available Systems for Identification of Members of the Family *Vibrionaceae*" (2003). *Public Health Resources*. 224.

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## Accuracy of Six Commercially Available Systems for Identification of Members of the Family *Vibrionaceae*

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Received 25 June 2003/Returned for modification 14 August 2003/Accepted 15 September 2003

Six commercially available bacterial identification products were tested with *Vibrio alginolyticus* (12 strains), *V. cholerae* (30 strains), *Photobacterium (Vibrio) damsela* (10 strains), *V. fluvialis* (10 strains), *V. furnissii* (4 strains), *V. hollisae* (10 strains), *V. metschnikovii* (9 strains), *V. mimicus* (10 strains), *V. parahaemolyticus* (30 strains), and *V. vulnificus* (10 strains) to determine the accuracy of each system for identification. The products included API 20E, Crystal E/NF, MicroScan Neg ID2 and Rapid Neg ID3, and Vitek GNI+ and ID-GNB. Each product was tested only with those species that were listed in its database. Overall, the systems correctly identified 63.9, 80.9, 63.1, 73.6, 73.5, and 77.7% of the isolates to species level, respectively. Error rates ranged from 0.8% for the API 20E to 10.4% for the Rapid Neg ID3. The API 20E gave “no identification” for 13.1% of the isolates, while the Neg ID2, GNI+, ID-GNB, and Crystal were unable to identify 1.8, 2.9, 5.0, and 6.9%, respectively. For *V. cholerae*, specifically, accuracy ranged from 50.0 to 96.7%, with the API 20E having the worst performance and Crystal having the best. *V. fluvialis* presented the biggest challenge for the API 20E and the GNI+, with probabilities averaging 10%, while *V. mimicus* was a major problem with the Crystal E/NF, which identified none of the strains correctly. With the Neg ID2, correct answers were often obtained only after a modified inoculation of the panel with a bacterial suspension prepared with 0.85% NaCl. Additional tests required for identification often included growth in the absence of NaCl, which is not readily available in most clinical laboratories. The only product to correctly identify at least 90% of *V. cholerae* strains was the Crystal E/NF, and only three of the six products, the API 20E and both of the Vitek cards, correctly identified more than 90% of the *V. parahaemolyticus* strains. Thus, extreme care must be taken in the interpretation of answers from these six commercially available systems for the identification of *Vibrio* species.

There are approximately 63 species of *Vibrio* ([www.bacterio.cict.fr](http://www.bacterio.cict.fr)), of which 12 occur in human clinical specimens (11). Of particular interest to clinical microbiology laboratories is *Vibrio cholerae*, which has been the cause of many epidemics and deaths and which is generally acknowledged to be of serious concern to those currently involved in bioterrorism. Indeed, when the list of critical agents for bioterrorism and civil preparedness was released (21), *V. cholerae* was classified as a biothreat level B, which is defined as an agent having a moderate ease of transmission and morbidity with a low rate of mortality (31). Thus, correct identification of this organism and its differentiation from other species of the genus are important.

Other species of *Vibrio* such as *V. vulnificus*, which has a fatality rate of approximately 50% in patients with primary septicemia, also cause significant human disease (11). *Photobacterium (Vibrio) damsela*, *V. metschnikovii*, and *V. cincinnatiensis* have all been isolated from patients with bacteremia or meningitis (7, 12, 14, 15, 29). *V. parahaemolyticus* is also the cause of many outbreaks (11).

Commercial bacterial identification systems are used in the vast majority of hospital-based clinical microbiology laboratories. To date, there has not been a comprehensive evaluation of the ability of these systems to accurately identify the 12

species of *Vibrio* known to infect humans. The database matrices of all six systems do not include all 12 of the species, but all systems claim the capability to identify *V. alginolyticus*, *V. cholerae*, *P. damsela*, *V. parahaemolyticus*, and *V. vulnificus*.

This study evaluated the ability of the six commercially available bacterial identification systems to accurately identify members of the family *Vibrionaceae*.

(This paper was presented in part at the 103rd Annual Meeting of the American Society for Microbiology, Washington, D.C., 19 to 22 May 2003 [C. M. O'Hara, E. G. Sowers, C. A. Bopp, N. A. Strockbine, and J. G. Wells, Abstr. Annu. Meet. Am. Soc. Microbiol., abstr. C008, p. 118, 2003]).

### MATERIALS AND METHODS

**Identification systems and software.** The six systems used in this study were the API 20E strip (bioMérieux Inc., Durham, N.C.) with profile index version 4.0, the Crystal E/NF panel (Becton Dickinson & Company, Sparks, Md.) with software version 4.0, the MicroScan Neg ID type 2 and the Rapid Neg ID type 3 panels (Dade Behring Inc., MicroScan Inc., West Sacramento, Calif.) with LabPro version 1.133, and the Vitek GNI+ and ID-GNB cards (bioMérieux Inc.) with software version 7.01 on the Vitek 32 instrument and version 2.03 on the Vitek 2 instrument. The MicroScan panels were analyzed on a MicroScan SI instrument. All products were inoculated and read according to the directions of the respective manufacturers.

All six cards and panels are products that have been the subject of extensive publications in the past. Readers are referred to prior reports for more details on the products (22–24, 30).

**Culture collection.** All 125 strains of biochemically typical and 10 atypical (3 strains each of *V. alginolyticus* and *V. damsela* and 4 strains of *V. hollisae*) members of the family *Vibrionaceae* were taken from the culture collections of

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TABLE 1. Accuracy of identification of isolates from 10 *Vibrio* species by six commercial systems<sup>a</sup>

Species	Accuracy (%) with:					
	API 20E	Crystal E/NF	MicroScan Neg ID2	MicroScan Rapid Neg ID3	Vitek GNI+	Vitek ID-GNB
<i>V. alginolyticus</i>	<b>91.6</b>	66.6	16.6	<b>100</b>	<b>91.6</b>	50.0
<i>V. cholerae</i>	50.0	<b>96.7</b>	86.6	73.3	66.6	73.3
<i>P. damsela</i>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	80.0	<b>100</b>
<i>V. fluvialis</i>	0	80.0	<b>90.0</b>	NA <sup>b</sup>	20.0	<b>90.0</b>
<i>V. furnissii</i>	NA	NA	NA	100	NA	NA
<i>V. hollisae</i>	0	<b>90.0</b>	10.0	<b>100</b>	NA	NA
<i>V. metschnikovii</i>	NA	88.8	NA	66.6	NA	33.3
<i>V. mimicus</i>	70.0	0	<b>90.0</b>	<b>100</b>	NA	<b>100</b>
<i>V. parahaemolyticus</i>	<b>96.6</b>	83.3	83.3	40.0	<b>96.6</b>	<b>90.0</b>
<i>V. vulnificus</i>	60.0	<b>90.0</b>	50.0	60.0	50.0	70.0
Totals	63.9	80.9	63.1	73.6	73.5	77.7

<sup>a</sup> Boldface indicates accuracy  $\geq 90\%$ .

<sup>b</sup> NA, not included in the database.

either the Diagnostic Microbiology Section (DMS), Epidemiology and Laboratory Branch, Division of Healthcare Quality Promotion, or the Foodborne and Diarrheal Diseases Laboratory Section (FDDLS), Foodborne and Diarrheal Diseases Laboratory Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention (CDC). An isolate is considered atypical if there are no more than three characteristics exhibited by that isolate that are present less than 5% of the time (e.g., a citrate-positive isolate of a species that is normally 95% citrate negative). The 10 atypical strains that were used in this study exhibited only one unusual characteristic per isolate. The cultures were maintained in either defibrinated sheep blood (DMS) or tryptic soy broth with 20% glycerol (FDDLS) at  $-70^{\circ}\text{C}$  and were passaged four times on tryptic soy agar with 5% sheep blood (TSA II; Becton Dickinson Biosciences Inc., Sparks, Md.) before testing. For studies with the MicroScan Rapid Neg ID3 panels, the fourth passage was on MacConkey agar. All incubations were at  $35 \pm 1^{\circ}\text{C}$ , unless otherwise indicated. If the isolate did not grow on MacConkey agar, the inoculum was taken from a fourth passage on a sheep blood agar plate. For the initial testing of any given isolate, all products were inoculated from the same blood or MacConkey agar plate.

Only the species that were included in a respective system's database were included in that part of the evaluation, i.e., not all isolates were tested in all systems. However, the number of isolates of a given species remained constant across the systems.

If an initial identification was in error, two additional tests were done simultaneously. The best two of three answers were used for the categorization of that isolate.

**Media, biochemical tests, and serology.** Biochemical tests for enteric identification were performed with conventional media and methods described by Edwards and Ewing (9), with some modifications by Hickman and Farmer (16) and Farmer et al. (10). Commercial media were used whenever possible.

If an identification required serology for confirmation of *V. cholerae* or *V. parahaemolyticus*, it was performed as described by Shimada et al. (28) with *V. parahaemolyticus* antiserum obtained from Denka-Seiken Co., Ltd. (Tokyo, Japan) and *V. cholerae* antiserum produced in-house.

**Additional tests.** If the probability level of an identification was below that established as the minimum acceptable level for a given product, additional tests were performed to achieve an identification. The additional tests were different for each product and often were not tests that are in common use in clinical microbiology laboratories. Those that were required in this study are listed below.

API 20E required tests for fermentation of glucose, cellobiose, trehalose, and xylose; growth on MacConkey agar; production of gas from glucose; esculin; motility; methyl red; growth in 4% sodium chloride (NaCl); and resistance to the vibriostatic agent O129.

The Crystal E/NF required tests only for the reduction of nitrate, Voges-Proskauer, production of  $\text{H}_2\text{S}$ , and ornithine decarboxylation.

The MicroScan conventional overnight product required tests for oxidase, Christensen's urea, fermentation of trehalose, production of gas from glucose, liquefaction of gelatin, methyl red, motility, DNase, and growth of the organism in 0, 6, or 8% NaCl.

The MicroScan rapid panel required tests for the fermentation of sucrose,

adonitol, rhamnose, and cellobiose; oxidase; Voges-Proskauer; arginine; flagella stain; *Vibrio* nitrate reduction (supplemented with 1% NaCl); and the ability to grow in the presence of 0, 8, or 10% NaCl.

The Vitek GNI+ card required only the test for the ability to grow in the presence of 6.5% NaCl or serologic confirmation of an identification.

The Vitek ID-GNB additional tests included oxidase, motility, indole, pigmentation, and hemolysis on sheep blood agar.

**Definitions.** "Correct" indicates that the product gave the correct genus and species at the end of the designated incubation time with no additional tests required.

"No ID" indicates that the product gave an identification of "no ID," "unacceptable ID," or "unidentified" at the end of the designated incubation time.

"Correct—low probability" means that the correct answer was given but at a probability level below that established as the minimum acceptable level for each respective product. This level differs for each product. For both of the Vitek products, it is 90%; for both of the MicroScan panels, it is 85%. The answer from the Crystal E/NF must be  $\geq 0.9000$  or additional tests are required for an answer to be generated. An API 20E answer must be categorized as "excellent," "very good," "acceptable," or "good" or additional tests are required for the completion of an identification.

"Inconclusive" means that the product gave an incorrect genus and species but at a probability level below that established as the minimum acceptable level for that product. It could also mean that the product gave three different answers on each of the three tests for a given isolate.

"Error" means that the product gave an incorrect genus and species at a probability level above that established as the minimum acceptable level for each respective product.

## RESULTS AND DISCUSSION

Table 1 shows the composite results from testing of all six products. These percentages represent the numbers of answers that were correct at the end of the initial incubation time for a given product without the use of additional tests.

**API 20E.** This manual product contains 20 conventional substrates that are inoculated with a 0.85% saline suspension of organism and that require overnight incubation with the addition of reagents before reading. Table 2 shows the test results for 122 strains from eight species that are included in the database of this product. The API performed best on the identification of *V. alginolyticus*, *V. parahaemolyticus*, and *P. damsela*, with accuracies of 91.6% or greater. At 24 h, it was able to identify only 50.0% of *V. cholerae* strains and none of the *V. fluvialis* or *V. hollisae* strains that were tested. Of the 15 *V. cholerae* strains that it could not identify, it rendered "no ID" on nine and erred only on one strain, calling it *Aeromonas*

TABLE 2. Results of testing eight species of *Vibrio* in the API 20E strip (read at 18 to 24 h)

Reference ID	n	No. (%) of strains with result of:				
		Correct	Correct— low probability	Incon- clusive	No ID <sup>a</sup>	Error
<i>V. alginolyticus</i>	12	11			1	
<i>V. cholerae</i>	30	15	5		9	1
<i>P. damsela</i>	10	10				
<i>V. fluvialis</i>	10		9		1	
<i>V. hollisae</i>	10		9		1	
<i>V. mimicus</i>	10	7	2		1	
<i>V. parahaemolyticus</i>	30	29			1	
<i>V. vulnificus</i>	10	6	1	1	2	
Totals	122	78 (63.9)	26 (21.3)	1 (0.8)	16 (13.1)	1 (0.8)

<sup>a</sup> No ID, no identification.

*hydrophila*. Nine strains of *V. hollisae* required additional tests for completion of the identification. One of these tests was growth on MacConkey agar. The matrix of the database was constructed with a 99% positive rate for this test. Because none of our strains of *V. hollisae* grew on MacConkey agar, all of the answers were correct, but categorized as “good likelihood, low selectivity.”

**Crystal E/NF.** This manual product contains 30 conventional and enzymatic substrates plus two off-line tests (oxidase and spot indole) and also requires 18 h of incubation but does not need to have reagents added before it is read. Once inoculated, it is a completely closed system, ensuring greater safety for the user. Table 3 shows the results of testing 131 strains from nine different species. Accuracy of identifications was 90% or greater with four species: *V. cholerae*, *P. damsela*, *V. hollisae*, and *V. vulnificus*. Of the *V. parahaemolyticus* strains, 83.3% were correctly identified. There is no single reason that none of the *V. mimicus* strains were correctly identified.

**MicroScan Neg ID type 2.** This panel contains 28 conventional biochemical substrates and six antimicrobial agents and requires overnight incubation with the addition of reagents before it is read. When the panel is inoculated according to the manufacturer's directions, the inoculum is made up in water with Pluronic, a proprietary substance that is believed to serve as a surfactant. As a result, many of the salt-requiring vibrios

TABLE 3. Results of testing nine species of *Vibrio* in the BD Crystal E/NF panel (read at 18 h)

Reference ID	n	No. (%) of strains with result of:				
		Correct	Correct— low probability	Incon- clusive	No ID <sup>a</sup>	Error
<i>V. alginolyticus</i>	12	8	3			1
<i>V. cholerae</i>	30	29	1			
<i>P. damsela</i>	10	10				
<i>V. fluvialis</i>	10	8	1		1	
<i>V. hollisae</i>	10	9			1	
<i>V. metschnikovii</i>	9	8				1
<i>V. mimicus</i>	10	0	1	2	4	3
<i>V. parahaemolyticus</i>	30	25	3		2	
<i>V. vulnificus</i>	10	9			1	
Totals	131	106 (80.9)	9 (6.9)	2 (1.5)	9 (6.9)	5 (3.8)

<sup>a</sup> No ID, no identification.

give an identification of “*Vibrio* sp. SF” (slow fermenter) at an accuracy averaging 98%. All of these identifications also contained the footnote “T,” explaining that the halophilic vibrios (*V. alginolyticus*, *P. damsela*, *V. fluvialis*, *V. hollisae*, *V. parahaemolyticus*, and *V. vulnificus*) require the addition of salt to biochemical tests for species identification. If a halophilic *Vibrio* is suspected, the isolate should be retested by emulsifying several colonies into 3 ml of sterile 0.85% saline, with a final turbidity equivalent to a 0.5 barium sulfate turbidity standard. The panel should then be rehydrated with 25 ml of uninoculated water with Pluronic and 50  $\mu$ l of the saline suspension and processed in the normal manner. For this reason, the results of testing 122 strains of eight species have been evaluated both without salt and then with the recommended additional salt (Table 4). The testing of *V. alginolyticus* was more accurate before the addition of salt. Once salt was added, 6 of the 12 answers were correct, but at probabilities ranging from 84.2 to 4.9%. The accuracy for *V. cholerae* increased from 63.3 to 86.6% with the addition of salt to the panels, and that for *P. damsela* increased from 70.0 to 100%. Accuracy for both *V. fluvialis* and *V. mimicus* increased from 0 to 90.0%, while accuracy for *V. parahaemolyticus* decreased from 83.3 to 42.1%. On the panels containing no salt, both *V. alginolyticus* and *V. parahaemolyticus* gave the “*Vibrio* sp. SF” identification at a high probability, which would lead the laboratorian to suspect a possible vibrio. The addition of salt to the panel made no difference in the identification of *V. hollisae*. Without salt, the 10 isolates gave an identification of “*Vibrio* sp. SF” at a probability of 23.8%. With salt added to the inoculum, 7 of 10 isolates were identified as *V. hollisae*, but only one probability exceeded 90.0%. One was called a “very rare biotype.” Overall, the accuracy of the panels was 51.6% without salt and 63.1%, a modest increase, with salt.

In an informal survey of seven clinical microbiology laboratories in both medium and large hospitals and one microbiology reference laboratory, five of the hospital-based laboratories and the reference laboratory would repeat the panel using the saline inoculum; two laboratories would send it on to their respective state laboratories with no further work-up.

**MicroScan Rapid Neg ID type 3.** This panel contains a combination of 35 conventional and enzymatic substrates that are read at 2 h 20 min after the automatic addition of the rapid indole reagent by the WalkAway. There are nine *Vibrio* species in this database, and 125 strains were tested (Table 5). The overall accuracy was 73.6%, with all *V. alginolyticus*, *P. damsela*, *V. furnissii*, *V. hollisae*, and *V. mimicus* strains identified correctly. *V. parahaemolyticus* was the biggest problem for this system, with only 40.0% correctly identified at the end of the initial incubation period. Of the remaining 18 strains, 14 were identified as *V. parahaemolyticus*, but at a very low probability.

The four strains of *V. cholerae* that were incorrectly identified were called *V. mimicus* at  $\geq 90\%$  probability. Two of the three misidentified *V. vulnificus* strains were called *V. metschnikovii*.

**Vitek GNI+.** This card contains 28 conventional biochemicals and requires at least 6 h of incubation time. No additional reagents are needed in this totally sealed system. Six species of *Vibrio* are included in the database, and 102 strains were tested with a Vitek 32 (Table 6). Overall accuracy was 73.5%, with only *V. alginolyticus* and *V. parahaemolyticus* having greater

TABLE 4. Results of testing eight species of *Vibrio* in the MicroScan Neg ID2 panel (read at 16 to 20 h)

Reference ID	n	No. (%) of strains with result of:					
		Correct in:		Correct—low probability	Inconclusive	No ID <sup>a</sup>	Error
		H <sub>2</sub> O with Pluronic	0.85% NaCl				
<i>V. alginolyticus</i>	12	12 <sup>b</sup>	2	6	2		2
<i>V. cholerae</i>	30	19	26	2			2
<i>P. damsela</i>	10	7 <sup>b</sup>	10				
<i>V. fluvialis</i>	10	0	9	1			
<i>V. hollisae</i>	10	0	1	7	1	1	
<i>V. mimicus</i>	10	0	9	1			
<i>V. parahaemolyticus</i>	30	25 <sup>b</sup>	8/19 <sup>c</sup>	9	1	1	
<i>V. vulnificus</i>	10	0	5	4	1		
Totals	122	63 (51.6)	70 (63.1)	30 (27.0)	5 (4.5)	2 (1.8)	4 (3.6)

<sup>a</sup> No ID, no identification.

<sup>b</sup> Panel identifications correct to genus.

<sup>c</sup> Only 19 panels repeated with salt.

than 90% accuracy. Seven of the 10 answers from testing *V. fluvialis* were *V. fluvialis*, but at probability levels below 90%, which is the minimum acceptable threshold for this product. Four of the five *V. cholerae* identifications that were in error were *V. alginolyticus* at probability levels  $\geq 92\%$ . The fifth error was a *V. cholerae* strain identified as *V. parahaemolyticus* at 92% probability.

**Vitek ID-GNB.** This card is the newest identification product on the market and is used with the Vitek 2 instrument. Table 7 shows the results of testing 121 strains from nine species. Strains of *P. damsela* and *V. mimicus* were all identified correctly, and the identifications of *V. fluvialis* and *V. parahaemolyticus* were 90% accurate. Four strains of *V. cholerae* were identified as *V. cholerae*, but at a “low discrimination” level, and would require supplemental testing.

Very few reports expressly concerning the ability of commercial systems to identify members of the genus *Vibrio* have appeared, although many evaluations have incorporated small numbers of these organisms within a larger study. Overman et al. (25) tested 13 strains from five species of *Vibrio* in the API 20E with an accuracy rate of 100%.

There have been at least eight published case reports in

which *V. metschnikovii* was the causative organism of infection. Of these, only once (8) was there an attempt to identify the isolates with a commercial system, in this case the API 20E. The report suggested that the results were not reproducible and resulted in some incorrect identifications.

Three biogroups of *V. vulnificus* are recognized in the literature. Biogroup 1 is positive in tests for indole, ornithine decarboxylase, and fermentation of D-mannitol and negative for the fermentation of D-sorbitol. This biogroup is generally considered to be an opportunistic pathogen for humans after the ingestion of raw shellfish or contamination of a wound after exposure to seawater or handling of shellfish (3). Biogroup 2 is pathogenic for eels, the natural host for this biotype, but articles citing it as the causative agent in human infections as well have now appeared (3). It is negative in tests for indole, ornithine, and D-mannitol but positive for the fermentation of D-sorbitol. Biogroup 3 has been reported in the literature as having been isolated from wound infections and bacteremia (5). It is positive in tests for ornithine and indole but negative for D-mannitol and D-sorbitol fermentations. After the selection and testing of *V. vulnificus* isolates were completed, we realized that we had picked three isolates of biogroup 1 and seven isolates of biogroup 2. Because the CDC generally receives only those isolates that cause problems in identification, it is not unusual that our collection would include a large

TABLE 5. Results of testing nine species of *Vibrio* in the MicroScan Rapid Neg ID3 panel (read at 2 h 20 min)

Reference ID	n	No. (%) of strains with result of:				
		Correct	Correct—low probability	Inconclusive	No ID <sup>a</sup>	Error
<i>V. alginolyticus</i>	12	12				
<i>V. cholerae</i>	30	22	4			4
<i>P. damsela</i>	10	10				
<i>V. furnissii</i>	4	4 <sup>b</sup>				
<i>V. hollisae</i>	10	10				
<i>V. metschnikovii</i>	9	6	1			2
<i>V. mimicus</i>	10	10				
<i>V. parahaemolyticus</i>	30	12	14			4
<i>V. vulnificus</i>	10	6	1			3
Totals	125	92 (73.6)	20 (16.0)			13 (10.4)

<sup>a</sup> No ID, no identification.

<sup>b</sup> Identification was *V. fluvialis* or *V. furnissii* with no additional tests given to separate the two species.

TABLE 6. Results of testing six species of *Vibrio* in the Vitek GNI+ card (read at 2 to 8 h)

Reference ID	n	No. (%) of strains with result of:				
		Correct	Correct—low probability	Inconclusive	No ID <sup>a</sup>	Error
<i>V. alginolyticus</i>	12	11	1			
<i>V. cholerae</i>	30	20		5		5
<i>P. damsela</i>	10	8	1	1		
<i>V. fluvialis</i>	10	2	7		1	
<i>V. parahaemolyticus</i>	30	29			1	
<i>V. vulnificus</i>	10	5	1	2	1	1
Totals	102	75 (73.5)	10 (9.8)	8 (7.8)	3 (2.9)	6 (5.9)

<sup>a</sup> No ID, no identification.

TABLE 7. Results of testing eight species of *Vibrio* in the Vitek ID-GNB card (read at 3 h)

Reference ID	n	No. (%) of strains with a result of:				
		Correct	Correct— low probability	Incon- clusive	No ID <sup>a</sup>	Error
<i>V. alginolyticus</i>	12	6	3	2		1
<i>V. cholerae</i>	30	22	4		4	
<i>P. damsela</i>	10	10				
<i>V. fluvialis</i>	10	9			1	
<i>V. metschnikovii</i>	9	3	3			3
<i>V. mimicus</i>	10	10				
<i>V. parahaemolyticus</i>	30	27	1	1		1
<i>V. vulnificus</i>	10	7	1		1	1
Totals	121	94 (77.7)	12 (9.9)	3 (2.5)	6 (5.0)	6 (5.0)

<sup>a</sup> No ID, no identification.

number of suspect biogroup 2 isolates. However, what is unusual is that the API 20E matrix has the indole reaction configured at 99% positive. All of our biogroup 2 isolates were positive for indole on the API 20E even though they were negative in our conventional Kovacs indole test. Four of these seven were correctly identified. No biogroup 3 strains were included in this study. However, Colodner et al. reported results obtained from testing 51 documented biogroup 3 strains in the ID-GNB card, which correctly identified 90.2% of the strains (R. Colodner, L. Lerner, J. Kopelowitz, I. Meir, Z. Lazarovich, Y. Keness, and R. Raz, Abstr. 12th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P637, 2002).

There are numerous case reports of *V. vulnificus* in the literature in which a commercial system was used for the initial identification of the organism. Bisharat and Raz (6) reported that only 12 of 25 *V. vulnificus* isolates from persons who had contact with fish were correctly identified with the API 20E. In 1999, Bisharat et al. further reported that 19 isolates of *V. vulnificus* from soft-tissue infections were identified as other genera by the API 20E (5). The authors indicated that the isolates were also tested in conventional MicroScan panels but did not report the identifications.

In 1993, Biosca et al. (4) tested 106 isolates in the API 20E system; however, they modified the manufacturer's directions so that the inoculum was made up in 0.85% NaCl and incubated at 25°C. Even with these alterations in the protocol, only 13 of 17 biotype 1 strains were identified correctly, with an accuracy of 76.5% with no additional tests. Of the 89 biotype 2 strains, none were identified correctly without additional tests. The investigators suggested that this finding was consistent with the database that was constructed with clinical strains. All of their biotype 2 strains were from diseased eels, the natural host for this biotype.

Abbott and Janda (1) reported on two cases of gastroenteritis associated with *V. hollisae*. In the first case, the API 20E misidentified the organism, which also failed to grow in a MicroScan conventional panel. In the second case, the API accurately identified the bacterium. As was discussed earlier, their isolates also failed to grow on MacConkey agar. Reina et al. (26) reported a case of gastroenteritis caused by *V. alginolyticus*, which was correctly identified by API 20E. Finally, Komarnicka et al. reported the first catheter site infection in a dialysis patient caused by *V. cholerae* non-O1, in which the

organism was correctly identified by the API 20E (J. Komarnicka, A. Samet, E. Dziemaszkiewicz, and L. Maumiuk, Abstr. 11th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P1065, 2001).

In evaluations of the Vitek 2, Funke et al. (13) tested seven isolates of *Vibrio* spp. Two isolates each of *V. cholerae* and *V. parahaemolyticus* were correctly identified, while only one of three strains of *V. alginolyticus* was correctly identified. Jossart and Courcol (18), however, had one strain each of these same three organisms correctly identified in their study on the Vitek 2, and Ling et al. (20) reported that the result of one strain of *V. vulnificus* was deemed "unidentified."

In an evaluation of the Crystal E/NF by Holmes et al. (17), five strains each of *V. cholerae* and *V. parahaemolyticus* were tested. Four strains each were correctly identified, and one strain each gave "no identification." Robinson (27) had one strain of *V. parahaemolyticus* correctly identified.

Another potential problem that must be taken into consideration involves the identification of aeromonads as vibrios. Abbott et al. (2) addressed this problem and reported on isolates of *Aeromonas schubertii* and *Aeromonas veronii* that were identified as *P. damsela* (Vitek GNI+) and *V. cholerae* (API 20E), respectively.

While it is a recognized fact that cholera is a potentially devastating worldwide disease, most of the reports in the literature do not address the identification of the organism. In many cases, selective medium is used for the initial isolation and, once the organism is isolated, identification is confirmed serologically. In a review of *V. cholerae*, Kaper et al. (19) state that commercially available enteric identification systems are suitable for identifying *V. cholerae*. Based on the study reported here, we cannot agree with this statement. None of the systems tested in our study achieved an overall accuracy of  $\geq 90\%$  for the identification of *Vibrio* species. Only the Becton Dickinson Crystal E/NF achieved an accuracy level of  $\geq 80\%$ . The only product to correctly identify at least 90% of *V. cholerae* strains was the Crystal E/NF, and only three of the six products, the API 20E and both of the Vitek cards, correctly identified more than 90% of the *V. parahaemolyticus* strains.

This study has shown that only 20 of 48 of the organism-product combinations will yield an accurate identification. The overall accuracy of all systems with all species that are included in their databases is 72.1%, even though in an additional 15.2% of the cases a correct identification would have been given, but at a low probability requiring additional confirmatory testing. *V. cholerae* would have been identified accurately in only 74.4% of the tests. In conclusion, caution must be taken in the interpretation of answers from these six commercially available systems for the identification of *Vibrio* species, with additional confirmatory testing being required in many cases.

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