MORLUC Numeric System for the Identification of Enterobacteriaceae

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Four hundred eighty-six members of the Enterobacteriaceae representing nine genera were identified by conventional methods, and the results were compared with MORLUC (Biotrol Company Inc., Jamaica, N.Y.). MORLUC, an acronym for melibiase, ONPG (o-nitrophenyl-β-D-galactopyranoside), rhamnose, lysine decarboxylase, urease, and citrate, are six prepackaged reagent-impregnated paper loops which are sealed within a plastic packet. The hydrogen sulfide reaction obtained from a triple sugar iron slant is coupled with MORLUC results and is readily converted into a three-digit numerical code, which is referenced on a preprinted single page listing. Additionally, the triple sugar iron is used to confirm the glucose fermentation by an unknown isolate. Comparisons of individual MORLUC tests and standard methods resulted in a better than 92% agreement, except for urease. Four hundred sixty-six of the 486 bacterial isolates, or 96% of the strains which were numerically identified by MORLUC, agreed with conventional diagnoses.

MORLUC, a proprietary product of the Biotrol Company, Inc., Jamaica, N.Y., is conceived as a scheme for the identification of Enterobacteriaceae. MORLUC is an acronym derived from the initial letters of the six major tests of the system. It represents melibiase, ONPG (o-nitrophenyl-β-D-galactopyranoside), rhamnose, lysine decarboxylase (LD), urease, and citrate. These diagnostic tests are represented as substrates impregnated on paper loops, sealed within a plastic packet. In addition to the six prepackaged tests, a conventional triple sugar iron (TSI) slant is required. A numerical value is assigned to each of the diagnostic indices. MORLUC results are interpreted and transferred into a three-digit number, the MORLUC numeric signature (MNS), which is referenced on a single page for identification of the organism.

MATERIALS AND METHODS

Of the total of 486 bacterial isolates analyzed, 468 were recovered from clinical specimens received in the diagnostic laboratory. Members of the Enterobacteriaceae that were studied are listed below. Species of Salmonella and Shigella not readily available as on-line clinical isolates were supplemented from stock cultures. Strains included: 119 Escherichiae, including 2 Shigella; 33 Salmonellae, comprising 16 Salmonella and 17 Citrobacter sp., of which 5 strains were Citrobacter diversus; 163 Klebsiellae, consisting of the various members of the Enterobacter genus, 18 Serratia sp., and 105 Klebsiella; and 171 Proteae, encompassing 27 strains of Providencia and the various species of Proteus. Clinical specimens were subcultured on an assortment of primary plating and holding media which included a MacConkey (MAC) agar plate. From this MAC plate, isolated colonies were selected and identified according to standard practices (3). Tests used consistently were oxidase, lactose, glucose, H2S, phenylalanine deaminase (PD), urease, LD, ornithine decarboxylase (OD), citrate, and indole. Other necessary biochemical and serological reactions were used as required based upon the identification schema of Edwards and Ewing (2). Individual tests, as well as the multiply reactive formulations of sulfide-indole-motility (SIM) and motility-indole-OD (MIO) were used to establish the conventional identification. Oxidase inactivity and glucose fermentation confirmed all isolates as members of the Enterobacteriaceae.

After the conventional identification, the known isolate maintained on a tryptic soy agar slant was restreaked onto a MAC plate to insure clonal individuality. A TSI slant inoculated from this MAC plate and incubated overnight was used to prepare a sufficient quantity of replicate inoculum for the comparative standard and MORLUC reactions. For this study we opted to first subculture the test isolates onto TSI, although simultaneous inoculation of the triple sugar and MORLUC tests is recommended. Conventional tests for melibiase and rhamnose incorporated 1% of the carbohydrate in phenol red agar base; Moeller's formulation of LD, Christensen urea agar, and Simmon's citrate agar slants were also used. Reactions of the MORLUC ONPG biolop were compared with the BBL Taxo ONPG disk.

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Figure 1 illustrates the configuration of the six MORLUC tests (bioloops) in each individual plastic packet. The left side represents the melibiose, ONPG, and rhamnose tests, and the right side represents the lysine, urease, and citrate reactions. When moistened with saline, the melibiose and rhamnose bioloops are red (ONPG is white). On the right side, the lysine, urease, and citrate tests are initially yellow. In the center of each biospace (reactant chamber) is an opening into which the inoculum is introduced.

Before inoculation the test packet was placed in the lid (cover) of a standard 95-mm diameter plastic plate which contained a premoistened circle of filter paper. Biospaces were filled with approximately 0.15 ml from a 2.0-ml saline emulsion of the TSI slant. Inoculum was added until the biospace was completely filled and a bubble formed. Essentially, there is no air in the reactant chamber, except that which is trapped in the inoculum at the time of introduction and that which diffuses into it. After inoculation the MORLUC plate was incubated over-night at 35 to 37°C, and the results were observed and tallied the next day.

MORLUC reactions were interpreted as in Table 1. Each positive result in this system is assigned a numeric equivalent of 1, 2, or 4; negative reactions are 0. The H2S result obtained from the TSI slant is assigned the value of 1 if positive. A melibiose-positive result is yellow-orange and is equivalent to a number 4; if negative, red and 0. ONPG when positive is yellow and assigned a value of 2; if negative, white and 0. Similarly, rhamnose is yellow-orange if positive and 1. Positive reactions on the right side, the lysine-urease-citrate side, are red-to-purple; yellow-orange, if negative. Positive reactions are lysine-4, urease-2, and citrate-1, respectively. All positive values are added to achieve a three-digit number. If all tests were positive, that is, if H2S from TSI were positive, and melibiose, ONPG, rhamnose, lysine, urease, and citrate were positive, then the addition would result in the MNS 177. (A three-digit number is always obtained; the first digit, either 0 or 1, negative or positive for H2S.)

After the reaction results were observed and the addition process was followed, the resulting MNS was compared with the preprinted listing (Fig. 2). The three-digit number encompasses the accumulated results of seven tests and represents an identity within the Enterobacteriaceae. In cases where the known reaction of a species is equivocal, then more than one three-digit code appears. If, on the other hand, two species yield the same MNS, then an annotation recommends the differential test or tests required to distinguish between two organisms yielding the identical MNS.

RESULTS

In the evaluation, the individual reactions of melibiose, ONPG, rhamnose, LD, urease, and citrate were compared with standard tests (Ta-
Table 1. Interpretation of MORLUC results

<table>
<thead>
<tr>
<th>Test</th>
<th>Reaction</th>
<th>Color</th>
<th>No.</th>
<th>Test</th>
<th>Reaction</th>
<th>Color</th>
<th>No.</th>
<th>Test</th>
<th>Reaction</th>
<th>Color</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S (TSI)</td>
<td>Positive</td>
<td>Black</td>
<td>1</td>
<td>M</td>
<td>Positive</td>
<td>Yellow-orange</td>
<td>4</td>
<td>L</td>
<td>Positive</td>
<td>Red-purple</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>None</td>
<td>0</td>
<td></td>
<td>Negative</td>
<td>Red</td>
<td>0</td>
<td></td>
<td>Negative</td>
<td>Yellow-orange</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Yellow</td>
<td>2</td>
<td>U</td>
<td>Negative</td>
<td>Red-purple</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>White</td>
<td>0</td>
<td></td>
<td>Negative</td>
<td>Yellow-orange</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Yellow-orange</td>
<td>1</td>
<td>C</td>
<td>Positive</td>
<td>Red-purple</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Red</td>
<td>0</td>
<td></td>
<td>Negative</td>
<td>Yellow-orange</td>
<td>0</td>
</tr>
</tbody>
</table>

Add for MNS

| M     | 7   | 7   |

*Abbreviations: M, Melibiose; O, ONPG; R, rhamnose; L, lysine; U, urease; C, citrate; MNS, MORLUC numeric signature.

The H₂S is a result obtained from the TSI slant.

MORLUC NUMERIC SIGNATURES

- 000 Shigella
- 001 Providencia
- 002 Proteus morganii
- 003 Proteus rettgeri
- 004 Alkaloescens-Dispar
- 005 Shigella
- 007 Shigella
- 010 Shigella
- 011 Citrobacter diversus
- 013 Proteus rettgeri
- 014 Enterobacter hafniae
- 020 Escherichia coli
- 020 Enterobacter agglomerans
- 060 Escherichia coli
- 061 Serratia liquefaciens
- 062 Serratia liquefaciens
- 064 Escherichia coli
- 065 Serratia liquefaciens
- 067 Serratia liquefaciens
- 070 Enterobacter agglomerans
- 070 Escherichia coli
- 070 Klebsiella
- 071 Enterobacter cloacae
- 071 Citrobacter diversus
- 072 Klebsiella
- 073 Enterobacter cloacae
- 073 Citrobacter diversus

Fig. 2. A partial listing of the three-digit MNS codes. (a) Serotype with appropriate antiserum. (b) Indole will differentiate H₂S-negative Proteus mirabilis from Proteus rettgeri: P. mirabilis, negative (0) and P. rettgeri, positive (+). (c) Indole will aid in differentiating Enterobacter from Escherichia and Citrobacter: Escherichia, +; Enterobacter, 0; Citrobacter, +. (d) Indole will aid in differentiating Escherichia and Klebsiella: Escherichia, +; Klebsiella, 0. (e) OD will aid in differentiating Enterobacter and Klebsiella: Enterobacter, +; Klebsiella, 0.

Table 3 compares the overall expression of these tests, i.e., the diagnoses achieved via conventional media with identifications derived from the MORLUC reactions and the resulting MNS. Of the total 466 strains, 466 or 95.9% of the identifications agreed. Twenty species did not correlate. No problem was encountered in identifying 116 Escherichia, and 42 of 48 Enterobacter strains were correctly identified. The six species that could not be identified were all Enterobacter agglomerans, and only 40% of those tested were identified correctly. Three Klebsiella were unidentifiable by the MORLUC numeric, and five Citrobacter strains were incorrectly identified. Six members of the Proteae were misidentified: one P. morganii, one P. rettgeri, three H₂S-negative strains of P. mirabilis, and one Providencia.

**DISCUSSION**

MORLUC differs from other kits designed for the identification of the Enterobacteriaceae in that it combines a conventional medium, the
TABLE 2. Correlation of individual MORLUC tests with conventional reactions

<table>
<thead>
<tr>
<th>Test</th>
<th>Agreement</th>
<th>Disagreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Melibiose</td>
<td>451</td>
<td>92.8</td>
</tr>
<tr>
<td>ONPG</td>
<td>460</td>
<td>94.6</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>474</td>
<td>97.5</td>
</tr>
<tr>
<td>LD</td>
<td>452</td>
<td>93.0</td>
</tr>
<tr>
<td>Urease</td>
<td>272</td>
<td>90.7</td>
</tr>
<tr>
<td>Citrate</td>
<td>450</td>
<td>92.6</td>
</tr>
</tbody>
</table>

* I, Indeterminable.

TSI slant, and reagent-impregnated paper tests. The inclusion of TSI to document the H2S reaction appears valid as it relies upon a widely accepted standard (2). Additionally, the TSI medium can confirm the glucose reactivity of the unknown isolate and provides a readily available reference for additional or confirmatory testing.

Substrate-impregnated paper tests have been noted for a variety of applications (4), as they offer certain conveniences that are not available in other test forms. Generally, they are easy to store, possess a long shelf life, are readily disposable, and are judged convenient to use. Inoculation of MORLUC required less time than conventional media, and reactions were clear-cut as only two differential colors, red or yellow (yellow or white for ONPG), were necessary for interpretation. One would expect the most accurate and precise reactions for those tests in which the color change results from the specific interaction between metabolic products or enzymes of the bacterial isolate and the indicator reagents incorporated in the paper. Reactions that rely upon pH shifts due to the action of organism on substrate impregnated into paper might generally be expected to be less satisfactory. Accordingly, ONPG should have yielded the best correlative results. However, in agreement percentage it was second to rhamnose.

The MORLUC numerical system correctly identified 466 of the 486 strains tested in this survey. However, additional tests were necessary. Of the 486 isolates, 373 or 77% were identified to the genus level, and 262 organisms within that group were identified to the species level, without supplementary tests. The additional tests most frequently required, OD, indole, and serotyping, were necessary to complete an identification or to differentiate between two species with the duplicate code number. Of the 466 correctly identified strains, 177 strains (38%) required indole, OD, or indole and OD for further confirmation. Indole and OD were obligatory 100 times; indole was obligatory alone in 71 identifications. The frequent requirement of OD coupled with indole was necessary to distinguish P. mirabilis and P. vulgaris. Considering that an additional day was required to obtain these results, it would seem logical for a potential user to inoculate conventional indole and OD media (e.g., MIO) when using this system or for the manufacturer to provide for these additional tests.

In all, 20 isolates of the Enterobacteriaceae were unidentifiable or misidentified by MORLUC. Three organisms that resulted in the MNS code of 073 could not be characterized, as the number did not appear on the coded chart. Reactions in the MORLUC system coincided with the conventional findings and could have been identified as Klebsiella pneumoniae if the MNS 073 was listed. Similarly, there was no MNS listing for the six isolates of Enterobacter agglomerans. Two strains proved to be ONPG negative with MORLUC, three resulted in contradictory H2S reactions, and a sixth strain yielded discordant melibiose results. Five strains routinely identified as Citrobacter freundii could not be diagnosed with MORLUC. In four cases the problem was due to negative MORLUC citrate reactions which were positive on Simmons citrate. In one instance, the problem stemmed from differential H2S reactions between TSI and SIM; the organism was H2S positive on SIM and H2S nega-
tive on TSI. One strain of *Proteus rettgeri* was not correctly diagnosed because the MORLUC ONPG was positive, although conventionally, PD, OD, and urease were positive, and the ONPG was negative. Another *Proteus* sp. was incorrectly identified due to a disagreement between MORLUC and conventional urease. Falsely negative MORLUC citrate reactions were responsible for misidentifying three H$_2$S-negative strains of *Proteus mirabilis* and one *Providencia*. There was no provision for the identification of *Yersinia* species in the registry. If *Yersinia* were included, its MNS would conflict with *Proteus morganii* and *Klebsiella* and require differential (25°C and 37°C) motility studies for final identification.

Based upon the data compiled here, MORLUC appears to be an effective test system utilizing seven tests, which is capable of identifying members of the *Enterobacteriaceae*. However, like other available commercial kits, it offers no significant time advantage over conventional methodologies in achieving a final diagnosis. To preclude any delay in identification with this system, our experience would suggest the simultaneous inoculation of MORLUC (including TSI), indole, and OD. Although it is generally conceded that a large number of pertinent tests will allow more accurate identification, and one system uses as many as 21 tests (5), no lower limit has been set. Theoretically, five tests ($2^5 = 32$) would be sufficient to identify all species included within the family *Enterobacteriaceae*. Reliance on a limited number of tests minimizes the cost and unnecessary confusion which arises when using a large number of characters to determine the identity of an organism. A numerical diagnostic key directed toward discriminating enteric organisms has previously been described (1). However, the system discussed here, as well as other commercial kits, uses a binary coded decimal device which converts reactions expressed in the radix two; (0, negative; 1, positive) into a decimal representation which is consecutively referenced as an organism registry. The application of a numeric identification system that has the capability to simultaneously consider reaction results represents a distinct advantage over conventional, intuitive methods of identification.

**LITERATURE CITED**


