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# Comparison of API 20E, API Rapid E, and API Rapid NFT for Identification of Members of the Family *Vibrionaceae*

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**Sixty isolates, from nine species of the family *Vibrionaceae*, were tested by the API 20E, API Rapid E, and API Rapid NFT systems. Results were compared with those obtained with standard biochemicals. Included were 7 *Aeromonas caviae* isolates, 27 *Aeromonas hydrophila* isolates, 10 *Aeromonas sobria* isolates, 3 *Plesiomonas shigelloides* isolates, 3 *Vibrio alginolyticus* isolates, 3 *Vibrio cholerae* isolates, 1 *Vibrio fluvialis* isolate, 5 *Vibrio parahaemolyticus* isolates, and 1 *Vibrio vulnificus* isolate. The API 20E correctly identified all the isolates within 24 h. The API Rapid E correctly identified only 77%, misidentified 8%, and failed to identify 2% of the isolates in 4 h. The remaining 13% of the isolates gave a low selectivity identification, with one of the choices being correct. The API Rapid NFT correctly identified 87%, misidentified 5%, gave a low selectivity identification for 8% of the isolates, and in some instances, required up to 48 h of incubation. The API 20E is a valid system for use in the identification of the more commonly occurring members of the family *Vibrionaceae* and the most accurate and efficient of the three systems tested.**

There has been a concerted effort to reduce the turnaround time for the identification of microorganisms isolated from clinical specimens by both manual and automated methods. Most of the effort has centered on the family *Enterobacteriaceae*, with the development of manual systems such as the Micro-ID (General Diagnostics, Div. Warner-Lambert Co., Morris Plains, N.J.) (1, 4, 7), the API 20E same-day identification (20E; Analytab Products, Plainview, N.Y.) (2, 8), and the recently introduced API Rapid E (RE; formerly DMS Rapid E) (9, 13, 15, 16). Automated systems, such as the Autobac-IDX (General Diagnostics) (5), the AutoMicrobic System (Vitek Systems, Inc., Hazelwood, Mo.) (11), and the Avantage and Quantum II systems (Abbott Diagnostics, Irving, Tex.) (12, 15), have also been introduced for the identification of members of the *Enterobacteriaceae*.

No systems have been developed specifically for the rapid identification of members of the family *Vibrionaceae*. The automated systems have made an attempt to include some members of the *Vibrionaceae* in their data bases. The Autobac-IDX identifies *Aeromonas* spp. (5). The AutoMicrobic System with the EBC+ card identifies *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* (11). The Avantage and Quantum II systems identify *A. hydrophila* and *P. shigelloides* (12, 15). However, since the primary emphasis of these instruments is identification of members of the *Enterobacteriaceae*, only 67 isolates of the family *Vibrionaceae* have been tested in recent evaluations of these four instruments (5, 11, 12, 15). In regard to manual systems, both the overnight 20E and the RE claim to be able to identify members of the *Vibrionaceae*. There are no reports in the literature to support this claim for either the 20E or the RE. There is another recently developed system, the API Rapid NFT (NFT; formerly DMS Rapid NFT), which identifies nonfermenting gram-negative bacilli (3) and is also supposed to identify oxidase-positive, fermentative, gram-negative bacilli. This report compares the abilities of the overnight 20E, the RE, and the NFT to identify the more commonly occurring members of the family *Vibrionaceae*.

## MATERIALS AND METHODS

Sixty isolates, from nine species of the family *Vibrionaceae*, were tested by the 20E, the RE, and the NFT procedures. Included in the 60 isolates were 7 *Aeromonas caviae* isolates, 27 *A. hydrophila* isolates, 10 *Aeromonas sobria* isolates, 3 *P. shigelloides* isolates, 3 *Vibrio alginolyticus* isolates, 3 *V. cholerae* isolates, 1 *Vibrio fluvialis* isolate, 5 *V. parahaemolyticus* isolates, and 1 *Vibrio vulnificus* isolate. The identity of each isolate was confirmed with standard biochemicals (10, 17, 18).

The 20E and the RE have been previously described (2, 4, 7, 9, 13, 15, 16) and will not be described here. The NFT is a plastic strip to which 8 microtubes for enzymatic tests and 12 microcupules for carbohydrate assimilation tests are attached. The assimilation cupules are similar to those on the API 20C used for yeast identification, while the enzymatic test microtubes are similar to those on the 20E. The inoculation and incubation procedures are described below. The tests are arranged in groups of three and, with octal numbers, a seven-digit profile number is generated for the 20 tests plus oxidase. The tests contained on the NFT strip are nitrate reduction, tryptophanase, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, beta-D-galactosidase with *p*-nitrophenyl-β-D-galactopyranoside as substrate, assimilation of glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconic acid, capric acid, adipic acid, malic acid, citric acid, and phenylacetic acid. The oxidase test is not performed on the strip. The Kovacs method (14) was used. All the required reagents are available from the manufacturer.

Each isolate was taken from the surface of a tryptic soy agar slant (Difco Laboratories, Detroit, Mich.), streaked onto the surface of a 5% sheep blood agar plate (tryptic soy agar base), and incubated overnight at 35°C. Culture purity was determined by examining the colony morphology and the oxidase activity (14) of the isolate on the sheep blood agar plate. One colony from the sheep blood agar plate was

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streaked onto a second sheep blood agar plate which was then incubated overnight at 35°C. Bacterial growth from the second sheep blood agar plate was used to inoculate the test systems as follows.

**20E.** Bacteria from one isolated colony were suspended in 5 ml of 0.85% sterile saline. This suspension was used to inoculate the 20E according to the instructions of the manufacturer. After 18 to 24 h of incubation at 35°C, reagents were added, and a seven-digit profile number was generated following the instructions of the manufacturer for test interpretation.

**RE.** Bacteria from one to two isolated colonies were suspended in 1.25 ml of 0.85% sterile saline to approximate the turbidity of a 0.5 McFarland barium sulfate standard. This suspension was used to inoculate the RE according to the instructions of the manufacturer. After 4 h of incubation at 35°C, reagents were added, and a seven-digit profile number was generated following the instructions of the manufacturer for test interpretation.

**NFT.** Bacteria from one to four isolated colonies were suspended in 2.0 ml of 0.85% sterile saline to approximate the turbidity of a 0.5 McFarland barium sulfate standard. This suspension was used to inoculate the NFT enzymatic tests according to the instructions of the manufacturer. Four drops of this suspension was also used to inoculate the NFT assimilation test medium. After thorough mixing, the assimilation test medium was used to inoculate the NFT assimilation tests according to the instructions of the manufacturer. After 24 h of incubation at 30°C, reagents were added, and a seven-digit profile number was generated following the instructions of the manufacturer for test interpretation. When the assimilation tests were weak or no identification was obtained after 24 h of incubation, the NFT was incubated for an additional 24 h at 30°C according to the instructions of the manufacturer. After the additional incubation period, a seven-digit profile number was determined following the instructions of the manufacturer for test interpretation at 48 h.

All identifications were made by finding the profile number in the appropriate API Analytical Profile index. When the profile number was not found, then the appropriate computer identification program was consulted. Each isolate was identified twice with each of the three test systems.

TABLE 1. Distribution of 20E identifications

| Organism | No. of isolates identified as: | | | | | | |
|---|---|---|---|---|---|---|---|
| | *A. hydrophila* | *P. shigelloides* | *V. alginolyticus* | *V. cholerae* | *V. fluvialis* | *V. parahaemolyticus* | *V. vulnificus* |
| *A. caviae* | 7 | | | | | | |
| *A. hydrophila* | 27 | | | | | | |
| *A. sobria* | 10 | | | | | | |
| *P. shigelloides* | | 3 | | | | | |
| *V. alginolyticus* | | | 3 | | | | |
| *V. cholerae* | | | | 3 | | | |
| *V. fluvialis* | | | | | 1 | | |
| *V. parahaemolyticus* | | | | | | 5 | |
| *V. vulnificus* | | | | | | | 1 |

TABLE 2. Distribution of RE identifications

| Organism | No. of isolates identified as: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Aeromonas* spp. | *P. shigelloides* | *V. alginolyticus* | *V. cholerae* | *V. fluvialis* | *V. parahaemolyticus* | *V. vulnificus* | Low selectivity, correct | No identification |
| *A. caviae* | 6 | | | | | | | 1 | |
| *A. hydrophila* | 23 | | | | | | | 4 | |
| *A. sobria* | 10 | | | | | | | | |
| *P. shigelloides* | | 3 | | | | | | | |
| *V. alginolyticus* | 1 | | 2 | | | | | | |
| *V. cholerae* | 3 | | | | | | | | |
| *V. fluvialis* | 1 | | | | | | | | |
| *V. parahaemolyticus* | | | | | | 2 | | 2 | 1 |
| *V. vulnificus* | | | | | | | | 1 | |

## RESULTS

None of the three identification systems was able to separate the three species of the genus *Aeromonas* because they do not contain all of the biochemical tests (10) necessary to do so.

The 20E correctly identified 100% (60 of 60) of the isolates, allowing for the fact that *A. caviae* and *A. sobria* were not in the 20E data base and were identified as *A. hydrophila* (*A. hydrophila* group, in the latest data base). Table 1 lists the distribution of the identifications of the 60 isolates as determined by the 20E.

The RE correctly identified 77% (46 of 60) of the isolates. The RE data base only contained *Aeromonas* species; therefore, genus-level identification had to be considered correct. The RE gave low selectivity identifications, in which the identification quality was <90.0% and one of the identification choices was correct, for 13% (8 of 60) of the isolates. The RE misidentified 8% (5 of 60) of the isolates. The RE was unable to identify the remaining 2% (1 of 60) of the isolates. Table 2 lists the distribution of the identifications of the 60 isolates as determined by the RE.

The NFT correctly identified 87% (52 of 60) of the isolates, allowing for the fact that *A. caviae* and *A. sobria* were not in the NFT data base and were identified as *A. hydrophila*. The NFT gave low selectivity identifications for 8% (5 of 60) of the isolates. The NFT misidentified 5% (3 of 60) of the isolates. For 15% (9 of 60) of the isolates, the NFT required 48 h of incubation to obtain a result. Table 3 lists the distribution of the identifications of the 60 isolates as determined by the NFT.

## DISCUSSION

The development of rapid methods in clinical microbiology has centered on the identification of members of the family *Enterobacteriaceae*. Rapid identification systems for members of the family *Vibrionaceae* would be desirable, as the types and incidence of clinically significant diseases caused by members of this family appear to be on the increase. Currently, the use of standard biochemicals (17, 18) for the identification of members of this family is considered the method of choice. A rapid screening procedure utilizing the immobilization of members of the genus *Vibrio*

TABLE 3. Distribution of NFT indentifications

| Organism | No. of isolates identified as: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *A. hydrophila* | *P. shigelloides* | *V. alginolyticus* | *V. cholerae* | *V. fluvialis* | *V. parahaemolyticus* | *V. vulnificus* | Low selectivity, correct | Low selectivity, incorrect[a] |
| *A. caviae* | 6 | | | | | | | 1 | |
| *A. hydrophila* | 27 | | | | | | | | |
| *A. sobria* | 10 | | | | | | | | |
| *P. shigelloides* | | 2 | | | | | | 1 | |
| *V. alginolyticus* | | | 1 | | | | | 2 | |
| *V. cholerae* | | | | 3 | | | | | |
| *V. fluvialis* | | | | | | | | 1 | |
| *V. parahaemolticus* | | | | | 1 | 3 | | | 1 |
| *V. vulnificus* | | | | 1 | | | | | |

[a] Identification quality <90.0%, all identification choices incorrect.

in distilled water has been described (6). However, members of the genera *Aeromonas* and *Plesiomonas* are not immobilized in distilled water.

The automated instruments Autobac-IDX, Avantage, Quantum II, and AutoMicrobic System with EBC+ cards have partially addressed the rapid identification of members of the *Vibrionaceae*. Published evaluations of these instruments (5, 11, 12, 15) have reported 100% agreement with conventional methods for 67 isolates of the four species of the *Vibrionaceae* which these instruments are programmed to identify.

The cost of automated instruments or the needed expendable supplies or both is prohibitive to many laboratories. Such laboratories may decide to use manual rapid systems such as Micro-ID or RE to identify members of the *Enterobacteriaceae*. Utilization of a system to rapidly identify the more commonly occurring members of the *Vibrionaceae* would be potentially very useful. Even the use of the 20E with overnight incubation could be considered a rapid method, when compared with the use of standard biochemicals.

The RE gave the lowest identification rate (77%), the highest low selectivity identification rate (13%), and the highest misidentification rate (8%) of the three systems. The data from this study suggest that, in its present state of development, the RE is not an acceptable method for the identification of the more commonly occurring members of the family *Vibrionaceae*.

The NFT was found to be better than the RE, with a higher identification rate (87%), a lower low selectivity identification rate (8%), and a slightly lower misidentification rate (5%). The NFT did not measure up to its name, Rapid NFT, for the 15% of the isolates that required 48 h of incubation to obtain a result. The data from this study suggest that, in its present state of development, the NFT is not an acceptable method for identification of the more commonly occurring members of the family *Vibrionaceae*.

Improvement in the RE and the NFT identification rates for members of the family *Vibrionaceae* should be expected when the number of species and the number of isolates of each species in the RE and the NFT data bases are increased. The RE and the NFT should be reevaluated at that time.

The 20E was found to be the best of the three systems tested with regard to accuracy and time efficiency. It identified 100% of the isolates after 18 to 24 h of incubation. The only problem was that all three species of the genus *Aeromonas* were identified as *A. hydrophila*. However, the RE and the NFT were also unable to identify the *Aeromonas* isolates to the species level. The data from this study suggest that the 20E is an acceptable method for the identification of the more commonly occurring members of the family *Vibrionaceae*.

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