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Preliminary Evaluation of Biolog, a Carbon Source Utilization Method for Bacterial Identification

J. MICHAEL MILLER* AND DWANE L. RHODEN
Centers for Disease Control, Atlanta, Georgia 30333

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The Biolog Identification System (Biolog, Inc., Hayward, Calif.) is a new bacterial identification method that establishes an identification based on the exchange of electrons generated during respiration, leading to a subsequent tetrazolium-based color change. This system tests the ability of a microorganism to oxidize a panel of 95 different carbon sources. We report on a preliminary investigation of the ability of the instrument to identify, using its computer-driven enzyme immunoassay reader, a diverse group of clinically relevant members of the family *Enterobacteriaceae* and gram-negative non-*Enterobacteriaceae*. The Biolog reported identifications (correct or incorrect) for 266 of 352 organisms tested (75.6%). Of the 266 identifications reported, 87.3% were correct at the genus level and 75.6% were correct at the species level at 24 h. In the total study of 352 strains, 46.6% were correct to the species level at 4 h and 57.1% were correct to the species level at 24 h. The error rate was 10.4% after 4 h and 9.6% after 24 h. The Biolog performed well with many genera, but problems were encountered with some strains of *Klebsiella*, *Enterobacter*, and *Serratia*. We found the system to be versatile and easy to use.

A large number of automated, semiautomated, and manual systems have been accepted by clinical laboratories for the identification of pathogenic bacteria. Most of these systems rely on bacterial growth and subsequent pH-directed color changes to establish an identification. Although these systems generally perform well for identifying most routinely encountered isolates, they are not as good at identifying some important genera such as *Acinetobacter*, *Moraxella*, *Aeromonas*, and *Haemophilus*, and they do not identify the rare or unusual human isolates and environmental contaminants that may be encountered in the clinical laboratory. The Biolog Identification System (Biolog, Inc., Hayward, Calif.) is a new instrument that establishes an identification based on the exchange of electrons produced during an organism's respiration, leading to a subsequent tetrazolium-based color change. This system tests the ability of a microorganism to oxidize a panel of 95 different carbon sources. Because Biolog panels contain 95 tests instead of the 20 to 30 tests found in most panels, the manufacturer has been able to compile a data base that includes 434 species or groups of mostly gram-negative bacteria.

Biolog's microplates use redox chemistry to colorimetrically indicate respiration of live cell suspensions. All wells are initially colorless. When a chemical in a well is oxidized, there is a burst of respiration and the cells reduce a tetrazolium dye, forming a purple color. A reference well contains no carbon source. The test yields a pattern of purple wells which constitutes a "metabolic fingerprint" of the organism. The microplate reader takes about 5 s to read each tray.

We report on a preliminary study of this system to test its performance limits.

MATERIALS AND METHODS

Cultures tested. The 352 isolates tested included 212 members of the family *Enterobacteriaceae*, 105 nonfermenters, and 35 oxidase-positive fermenters and consisted of not more than 10 strains in each of 91 species (Table 1). Most *Enterobacteriaceae* were stored at room temperature in nutrient agar stabs; other cultures were maintained at −70°C in sheep blood. The isolates, which consisted of both typical and atypical strains from collections at the Centers for Disease Control (CDC), had been identified by conventional biochemical and serologic techniques (3–7). After the study was completed, discrepancies noted between Biolog and conventional identifications were arbitrated by reidentification of the test cultures by using CDC's reference laboratory techniques.

Biolog Microstation system. The Biolog system consists of a microplate containing 95 different carbon sources and a control well, a turbidimeter, and a computer-driven automatic plate reader. Cultures to be tested were removed from storage, subcultured twice on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) containing 5% sheep blood, and incubated overnight at 35°C. The inoculum to be used for testing was prepared from the second subculture. The inoculum was prepared by rolling a cotton swab over the agar plate and preparing a suspension in 18 to 20 ml of 0.85% saline to establish the appropriate inoculum density relative to that of the milk of magnesia standard specified by the manufacturer (roughly equivalent to a range of 53 to 58% transmittance or 4×10^8 to 6×10^8 organisms per ml). The resulting suspension was poured into a multichannel pipette reservoir (Costar Corp., Cambridge, Mass.). By using an eight-channel repeating pipetter fitted with 1,500-μl-capacity sterile tips (Flow Laboratories, McLean, Va.), precisely 150 μl of the suspension was dispensed into each well of the microplate. The lid was replaced, and the microplate was incubated at 35°C for 4 h. After this initial incubation period, the plate was placed in the reader and read automatically. The plates were then returned to the incubator for an additional 14 to 20 h of incubation, and then a second and final reading was made. No additional biochemical tests were used. Serologic tests were used to confirm *Salmonella* and *Shigella* species when indicated on the Biolog report.

The instrument calculates a similarity index for the 10 strains in the data base most closely related to the test strain.

* Corresponding author.

TABLE 1. Biolog results for all taxa tested for genera and species

Organism	No. correct/no. tested at: 4 h	24 h
Achromobacter spp.[a]	3/7	5/7
Ochrabactrum anthropi[b]	1/2	2/2
A. xylosoxidans subsp. *xylosoxidans*	2/3	3/3
A. xylosoxidans subsp. *denitrificans*	0/2	0/2
A. faecalis	3/4	3/4
A. tumefaciens	2/2	2/2
Acinetobacter spp.[a]	5/6	3/6
A. baumanii[b]	4/4	3/4
A. lwoffii	1/2	0/2
Actinobacillus spp.	2/4	2/4
A. ureae	1/1	0/1
A. equuli	0/1	0/1
A. lignieresii	0/1	1/1
A. suis	0/1	0/1
Aeromonas hydrophila	1/3	2/3
Alteromonas (*Shewanella*) *putrefaciens*	2/2	2/2
Bordetella bronchiseptica	2/3	0/3
CDC IV C-2	1/2	1/2
Cedecea sp.	3/4	3/4
C. davisae	2/2	2/2
C. lapagei	1/1	0/1
C. neteri	0/1	0/1
Chromobacterium violaceum	2/2	2/2
Chryseomonas luteola	0/2	0/2
Citrobacter spp.	9/14	14/14
C. freundii	4/7	7/7
C. diversus	1/4	4/4
C. amalonaticus	3/3	3/3
Comamonas (*Pseudomonas*) *testosteroni*	2/2	1/2
C. (*Pseudomonas*) *acidovorans*	0/2	2/2
Edwardsiella tarda	3/4	2/4
Enterobacter spp.	20/25	20/25
E. aerogenes	2/3	2/3
E. agglomerans	6/7	6/7
E. asburiae	1/2	1/2
E. cloacae	2/7	1/7
E. gergoviae	1/2	2/2
E. sakazakii	1/2	0/2
E. taylorae	1/2	1/2
Escherichia spp.	32/34	28/34
E. coli	19/22	18/22
E. hermanii	3/4	3/4
E. fergusonii	3/4	3/4
E. vulneris	4/4	2/4
Flavimonas oryzihabitans	2/2	2/2
Flavobacterium sp.	9/11	4/11
F. breve	1/2	2/2
F. odoratum	1/2	0/2
F. indologenes	4/4	1/4
F. meningosepticum	3/3	3/3

Continued

TABLE 1—*Continued*

Organism	No. correct/no. tested at: 4 h	24 h
Hafnia alvei	0/6	5/6
Klebsiella spp.	14/14	12/14
K. pneumoniae	4/7	4/7
K. oxytoca	0/3	0/3
K. ozaenae	2/2	1/2
K. rhinoscleromatis	2/2	2/2
Kluyvera spp.	1/4	4/4
K. ascorbata	1/2	1/2
K. cryocrescens	0/2	2/2
Moraxella spp.	6/10	5/10
M. osloensis	2/2	1/2
M. phenylpyruvica	1/2	0/2
M. nonliquefaciens	1/2	1/2
M. lacunata	0/2	0/2
M. atlantae	1/2	1/2
Morganella morganii	4/4	4/4
Oligella ureolytica	0/2	0/2
Pasteurella spp.	6/9	5/9
P. aerogenes	1/2	2/2
P. multocida	3/3	1/3
P. haemolytica	0/2	1/2
P. pneumotropica	1/2	0/2
Plesiomonas shigelloides	0/3	1/3
Proteus spp.	16/16	15/16
P. mirabilis	10/10	7/10
P. penneri	2/2	2/2
P. vulgaris	3/4	4/4
Providencia sp.	12/12	12/12
P. rettgeri	4/5	5/5
P. stuartii	4/4	4/4
P. alcalifaciens	2/2	2/2
P. rustigiani	0/1	1/1
Pseudomonas spp.	32/34	32/34
P. aeruginosa	2/7	6/7
P. fluorescens	4/6	5/6
P. putida	0/2	2/2
P. cepacia	2/3	2/3
P. stutzeri	2/2	2/2
P. mendocina	0/2	1/2
P. paucimobilis	1/2	2/2
P. pickettii	2/2	2/2
P. diminuta	2/2	2/2
P. alcaligenes	1/2	2/2
P. pseudoalcaligenes	2/2	2/2
P. vesicularis	1/2	1/2
Salmonella spp.	21/27	22/27
Serratia spp.	13/17	11/17
S. liquefaciens	3/3	1/3
S. marcescens	5/6	4/6
S. rubidaea	1/2	1/2
S. odorifera	1/2	0/2
S. plymuthica	1/2	0/2
S. fonticola	2/2	2/2

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TABLE 1—*Continued*

Organism	No. correct/no. tested at:	
	4 h	24 h
Shigella spp.	10/15	11/15
Sphingobacterium multivorum	2/3	2/3
Vibrio spp.	15/16	16/16
V. alginolyticus	2/3	3/3
V. cholerae	0/3	2/3
V. fluvialis	1/2	2/2
V. vulnificus	2/2	1/2
V. mimicus	2/2	2/2
V. parahaemolyticus	2/4	4/4
Weeksella virosa	1/2	1/2
W. zoohelcum	1/2	1/2
Xanthomonas maltophilia	2/5	4/5
Yersinia spp.	10/16	14/16
Y. enterocolitica	4/6	5/6
Y. pseudotuberculosis	1/2	2/2
Y. frederiksenii	1/2	0/2
Y. ruckeri	1/2	1/2
Y. kristensenii	0/2	0/2
Y. intermedia	1/2	2/2

[a] Correct to the genus level.
[b] Correct to the species level.

At the 4-h reading, only an "excellent identification" (similarity index, >0.75) is acceptable. If a lower index is obtained, the user is instructed to continue the incubation for 24 h. At the 24-h reading, if the index is below 0.50, the instrument reports "poor identification" or "no identification." If the index is between 0.50 and 0.74 at 24 h, a "good identification" is reported along with a genus and species name. "Excellent identification" is reserved for indices of >0.75. The 10 closest matching species are printed on all reports, although only reports with an index of >0.5 are considered an acceptable genus and species identification.

For this study, "correct identification" means the correct genus and species compared with conventional biochemical identification and claimed by the manufacturer in the data base to be identifiable. *Salmonella* and *Shigella* species were accepted as "correct," even though the manufacturer claimed to be able to determine *Shigella* organisms to the species level and group the *Salmonella* species.

TABLE 2. Results of 4-h Biolog reading

Organism (no.)	%			
	Correct to genus level	Correct to species level	No identification, reincubate	Incorrect
Total study (352)	53.7	46.6	36.0	10.4
Enterobacteriaceae (212)	56.0	52.0	38.0	8.0
Non-*Enterobacteriaceae* (140)	51.5	38.6	33.6	15.0

TABLE 3. Results of 24-h Biolog reading

Organism (no.)	%			
	Correct to genus level	Correct to species level	Incorrect	No identification
Total study (352)	65.9	57.1	9.6	24.4
Enterobacteriaceae (212)	66.2	60.0	8.5	21.3
Non-*Enterobacteriaceae* (140)	78.7	55.8	10.8	20.8

RESULTS

When an instrument is evaluated in our laboratories, it is subjected to a "stress test" as well as a "weighted laboratory assortment." The stress test consists of hundreds of strains of usual and unusual isolates in proportions likely never to be seen in a routine clinical laboratory. The stress test is designed to test the limits of accuracy of any instrument or method. The weighted test, on the other hand, is one that uses commonly isolated clinical strains in relative proportions likely to be encountered in most laboratories.

In our stress test, Biolog provided a genus and species identification for 266 of 352 (75.6%) organisms tested. Of the 266 identifications reported, 87.3% were correct at the genus level and 75.6% were correct at the species level at 24 h. Tables 2 and 3 list the results of both the 4- and 24-h readings, respectively. In the total study, Biolog correctly identified 46.6% of the organisms to the species level in 4 h and 57.1% of the organisms to the species level in 24 h. The error rate after 4 or 24 h of incubation was 10.4 or 9.6%, respectively. The remaining strains (53.4%) either were prompted by Biolog to be reincubated after 4 h or a report of poor identification or no identification was generated at 24 h (42.9%). In the latter case, the system allows the user to interpret from a similarity list of species that most closely matches the unidentified strain tested, but it does not report an acceptable identification.

The study strains were evaluated on the basis of the fact that they belong to the family *Enterobacteriaceae* and on the basis of whether the more biochemically active lactose fermenters skewed the error rate. This analysis indicated that all of these categories had approximately the same rates of correct identifications and no identifications.

Table 4 shows that the overall performance would have improved if the first choice had been reported regardless of the generated report ($P < 0.01$). The data on the isolates classed as not identified or poor identification were studied to determine how accurate the instrument would be if the first choice (highest similarity index) were selected by the user (Table 5). Eighty-six strains were not identified. If the first choice were selected, 45.4% of the unidentified strains would have been correct at the species level, which would have improved the performance of the system. Overall, 79%

TABLE 4. Total potential at 24 h if first choice taken, regardless of report

Organism (no.)	% Correct to:	
	Genus level	Species level
Total study (352)	79	68.2
Enteric (212)	77.4	68.4
Non-enteric (140)	76.5	62.2

TABLE 5. Evaluation of nonidentified isolates, effects of first-choice selections

Organism (no.)	% Correct if first choice taken to:	
	Genus level	Species level
Total study (86)	53.6	45.4
Enterobacteriaceae (45)	53.4	40.0
Non-*Enterobacteriaceae* (29)	38.0	31.1

of the 352 study strains would have been correctly identified to the genus level and 68.2% would have been correctly identified to the species level.

The results of the 24-h weighted laboratory assortment (Table 6) were not significantly different from those of the stress test ($P > 0.2$). While the percent that was not identified failed to improve, the choices available were more accurate and could have resulted in an increase in accuracy to the species level at 24 h to 67.7%, which is better than the 52.2% reported. Table 1 presents the species-by-species results of the testing for each of the 91 taxa.

DISCUSSION

Carbon source utilization for the identification of bacteria is not a new technology, but application of this process in a familiar format similar to that of other commercially based identification systems is new (1). Biolog's entrance into the clinical microbiology marketplace is welcome; this system brings with it a potential for characterization of strains not enjoyed by systems whose sole biochemical procedure is built around carbohydrate utilization and pH changes.

Although Biolog appears to work well with some genera, there seem to be certain genera with which consistent problems occur. For example, some *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp. were so active that most of the carbon sources were utilized, giving too many positive reactions that led to erroneous results. We found, however, that in many cases there was a "time window" of 6 to 8 h of incubation during which the correct genus and species were determined but beyond which the results became inaccurate. Adjustments in test protocols may alleviate some of those problems. The new version of the manufacturer's instructions alert the user to read results for these enteric organisms at 4 h.

We found the visual reading option to be too difficult to judge true + and − wells compared with the very accurate and rapid automated Microstation reader. Generation of a 32-digit biotype number visually from the microtiter plate is time-consuming at best. After a trial of 50 organisms belonging to the family *Enterobacteriaceae*, we eliminated the visual reading and used only automated results. On the basis of our feedback, the manufacturer has revised the visual reading system to accommodate +, −, and +/− reactions. We have not evaluated this revised system.

TABLE 6. Evaluation of Biolog with a weighted laboratory assortment[a]

Time	%				
	Correct to genus level	Correct to species level	Incorrect	Reincubate	No identification
4 h	57.8	46.5	4.3	38.1	
24 h	62.0	52.2	15.2		22.6[b]

[a] A total of 71 organisms was tested.

[b] A total of 68% of these would have been correct if the first choice had been taken.

The computer program that drives the Biolog system is user-friendly, allows the technologist to check results, if necessary, and enhances research capabilities by providing a method for the user to create a unique data base of organisms being tested. The software can be purchased separately. It is designed to be run on any DOS-based IBM-compatible personal computer, including the XT, AT (286), 386, and 486 models with hard drives of 20 megabytes or larger and at least one floppy disk drive (5 1/4 or 3 1/2 in.). So much information is provided in the printout that some of it is irrelevant and not necessary for clinical laboratory applications. There has been no downtime after nearly 2 years of use, and the hardware has required no maintenance.

Although it reported an identification for 75.6% (266 of 352) of the strains in the 24-h stress test, the error rate for those identified was 12.8%. Of the 266 strains for which the Biolog reported at 24 h, 87.3% were correct at the genus level and 75.6% were correct at the species level. These results for the stress test could be comparable to those for other systems given the same test if the not identified group could be ignored. However, because 86 strains (32.4%) were not identified by the system, there are questions about the data base and software that could probably be answered by appropriate alterations. Indeed, the manufacturer is actively updating the present configuration to provide more accurate results.

In other studies in our laboratories, 13 strains of *Capnocytophaga canimorsus* (formerly DF-1) were tested. At 24 h, Biolog reported a name for 10 of the strains. All 10 were correct to the genus level, with 7 being correct to the species level. If the first choice had been taken from the lists printed on each report, all 13 genera would have been correct, with 10 being correct to the species level. There were no incorrect responses with this fastidious isolate. In another study of 64 strains of *Xanthomonas maltophilia* from a hospital outbreak, we found that Biolog correctly identified 63 strains at 4 h and 64 strains at 24 h. Studies with *Aeromonas* species have been reported (2) that reflect accurate results with this group.

The data base of Biolog was built to serve several microbiology disciplines: research laboratories, environmental laboratories, and clinical laboratories. Because of the array of strains in the data base, the potential exists for the slow-growing environmental strains to weaken the similarity indices of the common isolates.

A recent upgrade of the data base, as reported to us by the manufacturer, has resulted in identification by the system of 86.4% of the members of the family *Enterobacteriaceae* used in this study. Of this percent identified, 79.8% were correct to the species level and 89.9% were correct to the genus level. Of the non-*Enterobacteriaceae*, 80.4% were identified, 73.2% to the species level and 89% to the genus level.

The Biolog system may have potential for the clinical microbiology laboratory, especially for isolates that are difficult to identify. Further refinements in software are necessary to enable the system, in our hands, to compete with the systems already in the marketplace. The system is not yet ready, nor does the manufacturer intend it, to be used as the primary clinical data instrument for laboratories.

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