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Evaluation of Biolog for Identification of Members of the Family *Micrococcaceae*

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The Biolog Identification System (Biolog, Inc., Hayward, Calif.) was challenged at two separate laboratories with 113 coded isolates, including 33 type strains of staphylococci, 5 strains of *Micrococcus* spp., and 1 strain of *Stomatococcus mucilaginosus*. Test parameters between the sites were controlled as much as possible. Discrepancies were arbitrated by using conventional biochemicals. Overall accuracies (correct to the species level) upon initial testing were 47.7 and 59.3%, respectively, at the two laboratories. After repeat testing of isolates generating "no identification" responses or errors, the overall accuracies increased to 69.0 and 74.3% at the two sites, respectively, revealing no significant difference in the final results at the two laboratories (78 of 113 versus 84 of 113; $P > 0.05$). Error rates were 7.1% at one site and 9.7% at the other. The Biolog is not yet accurate enough to serve as a primary method for identifying staphylococci.

Systems available for bacterial identification in clinical laboratories rely on any one of a number of methods of measuring metabolic responses or by-products. The methods include pH changes, recognition of fatty acid composition, fluorometric readings for metabolizing cells, detection of preformed enzymes, and reduction of tetrazolium dyes. Identification instruments and packaged systems have been extensively evaluated in the literature, and a recent review summarized the automated methods (18). Identification of staphylococci has been addressed by many of these systems (2, 6–8, 10, 19). Because of the methods of presentation of data in many of those reports, side-by-side analysis of several systems is often difficult and confusing (13).

The Biolog Identification System (Biolog, Inc., Hayward, Calif.) identifies bacteria on the basis of the exchange of electrons produced during an organism's respiration, which causes a subsequent tetrazolium-based color change from clear to purple. This system tests the ability of a microorganism to oxidize a panel of 95 different carbon sources.

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, a burst of cellular respiration causes the reduction of a tetrazolium dye, forming a purple color. A reference well contains no carbon source. The test yields a pattern of purple wells that constitutes a metabolic fingerprint of the organism. The microplate reader takes about 5 s to read each tray.

The Biolog MicroPlate system is an alternative choice in the instrument-assisted bacterial identification market and is purported to be able to identify 569 species of gram-negative rods with the GN MicroPlate and 225 gram-positive species with the GP MicroPlate. Its effectiveness in identifying clinically significant gram-negative rods has been reported previously (3, 5, 14, 16, 17). The GP MicroPlate accuracy has been addressed (11, 12), but it has not been explored in the literature. In the present study, by two independent

laboratories, we examined the accuracy of the Biolog in characterizing staphylococci.

MATERIALS AND METHODS

Testing protocol. The blind study described here was performed at two institutions, the Centers for Disease Control and Prevention (CDC) and the University of New Mexico Hospital (UNMH), and was controlled so that each laboratory worked under the same test parameters. Each laboratory used the same set of cultures that were coded at the CDC and inoculated with a single colony pick by using disposable loops, the same lots of Biolog Universal Growth Medium (BUGM), and the same lot of Biolog test plates.

A standard quality control protocol was unavailable for testing the BUGM and the effects of different lots of medium on test outcome. All strains grew luxuriantly, and previous tests of staphylococci on different lots of BUGM showed no apparent difference in the ability of the other lots of medium to support growth, nor did the accuracy change substantially when subsets of staphylococci were tested with different lots (3a).

Cultures tested. The 113 isolates tested in the present evaluation included 33 type strains of coagulase-positive and coagulase-negative staphylococci, 5 strains of *Micrococcus*, and 1 strain of *Stomatococcus mucilaginosus*. Prior to testing by either laboratory, isolates were blinded at the CDC by assigning them arbitrary numbers. The code was broken only after all first-round testing was completed (prior to repeat testing). All strains analyzed were listed in the manufacturer's package insert as identifiable. Some strains not in the data base were included in the testing but were not included in the final analysis of the accuracy of the Biolog system. All isolates tested had been stored frozen at −70°C and had previously been identified by the CDC reference method (15). Discrepancies between the two testing laboratories were arbitrated by reidentifying single-colony isolates by this reference method.

Biolog Microstation system. The Biolog system consists of a microplate containing 95 different carbon sources and a

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control well, a turbidimeter, and a computer-driven automatic plate reader. The Biolog plates can be read at 4 or 24 h. For the present study, we chose to read plates only at the 24-h period because of the number of "no identification" responses initially recorded during several trial readings at 4 h.

Cultures to be tested were removed from storage, and single colonies were picked carefully and subcultured once on 5% sheep blood agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and once on BUGM containing 5% sheep blood and were incubated overnight at 35°C. BUGM is enriched and is especially helpful for fastidious isolates but is not required for performing the test. The inoculum for testing was prepared from the second subculture 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 "high-range" and "low-range" standards supplied by the manufacturer and measured by a turbidimeter (approximately 33 to 37% transmittance). The resulting suspension was poured into a multichannel pipette reservoir (Costar Corp., Cambridge, Mass.). An eight-channel repeating pipettor fitted with 1,500-μl-capacity sterile tips (Flow Laboratories, McLean, Va.) was used to dispense precisely 150 μl of the suspension into each well of the microplate. The lid was replaced, and the microplate was incubated at 35°C for 24 h. After the incubation period, the plate was placed into the reader and was read automatically. No additional biochemical tests were used. The plate used to prepare the inoculum was streaked to yield isolated colonies and was held for at least 5 days to check for the development of mixed colony types.

The microplate reader calculates a similarity index for the 10 strains in the data base most closely related to the test strain. At the 24-h reading, if the index is below 0.50, the instrument reports "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 a species name. "Excellent identification" is reserved for indices of 0.75 or greater. The 10 closest-matching species are printed on all reports, although only reports with an index of >0.5 are considered to represent acceptable genus and species identifications.

For the present 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. "Genus only" was used when the Biolog reported "*Staphylococcus* sp." If an incorrect genus or species was reported by Biolog, the report was considered an "error."

Internal controls. In order to control for technical error versus instrument error, all tests not reported as correct were repeated. When a first and a second test did not agree, a second repeat test was done to achieve a best two-out-of-three answer.

In order to study reproducibility, one strain of *Staphylococcus epidermidis* was inserted into the study five times, blinded along with all other isolates, and was tested at both sites.

Seventeen correct responses were selected arbitrarily and reread three times in succession on 1 test day to determine the stability of the instrument readings and the utility of the 32-number biotype.

Statistical analysis. Results of testing between the two laboratories were analyzed by chi-square analysis with Yates' correction.

TABLE 1. Accuracy of the Biolog system after repeat testing[a]

Organism tested	No. correct/no. tested	
	CDC	UNMH
Staphylococcus arlettae	1/1	0/1
Staphylococcus aureus	8/9	9/9
Staphylococcus auricularis	4/4	3/4
Staphylococcus capitis	3/3	3/3
Staphylococcus capitis subsp. ureolyticus	1/2	2/2
Staphylococcus caprae	1/1	1/1
Staphylococcus carnosus	2/2	2/2
Staphylococcus caseolyticus	0/1	0/1
Staphylococcus chromogenes	1/3	0/3
Staphylococcus cohnii	2/2	2/2
Staphylococcus cohnii subsp. ureolyticum	2/2	2/2
Staphylococcus epidermidis	13/16	14/16
Staphylococcus felis	1/1	1/1
Staphylococcus gallinarum	1/1	1/1
Staphylococcus hemolyticus	4/9	3/9
Staphylococcus hominis	7/10	7/10
Staphylococcus hyicus	2/2	2/2
Staphylococcus intermedius	1/3	2/3
Staphylococcus kloosii	0/1	1/1
Staphylococcus lentus	1/2	1/2
Staphylococcus lugdunensis	3/4	4/4
Micrococcus sp.	4/5	5/5
Staphylococcus saccharolyticus	0/1	0/1
Staphylococcus saprophyticus	2/5	2/5
Staphylococcus schleiferi	1/2	2/2
Staphylococcus sciuri	1/3	3/3
Staphylococcus simulans	4/4	4/4
Stomatococcus sp.	1/1	1/1
Staphylococcus warneri	3/8	3/8
Staphylococcus xylosus	4/5	4/5
Total	78/113 (69.0%)	84/113 (74.3%)

[a] All tests reported or determined to be no identification, error, or genus only were repeated.

RESULTS

In the present blind evaluation, 113 strains of the family *Micrococcaceae* were tested by the Biolog system. In addition, five strains of *S. epidermidis* were included in duplicate to determine the reproducibility of the system. The limited number of isolates of some species tested reflects the number of these isolates in the culture collection. For these strains, the data should be considered tentative and should be interpreted with caution. Table 1 lists the results of testing at CDC and UNMH.

Discrepancies between the overall number of correct responses at the two laboratories were not predictable. An identification response listed as an error reported on an isolate tested at one site was often reported as correct at the other. In a few cases, errors or no identification reports were generated on the same isolate from the two sites, but no pattern emerged.

When 33 type strains were tested (Table 2), the initial percentage of correct responses generated was lower than expected, primarily because of the large number of no identification responses after 24 h. One test site reported 45.5% as no identification with only 3% error, while the other site had only 15.2% responses with no identification but with 15.2% errors. After repeat testing of the strains initially labelled as no identification and errors, the correct responses increased to 78.8 and 84.9% for the two sites, respectively (Table 2). No statistically significant difference

TABLE 2. Ability of Biolog to identify type strains
of staphylococci

Test	Location (n = 33)	No. (%) of isolates			
		Correct	No identifi-cation	Error	Genus only
Initial	CDC	15 (45.5)	15 (45.5)	1 (3.0)	2 (6.1)
	UNMH	23 (69.7)	5 (15.2)	5 (15.2)	0
Repeat[a]	CDC	26 (78.8)	5 (15.2)	1 (3.0)	1 (3.0)
	UNMH	28 (84.9)	1 (3.0)	2 (6.1)	2 (6.1)

[a] Repeat tests were performed on all isolates reported as no identification or error.

existed between the results at the two test sites ($P > 0.05$). After repeat testing, all type strains were identified correctly by at least one test site with the exception of *Staphylococcus caseolyticus* and *Micrococcus lentus*. *M. lentus* was reported as no identification at both sites, but the first choice (with a low similarity index) printed on the report would have been correct. The first-choice result for *S. caseolyticus* would have been an error (*Staphylococcus hominis*) at both sites.

Table 3 shows the results of initial and repeat testing for all the taxa evaluated. After repeat testing of isolates initially reported as no identification and errors, the two test sites reported correct results for 69.0 and 74.3% of the isolates, respectively. In this category, no significant difference was noted between the results at the two test sites ($P > 0.05$). Repeat test results were significantly more accurate than initial results at both test sites ($P < 0.05$).

One strain of *S. epidermidis* was included five times within the test set to determine the internal reproducibility of the Biolog system. These five isolates were tested twice at each test site. Three of the five isolates were correctly identified. The disagreements were minor, in that upon retesting, one correct response changed to a no identification and one no identification response changed to correct. In both cases, the first choice from the no identification response would have been *S. epidermidis*.

Seventeen initially correct responses were retested and read three times within 1 h. Five of 17 (29.4%) readings changed from correct to no identification. For three of the five that changed to no identification, the first choice listed was correct, although in each case the similarity index was below the threshold for correct responses.

DISCUSSION

Because of the increased clinical significance of the staphylococci (1, 4, 9), many laboratory technicians find it impor-

TABLE 3. Ability of Biolog to identify members of the
family *Micrococcaceae*

Test	Location (n = 113[a])	No. (%) of isolates			
		Correct	No identification	Error	Genus only
Initial	CDC	54 (47.7)	50 (44.2)	6 (5.3)	3 (2.7)
	UNMH	67 (59.3)	29 (25.7)	14 (12.4)	3 (2.7)
Repeat[b]	CDC	78 (69.0)	23 (20.4)	8 (7.1)	4 (3.5)
	UNMH	84 (74.3)	11 (9.7)	11 (9.7)	7 (6.2)

[a] Includes five *S. epidermidis* blind duplicates.
[b] Repeat tests were performed on all isolates reported as no identification or error.

tant to identify and perform susceptibility testing on gram-positive cocci. Two critical issues must be addressed before clinical significance can be accurately attributed to isolates of coagulase-negative staphylococci and other members of the family *Micrococcaceae*. The first is understanding the importance of testing single-colony isolates of the coagulase-negative staphylococci (2, 9). Many of these species initially share similar colony characteristics within a routine 24- to 48-h incubation period. After this period, colony morphology begins to change enough so that the laboratory technician can recognize a mixed culture on what was initially thought to be a pure culture of staphylococci. Testing a "sweep" of several colonies, rather than a single colony pick, will likely lead to erroneous results that may obscure the clinical significance of opportunists among the population (2) and could lead to misleading susceptibility test results.

The second issue to be addressed is the development and use of identification methods of proven accuracy in identifying members of this important group of organisms. Published reports must specify that, for testing, the evaluation protocol use only carefully selected, single-colony isolates that were previously identified by the same careful isolation procedures.

In the present study, we were careful to select only single-colony isolates for testing by the Biolog system. In addition, plates were held for 5 to 7 days to ensure that mixed cultures, as recognized by different colony types, were not used. These well-characterized isolates were used in the study, and the same conventional procedure was used to reidentify strains whose test results did not agree between the two test sites.

In our hands, the Biolog system was simple to use and offered a wide range of organisms for comparison. The software is unique in that it allows two- and three-dimensional observations and analysis of dendrograms. This is especially useful as a research tool for studying cluster analyses of isolates and for comparing one strain with those genetically closest to it.

An analysis of the findings of this evaluation revealed that the two test sites had similar results. The errors and noncorrect (genus only, no identification, or error) responses at one site, however, did not necessarily match the errors and noncorrect responses at the other one. No predictable pattern emerged whereby problems could be anticipated and corrected.

In the present evaluation of the ability of the Biolog system to identify the type strains of staphylococci, only 14 of 33 type strains were identified correctly by the first test at both sites. In most of the other cases, the system at one site would generate a correct response and the system at the other site would generate a category of noncorrect. Repeat testing resolved all but two of the noncorrect responses. An identification method should be able to consistently identify upon initial testing the type strains of the organism groups in the data base.

On the basis of the upper range of accuracy reported here (74.3%), the results of the Biolog system were not significantly different from comparable results of the API StaphIdent system (3a, 6, 7), the ATB-32 system used for *Staphylococcus hyicus* (10), or even initial results of the Vitek system prior to additional tests (2). More recent evaluations of the MicroScan system suggest accuracy rates significantly higher than those that we found with the Biolog system (8, 19). Although the overall accuracy was higher with the other systems, the Biolog system was more accurate with some

species of coagulase-negative staphylococci than was the MicroScan (8) or the Vitek (2) system.

Overall, the accuracy (correct response) of the Biolog system did not exceed 74.3% for the 113 strains tested (Table 3). The error rate for the total study ranged from 7.1 to 9.7%. If one looks only at errors in the present evaluation, one might assume a somewhat impressive nonerror response of 91.3 to 92.9%. Unfortunately, the Biolog system placed many of the isolates that we tested into a no identification category, thereby offering little or no help to the diagnostic needs of the laboratory. We observed that in many cases the correct result rather than a no identification would have been reported if the similarity index had been lowered to below 0.5. Other results, however, would have emerged as errors.

Maintaining the integrity of a 32-number biotype over a series of tests with the same organism or even with repeat testing on the same MicroPlate is predictably difficult. While some individual biotype numbers within the profile can change without changing the final identification result, the biotype number cannot be used as an epidemiologic tool. We found that some alterations in biotype numbers could occur when testing the same isolate several times. We also found that both rereading and retesting of correct responses may produce different results.

Most published evaluations retest only organisms for which there are errors or noncorrect responses and ignore the possibility that some correct responses might change if they, too, were to be rechallenged. Instrument or method evaluations might be more valid if all analytes, correctly reported or not, were tested in three separate runs. While the cost and time involved might be greater, the resulting data might be more valid than those from single tests and limited analyses.

It appears from the results of the present study that either the Biolog data base is not broad enough to account for diverse strains of staphylococci or that the algorithm used in analysis does not allow the inclusion of many necessary metabolic profiles. The Biolog system is not yet accurate enough to serve as a primary method for identifying staphylococci in the clinical laboratory.

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