A new kit system, Micro-ID (General Diagnostics, Morris Plains, N.J.), provides for identification of organisms in the family Enterobacteriaceae within 4 h. Aldridge et al. (1) compared the original Micro-ID system with the API 20E overnight kit (Analytab Products, Inc., Plainview, N.Y.) and with a conventional system. They found an 83% correlation in identification between API and Micro-ID and an 82% correlation between the three systems. Since the time of that evaluation a new data base has been developed, based on Micro-ID reactions. We present here an evaluation of the new data base identification as compared to identification using API 20E. In addition, selected Micro-ID biochemical reactions were compared to conventional tests.

MATERIALS AND METHODS

Organisms. In the comparison of selected biochemical tests, 608 organisms of the Enterobacteriaceae were tested. Of these, 376 were fresh clinical isolates, and 232 were stock organisms that had been stored in Trypticase soy agar deeps in the dark at room temperature. The organisms used are shown in Table 1.

In the clinical evaluation of the new Micro-ID identification system, 304 organisms were tested; of these, 230 were consecutive fresh clinical isolates and 74 were stock cultures. These stocks were not the ones used in the biochemical study.

Prior to testing by any system, all stock cultures were subcultured two to three times on sheep blood agar.

Micro-ID. The Micro-ID system was supplied by General Diagnostics and consists of a molded styrene tray containing 15 chambers and a hinged lid. The first five chambers contain a paper substrate disk and a paper reagent disk in separate wells; the tests in these chambers are Voges-Proskauer, nitrate reduction, phenylalanine deaminase, H2S, and indole. The remaining chambers contain single paper disks containing both substrate and detection reagent; these include tests for ornithine decarboxylase, lysine decarboxylase, malonate utilization, urease, esculin hydrolysis, o-nitrophenyl-β-D-galactopyranoside, and arabinose, adonitol, inositol, and sorbitol fermentation.

For inoculation of the Micro-ID system, only oxidase-negative organisms were used. Organisms were taken from 18- to 24-h cultures on either 5% sheep blood agar or MacConkey agar. Growth was emulsified in 3.5 ml of 0.85% NaCl to match a no. 0.5 McFarland standard. With stock cultures, the suspension was made to match a no. 2 McFarland standard. Each well of the Micro-ID tray was inoculated with 0.2 ml of the standardized suspension. After inoculation the trays were placed upright in a plastic holder (five trays per holder) and were incubated in a 35°C incubator for 4 h. After incubation, 2 drops of 20% KOH were added to the Voges-Proskauer test. The trays were then rotated 90° to allow the suspension in the first five wells to wet the corresponding reagent disks. The trays were then set upright, and the reactions were read according to the manufacturer’s directions. The tests were divided into threes, and the first test in each group received a 4 for a positive result, the second test a 2, and the third test a 1. The totals for each group were recorded, and a five-digit octal number was obtained. In the clinical evaluation, the Micro-ID code book containing the octal numbers was consulted for identification of the organisms.

The API 20E strips were inoculated, incubated, and read according to the manufacturer’s directions. The seven-digit octal number obtained was used to find the identity of the organisms from the API Profile Register. All clinical isolates used in the study were inoculated into the API 20E from the primary culture plate by technologists in the Clinical Microbiology Laboratory of the University of Minnesota Hospitals. For the clinical evaluation of the Micro-ID system, the same plates were used to inoculate the trays, if sufficient growth was available. If there was insufficient growth a subculture was made to a MacConkey agar plate.

Conventional tests were performed as previously described (4).
When there was a disagreement between the API and Micro-ID identification, the cultures were checked for purity and the two systems were repeated. If there was still a discrepancy, appropriate conventional tests were inoculated to resolve the difference.

RESULTS

Table 2 shows the percent agreement between Micro-ID and conventional tests. All tests agreed at 95% or greater, except for urease (90%) and arabinose fermentation (91%). Klebsiella pneumoniae isolates accounted for most of the urease discrepancies; with these organisms there was only a 34% agreement between the Micro-ID urease and Christensen urea agar. Serratia marcescens, Proteus mirabilis, and Proteus vulgaris isolates accounted for the remaining disagreements.

Table 2. Comparison of Micro-ID biochemical reactions with conventional tests using 606 organisms

<table>
<thead>
<tr>
<th>Test</th>
<th>Percent agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adonitol</td>
<td>95</td>
</tr>
<tr>
<td>Lactose</td>
<td>91</td>
</tr>
<tr>
<td>H₂S</td>
<td>98</td>
</tr>
<tr>
<td>Indole</td>
<td>95</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>99</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>99</td>
</tr>
<tr>
<td>ONPG</td>
<td>99</td>
</tr>
<tr>
<td>Phenylalanine deaminase</td>
<td>99</td>
</tr>
<tr>
<td>Urease</td>
<td>90</td>
</tr>
</tbody>
</table>

*ONPG, o-Nitrophenyl-β-D-galactopyranoside.
additional reagent has to be added to the wells. In general, the reactions were very easy to read, although, as with any system, experience improves the ease of reading. With both the decarboxylase and the fermentation tests the colors should be either purple or yellow. Occasionally an in-between shade would be observed; in these cases, one could read the test as positive or negative and look up either number to be obtained. All the other reactions were clearly readable in 4 h. Extending the incubation time for 1 to 2 h would probably be acceptable, but longer periods would defeat the rapid nature of the test. Also, one could encounter contamination problems in lengthy incubation because the test does not require aseptic conditions.

Even though the Micro-ID system is easy to use and interpret, its accuracy is enhanced by having experienced technologists carrying out the testing. For example, there were three strains of *Serratia rubidaea* that were identified as *K. pneumoniae* by Micro-ID. These strains had a bright red pigment and thus should not be called *K. pneumoniae* by a microbiologist regardless of what the Micro-ID code number indicated.

The discrepancies between the Micro-ID system and the Micro-ID agree with *Klebsiella oxytoca.*

A limitation of the Micro-ID system is that it is not suited to identification of *Enterobacteriaceae.* An oxidase test must be performed first, and only oxidase-negative organisms are to be inoculated. In addition to the *Enterobacteriaceae,* these could include the relatively common
clinical isolates \textit{Acinetobacter calcoaceticus} and \textit{Pseudomonas maltophilia}. In this study we encountered seven such organisms; they were identified by API 20E, and no number for these was in the Micro-ID code book. In these cases one would have to then subculture the organism to an appropriate medium or system for the identification of nonfermenters. This would not delay identification, however, because the discovery is made on the same day the cultures are first read.

In this study we found that the Micro-ID could be used with 87.1\% of primary plates that contained gram-negative rods. Aldridge et al. (1) could use it in 74\% of clinical specimens on the first day. The applicability will vary with the types of specimens received. The usefulness of the system is not diminished, however, because it is a simple matter to pick up one colony for subculture and set up the Micro-ID the next day. The identification is not delayed as compared to an overnight identification system. Indeed, in 87\% (or 74\%) of cases, with Micro-ID the identification occurs 1 day earlier than with overnight systems.

LITERATURE CITED


