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# Comparison of the API 20E and Corning N/F Systems for Identification of Nonfermentative Gram-Negative Rods

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A total of 231 strains of nonfermenting gram-negative rods were tested on the API 20E system, the Corning N/F system, and conventional media. When the results of identification to species were compared, the API system agreed with the conventional system on 69% of the isolates, and the Corning system agreed with the conventional system on 79% of the isolates. Both kit systems were deficient in identifying *Pseudomonas cepacia* and the more unusual isolates.

A new kit for the identification of nonfermentative gram-negative rods, the Corning N/F system (Corning Medical Diagnostics, Corning Glass Works, Roslyn, N.Y.), has been developed and is now on the market. In this study, the new kit was compared with the API 20E system (Analytab Products, Inc., New York, N.Y.) and conventional methods for the identification of these organisms.

## MATERIALS AND METHODS

**Bacteria tested.** The nonfermentative organisms used included 231 clinical isolates and stock cultures. A total of 120 clinical isolates were obtained from the University of Minnesota Hospitals Diagnostic Microbiology Laboratory. In addition, 111 stock cultures which had been stored in agar deeps in the dark at room temperature for various periods of time were used. All of the *Pseudomonas aeruginosa*, *P. maltophilia*, and *Acinetobacter* species were clinical isolates; all other organisms tested were a combination of recent isolates and stock organisms. The stock organisms were transferred and subcultured to 5% sheep blood agar plates three times before the biochemical tests were inoculated.

**Conventional tests.** The conventional tests were performed as recommended by the Center for Disease Control (CDC) (1) and included the following: oxidase; triple sugar iron agar; $H_2S$ production in triple sugar iron agar and lead acetate strips; esculin hydrolysis; nitrate reduction; indole; urease; Simmons citrate; gelatin liquefaction; pigmentation on Tech (Baltimore Biological Laboratory, Cockeysville, Md.) and Mueller Hinton agars; motility at room temperature; growth on MacConkey agar; growth on Salmonella-Shigella agar; growth on cetrimide agar; growth in 0 and 6% NaCl; growth at 25, 35, and 42°C; oxidation of dextrose and maltose (OF medium); and arginine dihydrolase. All tests were inoculated from an overnight heart infusion agar slant or broth incubated at 35°C, except where noted above. All tests were read at 24 and 48 h, and after 7 days before they were called negative, with the following exceptions: the oxidase test was performed and read at 10 s; the *o*-nitrophenyl-β-D-galactopyranoside test was read at 24 h; the indole and nitrate reagents were added, and the results of those tests were read at 48 h; and the gelatin was held for 14 days before a final reading was made. If needed, a flagella stain, penicillin susceptibility, other OF sugars, and phenylalanine deaminase tests were performed. All OF sugars were read for acid production, alkalinization, or no change. The organisms were identified by the CDC charts (3).

**API.** The API 20E strips were inoculated and read according to the directions of the manufacturer. In addition, an oxidase test was performed and an API-OF dextrose and a MacConkey agar were inoculated from a suspension of organisms in sterile 0.85% saline. These tests and the strips were incubated for 48 h at 35°C. After 48 h, a wet mount was made from the $H_2S$ cupule to check for motility, and nitrate reagents were added to the glucose cupule after checking for gas bubbles.

After the tests were performed and interpreted, a 9-digit profile number was obtained. The API profile index was consulted for identification. All extra tests were performed where recommended, and in instances in which the number was not listed in the profile index the API computer center was called for the identification or for recommended extra tests.

**Corning.** The Corning N/F system consists of two tubes, the N/F screen, and a round plate called the Uni-N/F-Tek, which is divided into 12 sections (Fig. 1). One tube (the GNF tube) is divided by a constriction; the upper slant detects fluorescein production, and the bottom section detects nitrite reduction to gas and glucose fermentation. This tube was incubated at 30°C for 24 h. The other tube (the 42P tube) detects pyocyanin production and growth at 42°C. It was also incubated for 24 h. These tubes were inoculated only if the test organism was oxidase positive. The plate consisted of five carbohydrates (glucose, xylose, mannitol, lactose, and maltose), acetamide, esculin, urea, deoxyribonucleic acid, *o*-nitrophenyl-β-D-galactopyranoside, and media for $H_2S$ and indole production. The plates were inoculated with oxidase-negative organisms or with oxidase-positive organisms if the N/F


FIG. 1. *Uninoculated Corning Uni-N/F-Tek plate.*

screen failed to identify the organism after 24 h. A heavy suspension of the organism was made in 0.5 ml of sterile water, and a drop was inoculated into each well of the plate with a sterile Pasteur pipette. The center section was then stabbed with the pipette for detection of $H_2S$. The plate was incubated at 35°C and read after 24 h of incubation according to the instructions of the manufacturer.

A two-digit profile was constructed from the N/F screen tubes. There were six reactions, including the oxidase test, to be considered, and these were grouped into two sets of three reactions each. In each set, the first, second, and third reactions had values of 4, 2, and 1, respectively. The positive reactions in each set were added together to give a two-digit number, and the Corning profile screen book was consulted for identification. If the oxidase-positive organism could not be identified at this point, the Uni-N/F-Tek plate was inoculated. After the reading, the 12 tests on the plate were divided into sets of 3 reactions each and were allocated values based on the 4, 2, 1 scheme. The positive reactions were added for each set to obtain four additional digits, resulting in a final number of six digits. When the N/F screen was not performed, as with oxidase-negative organisms, the first two digits were 00. The Corning profile code book was consulted for identification. All recommended extra tests were performed, and the identification was obtained. The corning consulting number was called if the profile number was not listed.

## RESULTS

Table 1 shows the agreement of the conventional system with the API and Corning systems. There was 69% agreement in identification to species level with API, and 79% agreement with Corning. In general, Corning and API performed well with the same organisms and failed to correctly identify the same organisms, including the CDC groups, *Comamonas terrigena*, *P. cepacia*, and others.

TABLE 1. *Agreement among conventional, API, and Corning identification to species level*

| Conventional identification | No. tested | No. of agreements | |
|---|---|---|---|
| | | API | Corning |
| *Achromobacter xylosoxidans* | 8 | 7 | 6 |
| *Acinetobacter calcoaceticus* | 31 | 28 | 30 |
| *Alcaligenes denitrificans* (V-c) | 1 | 0 | 1 |
| *A. faecalis* | 1 | 0 | 1 |
| *A. odorans* | 6 | 1 | 6 |
| *Alcaligenes* sp. | 5 | 5 | 4 |
| *Bordetella bronchiseptica* | 6 | 6 | 6 |
| CDC group IV C-2 | 2 | 2 | 0 |
| CDC group V E-1 | 1 | 0 | 0 |
| CDC group II F | 1 | 0 | 0 |
| CDC group II K | 1 | 0 | 1 |
| CDC group II J | 1 | 0 | 0 |
| *Comamonas terrigena* | 6 | 0 | 2 |
| *Eikenella corrodens* | 1 | 0 | 0 |
| *Flavobacterium odoratum* (M4-F) | 1 | 1 | 1 |
| *Flavobacterium* sp. (IIB) | 8 | 5 | 6 |
| *Moraxella nonliquefaciens* | 1 | 0 | 1 |
| *M. osloensis* | 4 | 0 | 0 |
| *M. phenylpyruvica* | 3 | 0 | 2 |
| *M. urethralis* | 2 | 0 | 0 |
| *Moraxella* sp. | 8 | 8 | 5 |
| *Pseudomonas aeruginosa* | 52 | 40 | 47 |
| *P. alcaligenes* | 2 | 0 | 0 |
| *P. cepacia* | 13 | 6 | 7 |
| *P. diminuta* | 2 | 0 | 2 |
| *P. fluorescens* | 4 | 2 | 0 |
| *P. fluorescens* group/*P. putida* | 17 | 13 | 17 |
| *P. maltophilia* | 28 | 26 | 27 |
| *P. putrefaciens* | 1 | 1 | 1 |
| *P. stutzeri* | 6 | 5 | 5 |
| *Pseudomonas* sp. | 5 | 4 | 5 |
| Total | 231 | 160 (69%) | 183 (79%) |

Table 2 lists the major differences between API and conventional methods. Major differences were defined as misidentification at species or genus level, whereas a minor difference was the reporting of the genus only, even though the genus reported agreed with conventional identification. Of the 52 *P. aeruginosa* isolates identified by the conventional system, the API

TABLE 2. *Major identification differences between the conventional and API systems for 42 organisms identified to species level*[a]

| Conventional identification | API identification |
|---|---|
| *Achromobacter xylosoxidans* (1) | *Alcaligenes* sp. (1) |
| *Acinetobacter calcoaceticus* (3) | *Pseudomonas paucimobilis* (1), *Pseudomonas* sp. (2) |
| *Alcaligenes denitrificans* (V-c) (1) | *Pseudomonas* sp. (1) |
| CDC group II-F (1) | No identification |
| CDC group II-J (1) | CDC group II-F (1) |
| CDC group II-K (1) | *Pseudomonas* sp. (1) |
| CDC group VE-1 (1) | *P. maltophilia* (1) |
| *Comamonas terrigena* (3) | *Alcaligenes* sp. (3) |
| *Eikenella corrodens* (1) | *Moraxella* sp. (1) |
| *Flavobacterium* sp. (IIB) (3) | *F. odoratum* (M4-F) (1), *F. meningosepticum* (1), *P. fluorescens* (1) |
| *Moraxella phenylpyruvica* (1) | CDC group II-F (1) |
| *Pseudomonas aeruginosa* (12) | *P. fluorescens* (5), *P. fluorescens* group (3), *P. maltophilia* (1), *Pseudomonas* sp. (1), *P. stutzeri* (1), unacceptable profile (1) |
| *P. alcaligenes* (2) | *Alcaligenes* sp. (2) |
| *P. cepacia* (7) | CDC group VE-2 (1), *P. maltophilia* (1), *Pseudomonas* sp. (2), *P. stutzeri* (1), unacceptable profile (1) |
| *P. fluorescens* group/*P. putida* (1) | *P. stutzeri* (1) |
| *P. maltophilia* (2) | *A. calcoaceticus* (1), *P. cepacia* (1) |
| *Pseudomonas* sp. (1) | *Chromobacterium* sp. (1) |

[a] Number in parentheses indicates the number of organisms with this identification.

system identified only 40 to the species level. Six of 13 of the *P. cepacia* were identified correctly by API. In one other instance, an organism was identified as *P. cepacia* by API, but it was a *P. maltophilia*. In addition to the major differences listed in Table 2, minor differences were reported for 29 organisms. These included: one *Alcaligenes faecalis* and six isolates of *A. odorans* reported as *Alcaligenes* sp.; three *Comamonas terrigena* reported as *Pseudomonas* sp.; one *Moraxella nonliquefaciens* reported as *Moraxella* sp.; four *M. osloensis* reported as *Moraxella* sp.; two *M. urethralis* reported as *Moraxella* sp.; and two *M. phenylpyruvica* reported as *Moraxella* sp. In addition, three *Pseudomonas denitrificans*, one *P. diminuta*, two *P. fluorescens*, and one *P. stutzeri* were reported as *Pseudomonas* sp. Three *P. fluorescens* group/*P. putida* were reported as *P. fluorescens*.

Table 3 lists the major differences in identification between the Corning system and the conventional methods. Only 7 of 13 *P. cepacia* isolates were correctly identified by Corning. The only minor difference was with one *M. osloensis*

TABLE 3. *Major identification differences between the conventional and Corning systems for 47 organisms identified to the species level*[a]

| Conventional identification | Corning identification |
|---|---|
| *Achromobacter xylosoxidans* (2) | *Alcaligenes denitrificans* (2) |
| *Acinetobacter calcoaceticus* (1) | *A. odorans* (1) |
| *Alcaligenes* species (1) | *Pseudomonas testosteroni* (1) |
| CDC group II-F (1) | *A. odorans* (1) |
| CDC group II-J (1) | *A. odorans* (1) |
| CDC group IV-C (2) | *Bordetella bronchiseptica* (2) |
| CDC group VE-1 (1) | *P. maltophilia* (1) |
| *Comamonas terrigena* (4) | *A. odorans* (2), *A. denitrificans* (1), *P. diminuta* (1) |
| *Eikenella corrodens* (1) | *Moraxella osloensis* (1) |
| *Flavobacterium* sp. (IIB) (2) | M-4F (1), *A. odorans* (1) |
| *Moraxella osloensis* (3) | *E. corrodens* (1), *M. nonliquefaciens* (2) |
| *M. phenylpyruvica* (1) | *P. diminuta* (1) |
| *M. urethralis* (2) | *M. phenylpyruvica* (2) |
| *Moraxella* sp. (3) | *E. corrodens* (3) |
| *Pseudomonas aeruginosa* (5) | *P. stutzeri* (2), *P. acidovorans* (1), *P. mendocino* (1), *P. putida* (1) |
| *P. alcaligenes* (2) | *A. odorans* (1), *P. testosteroni* (1) |
| *P. cepacia* (6) | *A. calcoaceticus* (1), *A. denitrificans* (1), *A. odorans* (1), II K-1 (1), VE-2 (1), *P. maltophilia* (1) |
| *P. denitrificans* (3) | *A. denitrificans* (2), *P. pseudoalcaligenes* (1) |
| *P. fluorescens* (4) | *P. aeruginosa* (1), *P. fluorescens* group/*P. putida* (3) |
| *P. maltophilia* (1) | *A. calcoaceticus* (1) |
| *P. stutzeri* (1) | *P. pseudoalcaligenes* (1) |

[a] Number in parentheses indicates the number of organisms with this identification.

isolate, which was reported by Corning as *Moraxella* sp.

There were five organisms for which the identification by Corning and API agreed with each other but were different from the conventional results. Three of these were major disagreements and included one CDC group VE-1 identified by API and Corning as *P. maltophilia*, one *Flavobacterium* sp. (IIB) identified by API and Corning as M4-F, and one *P. aeruginosa* identified by the kits as *Moraxella* sp.

In 21 instances there was no agreement among the three methods. These differences were both major and minor (Table 4). If we exclude these 21 organisms from the total in Table 1, then the agreement for the API and Corning methods would be 76 and 87%, respectively. If we also exclude the five organisms for which the Corning and API methods agreed with each other but disagreed with the conventional system, the agreement then rises to 78% for API and 89% for Corning. The identity of these 26 organisms was not determined by an outside laboratory.

Of the organisms identified by the API system, 53 (23%) required extra tests, with the flagella stain most often required. Other supplemental tests included, in order of frequency: 42°C growth, acetamide, motility, cetrimide growth, Salmonella-Shigella agar growth, MacConkey growth, morphology, deoxyribonuclease production, and fluorescein production. In total, 90 extra tests were required.

The Corning system required supplemental tests on 57 (25%) of the organisms. The tests required, in order of frequency, were: motility; nitrate reduction; flagella stain; phenylalanine deaminase; arginine dihydrolase; growth at 42°C; gelatin liquefaction; morphology; growth on Salmonella-Shigella agar; growth on MacConkey agar; and polymyxin B susceptibility. In total, 115 extra tests were required.

Of the 231 organisms tested, 41 (18%) profiles were called to the API consulting center, and 4 were called to the Corning consulting center (less than 2%).

## DISCUSSION

In this study, agreement between API and the conventional method was 69% for identification to species level. However, with 29 additional organisms the differences were only minor and were related to the fact that API does not identify *Moraxella* sp., *Alcaligenes* sp., and some *Pseudomonas* sp. to species level. When this is considered, the percent agreement approaches that achieved by the Corning system. Both kits were able to satisfactorily identify the usual clinical isolates, but as in a previous study with the API system (2) they were not able to identify the more uncommon clinical isolates. API was able to identify 85% of the three common clinical isolates (*P. aeruginosa*, *A. calcoaceticus*, and *P. maltophilia*), whereas Corning correctly identified 94% of this group. For the balance of the organisms studied, i.e., the more unusual types, API identified 55%, whereas Corning identified 65% accurately.

Only six of the *P. cepacia* isolates were identified correctly by API. In contrast, one isolate of *P. maltophilia* was identified incorrectly as a

TABLE 4. *Identification differences among the conventional, Corning, and API systems*

| Conventional identification | Corning identification | API identification |
|---|---|---|
| *Acinetobacter calcoaceticus* | *Pseudomonas paucimobilis* | *Alcaligenes odorans* |
| CDC group II-F | Unacceptable profile | *A. odorans* |
| CDC group II-J | II-F | *A. odorans* |
| *Comamonas terrigena* | *Alcaligenes* sp. | *A. odorans* |
| *Eikenella corrodens* | *Moraxella* sp. | *M. osloensis* |
| *M. osloensis* | *Moraxella* sp. | *E. corrodens* |
| *M. phenylpyruvica* | II-F | *P. diminuta* |
| *M. urethralis* | *Moraxella* sp. | *M. phenylpyruvica* |
| *P. aeruginosa* | *P. fluorescens* | *P. acidovorans* |
| *P. alcaligenes* | *Alcaligenes* sp. | *A. odorans* |
| *P. alcaligenes* | *Alcaligenes* sp. | *P. testosteroni* |
| *P. cepacia* | *Pseudomonas* sp. | *A. denitrificans* |
| *P. cepacia* | VE-2 | *A. odorans* |
| *P. cepacia* | *P. stutzeri* | *P. maltophilia* |
| *P. cepacia* | Unacceptable profile | CDC group VE-2 |
| *P. cepacia* | *P. maltophilia* | *A. anitratus* |
| *P. denitrificans* | *Pseudomonas* sp. | *A. denitrificans* |
| *P. denitrificans* | *Pseudomonas* sp. | *A. denitrificans* |
| *P. denitrificans* | *Pseudomonas* sp. | *P. pseudoalcaligenes* |
| *P. fluorescens* | *Pseudomonas* sp. | *P. fluorescens/P. putida* |
| *P. stutzeri* | *Pseudomonas* sp. | *P. pseudoalcaligenes* |

*P. cepacia*, probably because these organisms are biochemically similar on API. *P. cepacia* is generally very resistant to antibiotics, and incorrect identification could be a major problem.

An inconvenient problem that arose with the API system was the necessity of calling the API computer center for aid on 41 organisms whose profile numbers were not present in the index. Some of these were identified by the call, but others required supplemental tests.

The Corning system also did not identify the *P. cepacia* organism very well, identifying only 7 of the 13. Another identification problem with the Corning system was that CDC group IV C-2 is not in the profile book and consequently is identified as *Bordetella bronchiseptica*. These two organisms are biochemically very similar, but CDC group IV C-2 does not grow on Salmonella-Shigella agar and usually does not reduce nitrate. Both organisms are rapidly urease positive. The API system does recognize both of these organisms.

One technical problem which arose with the Corning kit was reading nitrite reduction to gas in the GNF tube. Any bubble was read as gas positive, even though occasionally bubbles could inadvertently be introduced during inoculation. This was a problem particularly when the organism was identified as a *Moraxella* sp. by the conventional method, but a gas-positive reading in the N/F tube excluded *Moraxella* sp. and made *Eikenella corrodens* the only possibility. Most of these organisms did not resemble *E. corrodens* morphologically, and an astute microbiologist would probably recognize the problem and not identify the organisms incorrectly. All other biochemical reactions are similar for these two organisms.

The Corning identification system would be improved if the flagella stain were added more frequently to the supplemental tests required to help in the differentiation of *Alcaligenes* sp., *P. alcaligenes*, and *P. denitrificans*.

A problem which arises in any evaluation of nonfermentative organism identification systems is the necessity of using stock organisms. These tend to be much less reactive biochemically than fresh patient isolates. The fresh patient isolates we used, notably *P. aeruginosa*, *P. maltophilia*, and *Acinetobacter* sp., were the most likely to agree among all three methods in this study. However, since it was desired to compare how the more uncommon nonfermentative organisms reacted on these systems, it was necessary to use stock cultures.

It is difficult to determine which of these kit systems is better. The Corning system agreed with the conventional identification slightly more often, but the API system can be used for both fermenters and nonfermenters without a need to differentiate on the primary plate, and this must be considered an advantage.

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