Evaluation of the API 20E System for Identification of Nonfermentative Gram-Negative Bacteria

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The API 20E system for Enterobacteriaceae, recently broadened to include identification of nonfermentative gram-negative bacteria, was evaluated and compared with the conventional method for complete identification of 221 nonfermenters, which were well distributed into 48 species or biotypes and included organisms not listed in the API 20E data base. The results of 16 tests common to both systems were in close agreement. The API 20E system correctly identified 71 (43%) of the 165 organisms included in the API 20E data base. However, almost 90% of Acinetobacter calcoaceticus, three species of Pseudomonas, and Bordetella bronchiseptica were correctly identified to species.

The API 20E system is a plastic strip with microtubes containing dehydrated substrates, originally designed for the identification of Enterobacteriaceae. Later, API introduced the Profile Recognition System for numerical identification and then, using a computer-assisted program, developed the Analytical Profile Index, supplemented by the API Computer System Service. In 1976, the Analytical Profile Index for API 20E was expanded to include other fermentative and nonfermentative gram-negative bacteria. Five separate tests, not included in the strip, were then added to complete the system for identification of the nonfermenters.

Various investigators (1, 5, 10, 12) evaluated the API 20E system for the identification of Enterobacteriaceae and reported a high level of agreement with conventional methods in both biochemical reactions and identifications. The Analytical Profile Index (or Register) has also been mathematically evaluated as excellent (7). Two evaluations have been made of the complete API 20E system, including the five separate tests—fermentation of glucose (OFF), oxidation of glucose (OFO), motility, oxidase, and MacConkey—for identification of nonfermentative gram-negative bacteria (3, 6). Both reports found the system useful for identification of clinical isolates of Pseudomonas and Acinetobacter. We have now evaluated the system, which includes the API 20E and the five separate tests, for the complete identification (to species or biotypes) of 221 isolates of nonfermentative gram-negative bacteria as required by a reference laboratory.

MATERIALS AND METHODS

Bacteria. Of the 221 isolates used in this study, 166 were from a culture collection maintained by the Division of Laboratories and Research, New York State Department of Health. All isolates originated from clinical specimens that had been submitted to our laboratory for identification or confirmation. Forty-seven of the cultures were received through the courtesy of Analytab Products Inc., Plainview, New York. Eight were kindly provided by G. L. Gilardi, Hospital for Joint Diseases and Medical Center, New York City. In our laboratory all cultures were either lyophilized or maintained on blood agar slants.

The organisms used in the evaluation were nonfermentative gram-negative rods, well distributed among 48 species and including 56 isolates not listed in the API 20E data base. No fermentative organisms were used.

Conventional media and procedures. The media were prepared by the Division’s media section as described previously (9). The inoculated media were incubated for up to 5 days at 35 to 37°C. The organisms were identified by using generally accepted criteria (2, 4, 11, 13).

API 20E system for nonfermenters. The API 20E strip (this strip is the same one used for the identification of Enterobacteriaceae) contains 20 microtubes with substrates for the following 23 tests: O-nitrophenyl-β-D-galactosidase (ONPG); arginine dihydrolase; lysine and ornithine decarboxylase; citrate utilization; hydrogen sulfide; urease; tryptophan deaminase; indole; Voges-Proskauer (acetoin); gelatin liquefaction; fermentation of the carbohydrates glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose; nitrate reduction and nitrogen gas production, tested in the glucose microtube; and catalase production, in any other carbohydrate microtube. The catalase test was not used in this study. A complete description of the strip is given in other reports (1, 5, 10, 12).

Additional media are required for the five separate tests not on the strip. Media for three of these tests are available from the manufacturer in snap-open ampules: API M for the motility test and API OF for both the glucose oxidation and glucose fermentation tests. Also available is the API oxidase test kit con-
taining oxidase reagent (a 1% solution of tetrathiomethyl-
-p-phenylenediamine dihydrochloride) and plastic
chambers with filter paper. Conventional MacConkey
agar was used for the fifth separate test. In this study,
methodology for the use of the system and interpre-
tation of results were according to the manufacturer’s
recommendations.

Twenty-two of the initial tests were checked and
recorded after 24 h of incubation and again at 48 h. At
this point, reagents were added for the five remaining
tests (tryptophan deaminase, indole, Voges-Proskauer,
nitrate reduction, and nitrogen gas production), and
those results were recorded. The Analytical Profile
Index and (if necessary) the computer service were
consulted for identification of the isolates. When indi-
cated, supplemental tests recommended by the manu-
facturer for identification were done using conven-
tional media. When sufficient reactions were clear at
24 h, identification was determined on those readings.

RESULTS

Biochemical reactions of the 221 isolates in the
API 20E system (excluding fermentative carbohydrate
tests not used in the conventional method) were in close agreement with conven-
tional method results (Table 1). The lowest agreement was in the citrate util-
ization and motility tests.

The level of identification expected to be at-
tained by the API system for all 221 isolates was
compared with the actual identification achieved (Table 2). Of the organisms included in the API data base (165 isolates), 43% were correctly identified to the level expected by the manufacturer, and only 29% were completely identified to species and biotype. The system misidentified 22.4% of the organisms included in the API 20E charts and 32.2% of those not listed.

Unidentified by the system were 24.8% of the
organisms included and 26.8% of those not listed.

An analysis of the reactions and identification
obtained with the API system for each of the 48
conventionally identified species used in this evalua-
tion is given in Table 3. Of the 221 organi-
isms tested, 119 (53.8%) required from one to

**Table 1. Comparison of 16 biochemical reactions
of 221 isolates, using the API 20E and conventional (C) tests**

<table>
<thead>
<tr>
<th>Tests</th>
<th>No. in agreement</th>
<th>No. differing</th>
</tr>
</thead>
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<tr>
<td></td>
<td>API+, C+</td>
<td>API+, C+</td>
</tr>
<tr>
<td></td>
<td>API-, C+</td>
<td>API-, C+</td>
</tr>
<tr>
<td>ONPG</td>
<td>195</td>
<td>14</td>
</tr>
<tr>
<td>Arginine dihydrolosae</td>
<td>195</td>
<td>2</td>
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<td>Lysine decarboxylase</td>
<td>208</td>
<td>9</td>
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<tr>
<td>Ornithine decarboxylase</td>
<td>213</td>
<td>2</td>
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<tr>
<td>Citrate utilization</td>
<td>74</td>
<td>36</td>
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<td>Hydrogen sulfide</td>
<td>219</td>
<td>0</td>
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<tr>
<td>Urease</td>
<td>168</td>
<td>0</td>
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<tr>
<td>Tryptophan deaminase</td>
<td>195</td>
<td>23</td>
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<tr>
<td>Indole</td>
<td>210</td>
<td>0</td>
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<tr>
<td>Gelatin liquefaction</td>
<td>163</td>
<td>30</td>
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<tr>
<td>Oxidase</td>
<td>28</td>
<td>8</td>
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<tr>
<td>Nitrate to nitrate (NO₂ to NO₃)</td>
<td>148</td>
<td>6</td>
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<tr>
<td>Nitrate to gas (NO₂ to gas)</td>
<td>162</td>
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<tr>
<td>Motility</td>
<td>93</td>
<td>25</td>
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<tr>
<td>Glucose, oxidative (OFO)</td>
<td>150</td>
<td>32</td>
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<tr>
<td>Glucose, fermentative (OFF)</td>
<td>217</td>
<td>4</td>
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</table>

+ Positive reaction; −, negative reaction.

**Table 2. Extent of identification of 221 isolates by the API system**

<table>
<thead>
<tr>
<th>API's expected level of identification</th>
<th>No. of isolates</th>
<th>API identification [no. (%)]</th>
<th>Complete identification (as required by our reference lab) [no. (%)]</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>To expected level</td>
<td>Correct only to genus</td>
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<tr>
<td>Genus*</td>
<td>64</td>
<td>23 (35.9)</td>
<td>—b</td>
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<tr>
<td>Species†</td>
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<td>47 (54.0)</td>
<td>8 (9.2)</td>
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<tr>
<td>Biotype‡</td>
<td>14</td>
<td>1 (7.1)</td>
<td>8 (57.2)</td>
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<tr>
<td>Total</td>
<td>165</td>
<td>71 (43.0)</td>
<td>16 (9.7)</td>
</tr>
<tr>
<td>No expected identification (not included in API system)</td>
<td>56</td>
<td>23 (41.1)</td>
<td>18 (32.2)</td>
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<tr>
<td>Total</td>
<td>221</td>
<td>71 (32.1)</td>
<td>39 (17.6)</td>
</tr>
</tbody>
</table>

* Includes organisms designated “genus-like” when identified as that genus, e.g., Alcaligenes-like group IVc identified as Alcaligenes sp.

b For the 64 isolates in this category, “to genus only” was the expected level of identification.

c Unnamed organisms included in species and biotype whenever applicable.
<table>
<thead>
<tr>
<th>Organism</th>
<th>API’s expected level of identification</th>
<th>No. of isolates tested</th>
<th>No. requiring supplemental tests (tests per isolate)</th>
<th>No. correctly identified to: Genus* Species Bio-type</th>
<th>No. referred to computer</th>
<th>Incorrect identifications*</th>
<th>Incorrect reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achromobacter xylosoxidans IIIA</td>
<td>Species</td>
<td>10</td>
<td>5 (4), 5 (5)</td>
<td>0</td>
<td>0</td>
<td>1 Unidentified</td>
<td>Cetrimide –, gr. 41°C at 42°C</td>
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<td>A. xylosoxidans IIIB</td>
<td>Species</td>
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<td>1 (1), 2 (2)</td>
<td>1</td>
<td>4</td>
<td>5 Alcaligenes sp.</td>
<td>Cetrimide –</td>
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<td>Species</td>
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<td>1 (4), 1 (5)</td>
<td>1</td>
<td>2</td>
<td>5 Alcaligenes sp.</td>
<td>Cetrimide –</td>
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<td>Achromobacter sp. bio-type 2</td>
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<td>5</td>
<td>1 (2), 1 (5)</td>
<td>2</td>
<td>4</td>
<td>1 Alcaligenes sp.</td>
<td>Cetrimide –</td>
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<tr>
<td>Acinetobacter calcoaceticus subsp. anitratus</td>
<td>Species (sub-species)</td>
<td>10</td>
<td>1 (1), 1 (2)</td>
<td>2</td>
<td>7*</td>
<td>1 Unidentified</td>
<td>Acetamide +, gr. on SS –</td>
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<tr>
<td>A. calcoaceticus subsp. lwoffi</td>
<td>Species (sub-species) Genus</td>
<td>10</td>
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<td>0</td>
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<td>Unacceptable profile number</td>
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<td>Alcaligenes denitrificans</td>
<td>Genus</td>
<td>5</td>
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<td>0</td>
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<td>OPO –</td>
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<td>Genus</td>
<td>5</td>
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<td>4</td>
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<td>1 Pseudomonas sp.</td>
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<td>1 Unidentified</td>
<td>Gr. at 42°C –</td>
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<tr>
<td>Alcaligenes-like group IVc-2</td>
<td>Genus</td>
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<td>1</td>
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<td>1 Unidentified</td>
<td>Gr. at 42°C –</td>
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<tr>
<td>Bordetella bronchiseptica</td>
<td>Species</td>
<td>7</td>
<td>1 (3), 1 (4)</td>
<td>7</td>
<td>1</td>
<td>1 Unidentified</td>
<td>Gr. at 42°C –</td>
</tr>
</tbody>
</table>

*Genus* and *Species* refer to the identification level reached by the API 20E system. *Bio-type* indicates the level of specificity within the species. *Incorrect identifications* and *Incorrect reactions* list potential errors or reactions that did not match expected outcomes.
<table>
<thead>
<tr>
<th>Organism</th>
<th>API's expected level of identification</th>
<th>No. of isolates tested</th>
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<th>No. referred to computer</th>
<th>Incorrect identifications</th>
<th>Incorrect reactions</th>
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<td><em>Eikenella corrodens</em></td>
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<td>LDC -, ODC -, NO$_3$ to NO$_2$ -, MOT +</td>
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<td></td>
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<td>2 <em>Moraxella</em> sp.</td>
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<td><em>Moraxella</em> sp.</td>
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<tr>
<td><em>Flavobacterium meningosepticum</em></td>
<td>Genus</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>2 (4)</td>
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<td>Group M-1</td>
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<td>0</td>
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<td>MOT + ADH +</td>
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<td></td>
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<td>MOT + NO$_3$ to gas +</td>
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<td></td>
<td></td>
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<td>MOT +</td>
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<tr>
<td></td>
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<td>MOT +</td>
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<td><em>M. nonliquefaciens</em></td>
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<td>3</td>
<td>1 (5)</td>
<td>0</td>
<td>1</td>
<td>1 *CDC group IIk-1</td>
<td>MOT +</td>
</tr>
<tr>
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<td>1</td>
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<td>NO$_3$ to gas +</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 *B. bronchiseptica</td>
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<td>1 (3), 5 (4)</td>
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<td>Gr. on SS +</td>
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*Note: The table continues with additional data not fully visible in the image.*
<table>
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<tr>
<th>Organism</th>
<th>API's expected level of identification</th>
<th>No. of isolates tested</th>
<th>No. requiring supplemental tests (tests per isolate)</th>
<th>No. correctly identified to:</th>
<th>No. referred to computer</th>
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<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>P. testosteroni</strong></td>
<td>Genus</td>
<td>7</td>
<td>5 (4), 2 (5)</td>
<td></td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Pseudomonas-like group IIk, biotype 1</strong></td>
<td></td>
<td>5</td>
<td>1 (4)</td>
<td></td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
Table 3—Continued

<table>
<thead>
<tr>
<th>Organism</th>
<th>API's expected level of identification</th>
<th>No. of isolates tested</th>
<th>No. requiring supplemental tests (tests per isolate)</th>
<th>No. correctly identified to:</th>
<th>No. referred to computer</th>
<th>Incorrect identifications*</th>
<th>Incorrect reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas-like group Va, biotype 1</td>
<td>Not included</td>
<td>2</td>
<td>1 (4)</td>
<td>0</td>
<td>2</td>
<td>1 Achromobacter sp.</td>
<td>ONPG +, OPO -</td>
</tr>
<tr>
<td>Pseudomonas-like group Va, biotype 2</td>
<td>Not included</td>
<td>1</td>
<td>1 (5)</td>
<td>0</td>
<td>1</td>
<td>1 CDC group Ve-2</td>
<td>OXI -</td>
</tr>
<tr>
<td>Pseudomonas-like group Ve, biotype 1</td>
<td>Biotype</td>
<td>2</td>
<td>1 (2), 1 (4)</td>
<td>2</td>
<td>2</td>
<td>1 P. cepacia</td>
<td>OXI + (weak)</td>
</tr>
<tr>
<td>Pseudomonas-like group Ve, biotype 2</td>
<td>Biotype</td>
<td>3</td>
<td>1 (1)</td>
<td>2</td>
<td>2</td>
<td>1 Pseudomonas sp.</td>
<td>Gr. at 42°C -</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>A. calcoaceticus</td>
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</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>subsp. anitratus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 F. maltophilia</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td>1 P. putida</td>
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</tr>
<tr>
<td>Camphylobacter fetus (Vibrio fetus)</td>
<td>Not included</td>
<td>1</td>
<td></td>
<td>0</td>
<td>1</td>
<td>A. calcoaceticus</td>
<td></td>
</tr>
</tbody>
</table>

* Incorrect identifications found by the system combined with the supplemental tests.
* Includes organisms designated "genus-like" when identified as that genus, e.g., group IVe identified as Alcaligenes sp.
* gr., Growth.
* ADH, arginine dihydrolase; CIT, citrate; GEL, gelatin; LDC, lysine decarboxylase; MAC, growth on MacConkey agar; MOT, motility; NO₃ to NO₂, reduction of nitrate to nitrite; NO₂ to NO₃, reduction of nitrite to nitrogen gas; ODC, ornithine decarboxylase; OXI, oxidase.
* 7/10 isolates correctly identified to variety, 9/10 correct to species.
* In the API 20E system, commonly isolated species of Pseudomonas are usually identified to species, but they may be identified by a group designation. All Pseudomonads in the system are categorized as members of three groups, two of which overlap. The less commonly isolated species are identified by their group designation only.
five supplemental tests. Eighty-one isolates generated profile numbers not found in the Analytical Profile Index and had to be referred to API's computer service, yet 63 of these were in categories included in the API 20E system. Of the 63, only 26 were correctly identified to the API system's expected level of identification (15 requiring supplemental tests), while 23 were misidentified (12 requiring supplemental tests) and 14 remained unidentified (13 requiring supplemental tests).

DISCUSSION

We used 221 isolates which belong to 48 species and biotypes of nonfermentative gram-negative rods, 15 of which are not included in the API 20E system charts. The results of the initial API 20E biochemical tests (excluding the carbohydrate fermentation tests) showed close agreement with the conventional method results. This compares with the close agreement for the same common tests as reported by Smith et al. (10) in a study using only Enterobacteriaceae.

Despite this close agreement in biochemical reactions, only 110 (50%) of the 221 isolates were correctly identified to genus, and only 48 (22%) to species and biotype. However, the API 20E system does not claim to completely identify all nonfermenter isolates used in this study to genus, species, and biotype. When the expected level of identification was based on the Analytical Profile Index and reaction chart, 43% of the isolates included in the system were correctly identified.

All isolates of Pseudomonas aeruginosa, P. cepacia, and Bordetella bronchiseptica and most of the two subspecies of Acinetobacter calcoaceticus and P. maltophilia isolates were correctly identified to species by the API 20E system. Three of these organisms (P. aerugi-nosa and Acinetobacter species) are among those more commonly found in clinical specimens.

These findings suggest that the API 20E may be useful for the identification of the nonfermentative gram-negative bacteria more commonly encountered in the clinical laboratory, but, as with the Oxi/Ferm tube system (8) the API system is not suitable for use by reference laboratories.

LITERATURE CITED