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# Evaluation of Biolog System for Identification of Some Gram-Negative Bacteria of Clinical Importance

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**The Biolog system (Biolog, Inc., Hayward, Calif.) was evaluated for the identification of 55 gram-negative taxa (789 strains) likely to be encountered commonly in clinical laboratories. The Biolog system performed best with oxidase-positive fermenters and biochemically active nonfermenters and had the most problems with unreactive nonfermenters. It gave significantly better results when the MicroPlates were read manually rather than when they were read by the automated reader. Plates read manually gave the following performances: oxidase-positive fermenters, five taxa, 64 strains, 92% correct, 3% not identified, and 5% incorrect; biochemically active nonfermenters, eight taxa, 122 strains, 88% correct, 6% not identified, and 6% incorrect; members of the family *Enterobacteriaceae*, 35 taxa, 511 strains, 77% correct, 8% not identified, and 15% incorrect; unreactive nonfermenters, seven taxa, 92 strains, 38% correct, 24% not identified, and 38% incorrect. We found the system easy to use, but while for 39 of 55 of the taxa an identification rate of >70% was achieved, problems were encountered, particularly with identification of capsulated strains of some *Enterobacter* and *Klebsiella* taxa, as well as the least biochemically active *Moraxella* and *Neisseria* strains.**

The Biolog system (Biolog, Inc., Hayward, Calif.) has been introduced only recently to the United Kingdom. It is based on tests for the oxidation of 95 substrates in a 96-well microtiter plate. Each well contains a redox dye, tetrazolium violet, that permits colorimetric determination of the increased respiration that occurs when cells are oxidizing a carbon source. Reactions are read after 4 h and/or after overnight incubation. With so many test results, computer processing is necessary. The test results may be read by eye and recorded manually or read with an automated plate reader. Biolog gram-negative (GN) database release 3.01A comprises 569 taxa. Similarity and distance (including average and maximum) are calculated, and all four values are displayed on the screen and printout. In addition, MicroPlates and databases for gram-positive organisms and yeasts are also now available.

The large number of characters makes this system particularly suitable for reference or research laboratories. In an evaluation (3) of an earlier release of the GN database, 114 strains representing 22 *Pseudomonas* or *Pseudomonas*-like species were processed through the GN system. With the automated plate reader, rates of 74% correctly identified (CI), 9% not identified (NI), and 17% incorrectly identified (II) were obtained. When the results were read visually and entered into the computer manually, the corresponding figures were 79, 7, and 14%. The improved identification rate may have been due to the ability to enter indeterminate results as borderline, an option then available only when results were entered manually. With the automated reader, all isolates of the following organisms were CI by the system: *Chryseomonas luteola*, *Comamonas acidovorans*, *Pseudomonas fragi*, *P. fluorescens*, *P. pickettii*, *P. pseudoalcaligenes*, and *P. stutzeri*. With manual entry of results, all strains of *P. aeruginosa*, *P. cepacia*, *P. diminuta*, and *P. mendocina*, but not all strains of *P. fluorescens*, were also correctly identified. By either method of data entry, none of the strains of *Comamonas testosteroni* were correctly identified. Thus, with the automated reader, the system correctly identified all isolates of 7 species but with manual entry of results, all strains of 10 species were correctly identified.

Miller and Rhoden (8) reported identification rates at 24 h of 65.9% CI to the genus level and 57.1% CI to the species level for a range of members of the family *Enterobacteriaceae* (212 strains), nonfermenters (105 strains), and oxidase-positive fermenters (35 strains). Klingler et al. (7) tested 39 American Type Culture Collection reference strains and 45 water isolates; for the American Type Culture Collection strains, they reported rates of 98% CI to the genus level and 76% CI to the species level (93% of the water isolates were CI). The Biolog system was found to be useful in the identification of clinical *Aeromonas* isolates (1) to the species level, and it has also been employed in the differentiation of *Legionella* species (see Stager and Davis [9]).

We report here an evaluation of the ability of the GN system (release 3.01A) to identify 55 GN taxa likely to be encountered commonly in clinical laboratories.

## MATERIALS AND METHODS

**Bacterial strains.** About 15 strains of each taxon were chosen to represent 55 GN taxa (listed in Table 1) considered to be encountered commonly in clinical laboratories; altogether, 789 strains were tested. The taxa comprised 35 *Enterobacteriaceae* (511 strains), 15 nonfermenters (214 strains), and five oxidase-positive fermenters (64 strains). When available, the type strain for each taxon was included, together with other National Collection of Type Cultures reference strains and field strains.

**Biochemical tests.** All strains had been previously examined in a range of up to 66 conventional tests, and the identities of the field strains had been determined and those of the reference strains had been confirmed by processing the results through the appropriate probability matrices of Holmes et al. (5, 6).

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TABLE 1. Identification of GN bacteria by the Biolog system (release 3.01A)

| Taxon or parameter | Total no. of strains | No. of strains with result indicated obtained with following identification parameters[a]: Automated reading, 4 h | | | Automated reading, 4 and 24 h[b] | | | Automated reading, 24 h | | | Manual reading, 24 h | | | % Identified |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CI | NI | II | CI | NI | II | CI | NI | II | CI | NI | II | |
| *Enterobacteriaceae* | | | | | | | | | | | | | | |
| *Citrobacter amalonaticus* | 15 | 4 | 11 | 0 | 15 | 0 | 0 | 15 | 0 | 0 | 15 | 0 | 0 | 100 |
| *Citrobacter diversus* | 15 | 0 | 15 | 0 | 15 | 0 | 0 | 15 | 0 | 0 | 15 | 0 | 0 | 100 |
| *Citrobacter freundii* | 15 | 2 (1) | 13 | 0 | 15 | 0 | 0 | 15 | 0 | 0 | 15 | 0 | 0 | 100 |
| *Edwardsiella tarda* | 13 | 0 | 13 | 0 | 3 | 9 | 1 | 3 | 9 | 1 | 13 | 0 | 0 | 100 |
| *Enterobacter aerogenes* | 15 | 1 | 14 | 0 | 4 | 5 | 6 | 3 | 5 | 7 | 1 | 1 | 13 | 7 |
| *Enterobacter agglomerans* | 15 | 6 | 9 | 0 | 12 | 2 | 1 | 11 | 3 | 1 | 13 | 1 | 1 | 87 |
| *Enterobacter cloacae* | 12 | 1 | 11 | 0 | 6 (1) | 5 | 1 | 5 (1) | 5 | 2 | 5 | 2 | 5 | 42 |
| *Enterobacter sakazakii* | 15 | 8 | 7 | 0 | 11 | 4 | 0 | 8 | 7 | 0 | 15 | 0 | 0 | 100 |
| *Escherichia coli* | 15 | 7 (2) | 8 | 0 | 12 | 2 | 1 | 11 | 2 | 2 | 12 (1) | 0 | 3 | 80 |
| *Hafnia alvei* | 15 | 0 | 15 | 0 | 7 | 0 | 8 (2) | 7 | 0 | 8 (2) | 7 | 3 | 5 (1) | 47 |
| *Klebsiella oxytoca* | 15 | 10 (5) | 3 | 2 | 10 (1) | 1 | 4 | 8 (1) | 2 | 5 | 4 (1) | 2 | 9 | 27 |
| *Klebsiella ozaenae* | 18 | 9 (4) | 9 | 0 | 9 | 1 | 8 | 5 | 1 | 12 | 13 (1) | 0 | 5 | 72 |
| *Klebsiella pneumoniae* | 15 | 3 (1) | 4 | 8 (2) | 5 (2) | 8 | 2 | 4 (2) | 8 | 3 | 14 | 0 | 1 | 93 |
| *Klebsiella rhinoscleromatis* | 8 | 2 | 6 | 0 | 4 | 3 | 1 | 4 | 3 | 1 | 2 | 6 | 0 | 25 |
| *Morganella morganii* | 15 | 3 | 12 | 0 | 7 | 8 | 0 | 7 | 8 | 0 | 15 | 0 | 0 | 100 |
| *Proteus mirabilis* | 15 | 2 | 13 | 0 | 7 (2) | 6 | 2 | 5 (2) | 7 | 3 | 11 | 1 | 3 | 73 |
| *Proteus penneri/vulgaris* | 15 | 8 | 7 | 0 | 11 (1) | 3 | 1 | 8 (1) | 6 | 1 | 13 | 2 | 0 | 87 |
| *Providencia alcalifaciens* | 15 | 4 | 11 | 0 | 11 (1) | 4 | 0 | 11 (1) | 4 | 0 | 15 | 0 | 0 | 100 |
| *Providencia rettgeri* | 15 | 3 | 12 | 0 | 14 | 1 | 0 | 14 | 1 | 0 | 15 | 0 | 0 | 100 |
| *Providencia stuartii* | 15 | 5 | 10 | 0 | 12 | 3 | 0 | 12 | 3 | 0 | 14 | 1 | 0 | 93 |
| *Salmonella arizonae* | 15 | 4 | 11 | 0 | 5 | 5 | 5 | 1 | 6 | 8 | 1 | 2 | 12 | 7 |
| *Salmonella choleraesuis* | 9 | 0 | 9 | 0 | 9 (2) | 0 | 0 | 9 (2) | 0 | 0 | 9 | 0 | 0 | 100 |
| *Salmonella gallinarum* | 15 | 0 | 15 | 0 | 11 (1) | 4 | 0 | 11 (1) | 4 | 0 | 14 | 1 | 0 | 93 |
| *Salmonella paratyphi* A, B, C | 15 | 0 | 15 | 0 | 13 (2) | 2 | 0 | 13 (2) | 2 | 0 | 13 (2) | 2 | 0 | 87 |
| *Salmonella typhi* | 15 | 1 (1) | 14 | 0 | 13 | 2 | 0 | 13 | 2 | 0 | 14 | 1 | 0 | 93 |
| *Salmonella typhimurium* | 15 | 0 | 15 | 0 | 7 | 6 | 2 | 7 | 6 | 2 | 3 | 2 | 10 | 20 |
| *Salmonella* sp. | 16 | 0 | 16 | 0 | 10 | 3 | 3 | 10 | 3 | 3 | 12 (1) | 0 | 4 | 75 |
| *Serratia liquefaciens* | 15 | 10 | 5 | 0 | 15 | 0 | 0 | 15 | 0 | 0 | 14 | 0 | 1 | 93 |
| *Serratia marcescens* | 16 | 10 | 6 | 0 | 15 | 0 | 1 | 14 | 0 | 2 | 13 | 0 | 3 | 81 |
| *Shigella boydii* | 15 | 4 (1) | 10 | 1 | 11 (2) | 2 | 2 | 10 (2) | 3 | 2 | 10 | 4 | 1 | 67 |
| *Shigella dysenteriae* | 15 | 5 (3) | 10 | 0 | 9 (3) | 6 | 0 | 4 | 11 | 0 | 7 (2) | 7 | 1 | 47 |
| *Shigella flexneri* | 15 | 1 | 13 | 1 (1) | 12 | 3 | 0 | 12 | 3 | 0 | 14 (1) | 1 | 0 | 93 |
| *Shigella sonnei* | 15 | 1 | 12 | 2 | 12 | 1 | 2 | 12 | 1 | 2 | 13 | 0 | 2 | 87 |
| *Yersinia enterocolitica* | 14 | 8 (1) | 6 | 0 | 12 (2) | 1 | 1 | 12 (2) | 1 | 1 | 14 (1) | 0 | 0 | 100 |
| *Yersinia pseudotuberculosis* | 15 | 4 | 11 | 0 | 12 | 3 | 0 | 10 | 5 | 0 | 15 | 0 | 0 | 100 |
| Subtotal, release 3.01A | 511 | 126 | 371 | 14 | 356 | 103 | 52 | 324 | 121 | 66 | 393 | 39 | 79 | |
| %, release 3.01A | | 25 | 72 | 3 | 70 | 20 | 10 | 64 | 23 | 13 | 77 | 8 | 15 | |
| %, release 3.60[c] | | 44 | 52 | 4 | | | | 77 | 12 | 11 | | | | |
| Oxidase-positive fermenters | | | | | | | | | | | | | | |
| *Aeromonas hydrophila* | 14 | 9 (5) | 5 | 0 | 11 | 2 | 1 | 11 | 2 | 1 | 11 (3) | 1 | 2 | 79 |
| *Pasteurella haemolytica* | 13 | 1 | 12 | 0 | 5 | 7 | 1 | 5 | 7 | 1 | 13 | 0 | 0 | 100 |
| *Pasteurella multocida* | 15 | 6 (1) | 9 | 0 | 10 (3) | 2 | 3 | 10 (3) | 2 | 3 | 15 | 0 | 0 | 100 |
| *Vibrio cholerae* | 10 | 3 (2) | 4 | 3 | 8 (2) | 2 | 0 | 6 (1) | 4 | 0 | 9 (1) | 0 | 1 | 90 |
| *Vibrio parahaemolyticus* | 12 | 9 (1) | 3 | 0 | 12 | 0 | 0 | 10 | 2 | 0 | 11 | 1 | 0 | 92 |
| Subtotal, release 3.01A | 64 | 28 | 33 | 3 | 46 | 13 | 5 | 42 | 17 | 5 | 59 | 2 | 3 | |
| %, release 3.01A | | 44 | 52 | 4 | 72 | 20 | 8 | 66 | 26 | 8 | 92 | 3 | 5 | |
| %, release 3.60 | | 64 | 30 | 6 | | | | 89 | 4 | 7 | | | | |
| Biochemically active nonfermenters | | | | | | | | | | | | | | |
| *Acinetobacter calcoaceticus* | 15 | 1 | 14 | 0 | 10 (1) | 5 | 0 | 9 (1) | 6 | 0 | 14 (2) | 1 | 0 | 93 |
| *Acinetobacter lwoffii* | 15 | 6 (2) | 9 | 0 | 10 | 4 | 1 | 11 (1) | 4 | 0 | 8 (3) | 3 | 4 | 53 |
| *Alcaligenes faecalis* | 17 | 6 | 11 | 0 | 15 | 1 | 1 | 13 | 2 | 2 | 17 (1) | 0 | 0 | 100 |
| *Flavobacterium meningo-septicum* | 15 | 2 | 13 | 0 | 10 | 5 | 0 | 9 | 6 | 0 | 14 | 1 | 0 | 93 |
| *Pseudomonas aeruginosa* | 15 | 3 | 12 | 0 | 9 (1) | 2 | 4 | 7 (1) | 4 | 4 | 13 | 0 | 2 | 87 |
| *Pseudomonas fluorescens* | 15 | 0 | 13 | 2 | 12 | 2 | 1 | 12 | 2 | 1 | 13 | 1 | 1 | 87 |
| *Pseudomonas putida* | 14 | 3 (1) | 11 | 0 | 11 | 3 | 0 | 11 | 3 | 0 | 12 | 1 | 1 | 86 |
| *Xanthomonas maltophilia* | 16 | 0 | 16 | 0 | 15 | 1 | 0 | 15 | 1 | 0 | 16 | 0 | 0 | 100 |

*Continued on following page*

TABLE 1—*Continued*

| Taxon or parameter | Total no. of strains | No. of strains with result indicated obtained with following identification parameters[a]: Automated reading, 4 h | | | Automated reading, 4 and 24 h[b] | | | Automated reading, 24 h | | | Manual reading, 24 h | | | % Identified |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CI | NI | II | CI | NI | II | CI | NI | II | CI | NI | II | |
| Subtotal, release 3.01A | 122 | 21 | 99 | 2 | 92 | 23 | 7 | 87 | 28 | 7 | 107 | 7 | 8 | |
| %, release 3.01A | | 17 | 81 | 2 | 75 | 19 | 6 | 71 | 23 | 6 | 88 | 6 | 6 | |
| %, release 3.60 | | 34 | 65 | 1 | | | | 79 | 13 | 8 | | | | |
| Biochemically inactive nonfermenters | | | | | | | | | | | | | | |
| *Moraxella catarrhalis* | 15 | 1 (1) | 13 | 1 | 2 (2) | 5 | 8 | 1 (1) | 6 | 8 | 5 (5) | 1 | 9 (1) | 33 |
| *Moraxella lacunata* | 12 | 1 (1) | 9 | 2 | 3 (3) | 6 | 3 | 2 (2) | 6 | 4 | 2 (2) | 8 | 2 | 17 |
| *Moraxella nonliquefaciens* | 9 | 3 (2) | 6 | 0 | 3 | 1 | 5 | 1 | 2 | 6 | 0 | 8 | 1 | 0 |
| *Moraxella osloensis* | 16 | 10 | 6 | 0 | 15 (2) | 1 | 0 | 15 (2) | 1 | 0 | 16 (1) | 0 | 0 | 100 |
| *Moraxella phenylpyruvica* | 16 | 4 (1) | 12 | 0 | 7 (1) | 7 | 2 | 4 (1) | 9 | 3 | 9 (4) | 3 | 4 | 56 |
| *Neisseria gonorrhoeae* | 13 | 0 | 9 | 4 | 0 | 6 | 7 | 0 | 6 | 7 | 0 | 13 | 0 | 0 |
| *Neisseria meningitidis* | 11 | 2 | 9 | 0 | 5 | 3 | 3 (1) | 5 | 3 | 3 (1) | 3 (3) | 2 | 6 | 27 |
| Subtotal, release 3.01A | 92 | 21 | 64 | 7 | 35 | 29 | 28 | 28 | 33 | 31 | 35 | 35 | 22 | |
| %, release 3.01A | | 23 | 69 | 8 | 38 | 32 | 30 | 30 | 36 | 34 | 38 | 38 | 24 | |
| %, release 3.60 | | 15 | 78 | 7 | | | | 22 | 39 | 39 | | | | |
| Release 3.01A | | | | | | | | | | | | | | |
| Genus level grand total | 789 | 196 | 567 | 26 | 529 | 168 | 92 | 481 | 199 | 109 | 594 | 83 | 112 | |
| % | | 25 | 72 | 3 | 67 | 21 | 12 | 61 | 25 | 14 | 75 | 11 | 14 | |
| Species level grand total | 789 | 160 | 606 | 23 | 494 | 206 | 89 | 451 | 232 | 106 | 559 | 120 | 110 | |
| % | | 20 | 77 | 3 | 63 | 25 | 12 | 57 | 29 | 14 | 71 | 15 | 14 | |
| Release 3.60 | | | | | | | | | | | | | | |
| Genus level grand total | 789 | 319 | 435 | 32 | | | | 558 | 113 | 108 | | | | |
| % | | 41 | 55 | 4 | | | | 72 | 14 | 14 | | | | |

[a] Each value in parentheses shows the proportion of the total number of strains either CI or II at the genus level.
[b] When strains were reported as identified after the 4-h automatic reading, these results are included as correct in place of further readings at 24 h.
[c] Summary of results produced by the manufacturer by using software release 3.60 and those based on our raw data.

**Whole-cell protein electrophoresis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.** Whole-cell protein patterns were obtained for the strains representing the majority of the taxa studied by the methods described by Costas (2). These patterns were compared visually for each taxon as a further confirmation of identity, to ensure within-taxon homogeneity and as a method of detecting contaminated cultures. The protein patterns were compared prior to testing of the strains with the Biolog system so that isolates with atypical patterns could be excluded. In some cases, however, this was not possible because certain taxa are known to be genomically heterogeneous and variability in protein patterns was therefore expected. In such cases, assessment of identification rates obtained with the Biolog system could be difficult and these problems are discussed below.

**Inoculation of Biolog MicroPlates.** Stock cultures were subcultured several times to increase their levels of metabolic activity. Inocula for the Biolog system were prepared from overnight growth on tryptic soy agar plates incorporating 5% (vol/vol) horse blood. A plastic disposable loop was used to collect colonies carefully so that there would be minimum carryover of nutrients from the agar when the growth was suspended in 0.85% saline. The total inoculum volume required is large (18 ml), and to accommodate this volume, test tubes measuring 6 by 0.75 in. (1 in. = 2.54 cm), which still fit into the turbidimeter, were used.

The turbidimeter (which measured optical density at 590 nm) was blanked with a tube of uninoculated saline. The suspension was then adjusted to fall within the low-limit and high-limit GN MicroPlate turbidity standards supplied by the manufacturer. The inoculum, which was always used within 10 min of preparation, was poured into a disposable plastic reservoir just prior to use. MicroPlates were inoculated with an eight-channel multipipette, with 150 μl of the inoculum being dispensed per well; plates were then generally incubated at 35°C, although for a few organisms that prefer lower growth temperatures, incubation was at 30°C (as recommended by the manufacturer).

**Interpretation of metabolic profiles.** MicroPlates were read at both 4 and 24 h with a computer-controlled MicroPlate reader. The software subtracts the control well reading from the readings of the other wells and expresses their individual activities as a percent change with respect to the control well. Reactions are then interpreted as positive or negative if the corrected readings exceed a predetermined threshold value. In addition, at 24 h, MicroPlates were read visually (well by well) and reactions were recorded on the computer as positive, negative, or borderline (indeterminate reactions). The metabolic profile of each organism was then compared automatically, by using the MicroLog software, with the MicroLog GN database (release 3.01A). Profile data were saved permanently as computer files (ASCII) to facilitate subsequent analyses.

Biolog identifications were reported if the similarity index of the genus or species was 0.750 or greater at 4 h. When a lower similarity value is obtained, the user is prompted to continue the incubation for 24 h. In this study, all MicroPlates were read at both 4 and 24 h even when an identification was reported at 4 h. At 24 h, a similarity index of less than 0.500 results in an instrument report of NI. Similarity indices of ≥0.500 result in a computer report of identification to either the genus or the

species level. In this study, we used the term CI when the correct genus or species was reported and this concurred with the identification produced by using conventional phenotypic tests, and when possible, this was supported by protein profile data. II is recorded when an identification made to either the genus or the species level by the Biolog system does not agree with the phenotypic-protein profile identification.

Some taxa presented particular problems. For the genus *Acinetobacter*, identification of whose species is especially difficult, nonoxidizing strains were considered CI if the identification suggested by the Biolog system was any one of the following: *Acinetobacter junii*, *A. johnsonii*, *A. lwoffii*, *A. radioresistens*, or *Acinetobacter* genospecies 9 or 11. Oxidizing strains were considered CI if they were identified as belonging to any of the remaining genospecies. *Aeromonas* is another genus that contains several genomic species, and as the precise taxonomic status of the strains we used in the evaluation was unknown, we accepted such strains as CI by the Biolog system if the strains were identified with any *Aeromonas* taxon. When starting the evaluation, we assumed that the *Pasteurella haemolytica* in the Biolog database comprised both biotypes A and T. However, strains of *P. haemolytica* T were not identified by the Biolog system, so we excluded these strains from the analysis on the assumption that *P. haemolytica* in the Biolog database was used in its strict sense to include only those strains formerly included in biotype A (biotype T is now *P. trehalosi*). Since the *Salmonella* taxa in the Biolog database do not correspond to the biogroups traditionally recognized by conventional biochemical tests, strains were considered CI in the present study if they were identified with any of the Biolog *Salmonella* subspecies 1 groups (A through G). However, strains of *Salmonella arizonae* were only considered CI with *Salmonella* subspecies 3. For both *Salmonella* and *Shigella* species, the manufacturer recommends that the identification be confirmed serologically; when a strain of either genus was II to a particular taxon within the appropriate genus, it was considered NI. Although the serological examination was not carried out, it was assumed that it would have refuted the II result.

## RESULTS

The numbers of CI, II, and NI strains for each taxon are given in Table 1. The numbers in the first column are the results obtained after 4 h with automatic readings. Strains that were NI at 4 h were incubated further, and the results were read automatically at 24 h. The second column thus represents the combined results of these 4- and 24-h readings. In addition, the third column presents results obtained after 24-h automatic readings, even when strains had already been identified at 4 h. This strategy was adopted because in clinical laboratories, strains which are identified after 4 h would not need to be incubated further. However, it was of interest to determine whether strains identified after 4 h and incubated further to 24 h would still produce the same identification result. All plates were also read after 24 h manually, and these results are shown in the fourth column. Since the highest CI rates were obtained by reading the MicroPlates manually at 24 h, the final column of Table 1 contains the percentages of strains of individual taxa that were CI by these manually read data.

All of the strains of 16 taxa were CI, in 39 of 55 taxa a CI rate of >70% was achieved, and in only 4 taxa were <10% of the strains CI.

After 4 h of incubation, 25% of all of the strains were CI to the genus level and for 82% of these the correct species or subspecies was identified. Of the *Enterobacteriaceae* taxa, 25% were CI, while 44% of the oxidase-positive fermentative taxa and 20% of the nonfermentative taxa were CI (17% of the biochemically active and 23% of the inactive taxa were CI).

The summary results obtained by combined automated readings after 4 and 24 h of incubation show that 67% of the strains were CI to the genus level and 93% of these were CI to the species or subspecies level. Of the *Enterobacteriaceae* taxa, 70% were CI, while 72% of the oxidase-positive fermentative taxa and 59% of the nonfermentative taxa were CI.

By manual reading after 24 h of incubation, 75% of the strains were CI to the genus level and of these, 94% were CI to the species or subspecies level. Of the *Enterobacteriaceae* taxa, 77% were CI, while 92% of the oxidase-positive fermentative taxa and 66% of the nonfermentative taxa were CI (88% of the biochemically active and 38% of the inactive taxa were CI).

Also presented in Table 1 (third column) are the summary results obtained by automated reading of all plates after 24 h, which show that 61% of the strains were CI to the genus level and of these, 94% were CI to the species or subspecies level. Of the *Enterobacteriaceae* taxa, 64% were CI, while 66% of the oxidase-positive fermentative taxa and 54% of the nonfermentative taxa were CI.

The information obtained in this study was used to update both the database and threshold algorithms used by the Biolog software to determine positive, negative, and borderline reactions. The ASCII files containing the raw data were then reprocessed by the manufacturer through the revised software (release 3.60). The summary results for each group are presented for comparison below those of release 3.01A (Table 1). These results show an increase in the CI rates for automatic readings at both 4 and 24 h for *Enterobacteriaceae*, oxidase-positive fermenters, and biochemically active nonfermenters.

## DISCUSSION

Holmes et al. (5, 6) described two probability matrices for the identification of atypical or rare GN rods in a reference laboratory: one for fermenters (110 taxa, 66 conventional tests), for which a rate of 89.2% CI was obtained, with 10.8% NI and 0% II, and one for nonfermenters (66 taxa, 83 conventional tests), for which the corresponding identification rates were 91.5, 8.5, and 0%. A successful identification system should thus yield, for organisms which may include atypical or rare isolates, a CI rate of about 90% but a low misidentification rate. As can be seen from Table 1, the automated readings of Biolog release 3.01A gave an overall unsatisfactory performance. However, the manual readings were satisfactory for some of the groups. CI rates of 92 and 88% were obtained for oxidase-positive fermenters and biochemically active nonfermenters, respectively. II rates of less than 10% were obtained for these same groups.

Conventional and commercial systems other than the Biolog system differ fundamentally in the approach taken and the methodology used to identify isolates, and these may have influenced the results obtained in this evaluation. We discuss below possible reasons for the performance of the Biolog system and ways in which the system may be improved to realize its full potential.

It should be remembered that good identification relies on sound classification. Certain species, such as *Enterobacter agglomerans*, *E. cloacae*, and *P. fluorescens*, are known to be genomically heterogeneous and are therefore likely also to be phenotypically heterogeneous. For such species, a high CI rate may not be possible whichever system is used. For example, in the National Collection of Type Cultures matrix for fermenters (5), *E. cloacae* gave one of the worst identification perfor-

mances, with a CI rate of only 53% (42% in the present study); this confirms the findings of Grimont and Grimont (4).

The best Biolog performance was obtained by manual reading after 24 h of incubation and concurs with the results of a previous evaluation of the system (3). This is in contrast to the findings of Miller and Rhoden (8), who reported "the visual reading option to be too difficult to judge true + and − wells compared with the very accurate [sic] and rapid automated Microstation reader." We encountered little difficulty in interpreting results as positive or negative, but it was following the evaluation of Miller and Rhoden (8) that the manufacturer introduced the borderline facility, which was available for the present study, for manual entry of indeterminate reactions. It is possibly the availability of the borderline facility for manual input of results, as opposed to a strict cutoff level between positive and negative results on automated reading, that accounts for the higher CI rate obtained in this study. The inconsistency noted with respect to the results obtained by automatic and manual interpretations of data (release 3.01A) is being addressed by the manufacturers, who have modified the threshold algorithm for release 3.60.

It was apparent, particularly among strains of reactive enteric taxa, that some strains which were CI after 4 h of incubation could be II if incubated for 24 h. The CI rate at 24 h improved from 61 to 67% when those strains that were CI at 4 h were not further incubated and read at 24 h. However, even this improved rate was still not as good as that obtained with manual reading of results (75%).

In operating a reference service, it is important that strains which cannot be CI remain NI rather than be II. With the Biolog system, a low II rate of 3% was obtained at 4 h, which indicates that when an identification is obtained at this time, it is generally reliable. However, higher II rates are reported at 24 h.

No other commercial bacterial identification system has as many taxa in a single database as that supplied by Biolog, but because there are so many, even the large number of tests available may not be adequate, in practice, for discriminating all pairs of taxa. There did appear to be excessive subdivision of some taxa. Within *Salmonella* subspecies 1, for example, Biolog subgroups A to G do not correspond to the biogroups traditionally recognized by conventional biochemical tests. Fifteen strains of *S. paratyphi* were tested, but the Biolog system identified them as belonging to four different subgroups and identified two to the genus level. The identification rate for salmonellas as a whole might be improved if there were but a single taxon, *Salmonella* sp., in the database, leaving the user to go on to serology for confirmation of the identification, as recommended by the manufacturer. Furthermore, some II isolates were identified only to subspecies level, also suggesting that some pairs of taxa should be combined, e.g., the various *Klebsiella* species and subspecies included in this study. Similarly, when *Shigella* strains were II, they were often II as other *Shigella* species (these were treated as NI in this study, as performance of the recommended serology tests should have prevented the II finding). Again, a combined single taxon for *Shigella* spp. may be more appropriate, with identification to the species level achieved largely via serologic testing.

In providing the National Collection of Type Cultures reference identification service, some initial tests are performed to determine if an unknown has a fermentative or nonfermentative mode of metabolism. Appropriate tests and the appropriate database can then be chosen, and because they are in separate databases, there is no possibility of a fermentative organism interfering with the identification of a nonfermentative one. With a single database, as in the Biolog system, it is theoretically possible for organisms with quite different modes of metabolism to interfere with each other's identification. Introduction of some basic initial screening tests and subdivision of the Biolog GN database into a number of smaller databases could improve the CI rate, especially when fundamentally different taxa have similar metabolic patterns. However, such an approach would unfortunately increase turnaround times in clinical laboratories. It appears from the data presented in Table 1 that, in general, the Biolog system is better at identifying fermentative organisms than nonfermenters. Indeed, many other commercial identification system manufacturers have separate products appropriate for fermenters and nonfermenters. However, it should be noted that biochemically active nonfermenters do achieve high identification rates (88%) in the Biolog system, so a different product may be more suitable for inactive nonfermenters, a course followed by some other manufacturers of commercial identification systems.

In release 3.01A, when automated readings are taken, the software subtracts the control well reading from the readings of the other wells and expresses their individual activities as a percent change with respect to the control well. However, a positive result in the control well is sometimes evident and consequently the other wells are interpreted as negative, and either an NI or an II result is obtained. Visual inspection of the plate may nevertheless reveal several clearly positive results. The positive result in the control well may be overridden, and when the positive results in the other wells have been recorded as such, a CI result may be achieved. This situation tends to arise with capsulated organisms, such as *Enterobacter* and *Klebsiella* spp., which apparently respire by using their capsular material. Release 3.60 of the Biolog software appears to rectify some of the problems observed in the way the automated software determines positive and negative results.

Most prominent among the taxa whose identification fared badly in this study were *E. aerogenes* and several of the *Klebsiella* species (subspecies) which were not differentiated well from each other. The differentiation of these organisms may not, however, prove easy if the problem lies with the respiration of capsular material, as positive results are likely to be obtained in all tests for all strains. Klingler et al. (7) also found unreliable identifications, particularly with *Enterobacter*, *Klebsiella*, and *Serratia* strains. The manufacturer is aware of this problem and has since modified the protocol so that capsule-producing strains are inoculated at a lower density.

Just as discrimination is lost between very reactive taxa, yielding many positive results, so discrimination is lost between highly unreactive taxa. This happens with any system, so it is not surprising that the CI rate for *Moraxella* and *Neisseria* isolates in the present study was 38%; however, all strains of *Moraxella osloensis* were CI. The makers of the Biolog system have since amended their protocol so as to increase the inoculation density of unreactive species and thereby yield a strong and complete pattern of reactions.

The Biolog system presented few obvious hazards beyond the mixing and use of a large inoculum volume, which requires care to avoid spillage and creation of aerosols.

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