Comparison of Identifications of Human and Animal Source Gram-Negative Bacteria by API 20E and Crystal E/NF Systems

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This study compared the abilities of API 20E and BBL Crystal E/NF identification systems to correctly identify human and animal source gram-negative bacilli of known identifications, as provided by the American Type Culture Collection, Rockville, Md., and the Research Diagnostic and Investigative Laboratory, Columbia, Mo. Also addressed in the comparison are the cost, the relative ease of performing and interpreting the tests, and the potential problems surrounding each system. The two systems were comparable in terms of their respective costs and abilities to identify the bacteria tested. The cost per test was calculated as $4.69 for API 20E and $4.62 for Crystal E/NF. Of the animal source bacteria tested, Crystal E/NF identified 68% to the correct genus and species and 90% to the correct genus or group. The remaining 10% of the animal source bacteria were unidentified by Crystal. Human source bacteria tested by BBL Crystal E/NF gave very similar results: 47% correctly identified to genus and species, 90% correctly identified to genus or group, 7% unidentified, and 3% incorrectly identified. API 20E results were as follows for animal source bacteria: 53% correctly identified to genus and species, 76% correctly identified to genus or group, and 24% unidentified; the results for human source bacteria were as follows: 40% correctly identified to genus and species, 83% correctly identified to genus or group, and 17% unidentified. API 20E has a slightly more labor-intensive protocol for setting up the test than BBL Crystal E/NF but produced fewer questionable results.

Rapid and accurate identification of gram-negative bacilli is an important consideration in any clinical laboratory. In the veterinary diagnostic laboratory, the microbiologist must also determine the ability of the tests to correctly identify gram-negative organisms from animal sources. The diversity of the bacteria due to source may provide a difficult test for the identification systems since bacterial strains of the same species may vary slightly in their biochemical reactions and animal source bacteria may be less commonly tested and incorporated in the identification databases of the systems. Microbiologists involved in veterinary microbiology, like those involved in human clinical microbiology, must evaluate rapid-identification systems on the basis of not only their costs, their abilities to give reliable results, and their relative ease of performing and interpreting the tests, but also on their incorporation of a diverse database that accounts for variations in biochemical reactions in different strains of the same bacterium. An identification system that has low cost and simple instructions but unclear biochemical reactions, or reactions that do not yield a reliable identification, is of little value to the clinical microbiologist.

Among the numerous commercial identification systems available today for identification of gram-negative bacilli are the API 20E (bioMerieux Vitek, Inc., Hazelwood, Mo.) and the Crystal E/NF (Becton Dickinson, Inc., Cockeysville, Md.). Data have been published that compare the abilities of these systems to accurately identify gram-negative bacilli from human clinical specimens (1, 3). However, the accuracy of the Crystal E/NF system compared to that of the API 20E system to correctly identify gram-negative bacilli of animal origin has not been reported. These two kits are routinely used for the identification of clinical bacterial isolates in our laboratory,

and it has been our observation that on occasion these systems are unable to identify some animal clinical isolates. Our facility has also noticed differences in the ease of setting up the test kits and in interpreting the results. This study presents the results of a controlled comparison between these two identification systems with American Type Culture Collection (ATCC; Rockville, Md.) human and animal clinical isolates which reflects cost, ability to produce correct, reliable results, and relative ease in performing and interpreting the tests.

MATERIALS AND METHODS

The animal source bacteria selected are pathogens, or potential pathogens, in animals and are often isolated in the veterinary diagnostic laboratory (4).

Organisms tested. The following bacteria were obtained from ATCC as freeze-dried clinical samples: mouse *Pasteurella pneumotropica* ATCC 35149, human *P. pneumotropica* ATCC 12555, dog *Pasteurella multocida* ATCC 12947, human *P. multocida* ATCC 8747, dog *Escherichia coli* ATCC 35322, human *E. coli* ATCC 35323, mouse *Pseudomonas aeruginosa* ATCC 29511, human *P. aeruginosa* ATCC 14218, guinea pig *Salmonella choleraesuis* ATCC 49223, human *S. choleraesuis* ATCC 49222, monkey *Yersinia enterocolitica* ATCC 29913, human *Y. enterocolitica* ATCC 49397, beaver *Klebsiella pneumoniae* ATCC 4727, human *K. pneumoniae* ATCC 4208. *Citrobacter freundii* (4280) and *Citrobacter freundii* (not 4280) from dogs were provided by the Research Animal Diagnostic and Investigative Laboratory, University of Missouri, School of Veterinary Medicine, Columbia, Mo. (RADIL).

Bacterial preparation. All organisms obtained from ATCC were reconstituted in the appropriate medium according to ATCC guidelines and subcultured onto Trypticase soy agar (TSA) with 5% sheep blood (BBL TSA II; Becton Dickinson Microbiology Systems). After overnight incubation at 37°C in ambient atmosphere, the pure cultures were used to inoculate both the API 20E and BBL Crystal E/NF identification systems. Organisms received from the RADIL were subcultured from TSA slants onto BBL TSA II plates and incubated overnight at 37°C with air. Pure, 24-h cultures were used to inoculate the API 20E and BBL Crystal E/NF systems.

Identification by API 20E. One well-isolated colony from each culture was used to inoculate 5 ml of 0.85% NaCl medium, pH 5.5 to 7.0. A humid atmosphere was provided, and the kit was used as directed by the manufacturer to identify the organisms. After 18 to 24 h, all reactions were analyzed according to the interpretation chart included in the package insert. Reagents were added appropriately to the TDA, Voges-Proskauer, and IND tubes, and the reactions were recorded. The oxidase test was also performed with BBL oxidase reagent. After the results for all biochemicals were obtained, the identification of the

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TABLE 1. Comparison of identification data produced by API 20E and BBL Crystal E/NF systems

Organism	Crystal E/NF					API 20E			
	No.[a]	No. of correct genus and species ID[b]	No. of correct genus or group ID	No. of unidentified isolates	No. of incorrect isolates	No.	No. of correct genus and species ID	No. of correct genus or group ID	No. of unidentified isolates
Animal source bacteria									
P. aeruginosa	10	10	10	0		9	0	9	0
P. pneumotropica	10	0	10	0		9	0	0	9
P. multocida	10	10	10	0		10	7	7	3
C. freundii	10	10	10	0		9	9	9	0
C. freundii 4280	10	3	3	7		9	1	1	8
K. pneumoniae	5	4	4	1		5	5	5	0
E. coli	5	5	5	0		5	5	5	0
S. choleraesuis	5	0	5	0		5	0	5	0
Y. enterocolitica	5	5	5	0		5	5	5	0
Total (%)		47 (68)	62 (90)	8 (10)			32 (53)	46 (76)	20 (24)
Human source bacteria									
P. aeruginosa	5	5	5	0	0	5	0	5	0
P. pneumotropica	10	0	10	0	0	10	0	0	10
K. pneumoniae	5	0	3	1	1	5	2	5	0
E. coli	5	4	4	1	0	5	5	5	0
S. choleraesuis	5	0	5	0	0	5	0	5	0
Y. enterocolitica	10	10	10	0	0	9	9	9	0
Total (%)		19 (47)	37 (90)	2 (7)	1 (3)		16 (40)	29 (83)	10 (17)

[a] Number of times the same isolate was tested.
[b] ID, identifications.

organism was made by using the seven-digit number generated (e.g., 5205573) and the analytical profile index.

Identification by BBL Crystal E/NF. One well-isolated colony from each 24-h culture was suspended in a tube of BBL Crystal E/NF inoculum fluid. The tube was vortexed for 15 s, and the entire inoculum was emptied into the target area of the BBL Crystal base. The fluid was then rolled gently along the tracks until all wells were filled. The lid was placed on the base, and the tube was incubated at 37°C overnight in a humid atmosphere (average relative humidity, 48%). After 18 to 24 h, all biochemical reactions were interpreted according to the color reaction chart provided with the system by using the BBL Crystal viewing box. Biochemicals tested included gamma-L-glutamyl p-nitroanilide, esculin, p-nitro-DL-phenylalanine, urea, glycine, citrate, malonate, tetrazolium, arginine, lysine, p-nitrophenyl alpha-arabinoside, p-nitrophenyl phosphorycholine, p-nitrophenyl-beta-glucuronide, p-nitrophenyl-N-acetylglucosaminide, arabinose, mannose, sucrose, melibiose, rhamnose, sorbitol, mannitol, adonitol, galactose, and inositol (INO). Questionable biochemical reactions were evaluated carefully. Oxidase and indole tests were performed with BBL oxidase and indole reagents (Becton Dickinson, Inc.). A 10-digit number (e.g., 5775475551) was generated, and an identification was obtained by using the BBL Crystal E/NF code book and electronic database.

Data interpretation. The API 20E and BBL Crystal E/NF systems were evaluated by using the organisms described earlier to determine the abilities of the systems to accurately identify the organisms. Identifications were performed by two individuals, including a medical technologist certified by the American Society for Clinical Pathologists. Every isolate was tested separately by each technologist according to the outline described above. Following identification of the isolate as a known culture, the procedures for identification by the API 20E and BBL Crystal E/NF systems were performed in a blinded study for 5 days ($n = 5$ to 11, where n is the number of tests performed for an individual isolate) on 24-h cultures of each organism. All questionable biochemical reactions were evaluated by two individuals, who did not know the identity of the isolate, to determine the best result for that biochemical. In cases where multiple identification numbers were possible, all codes were tried for a positive identification. If none of the combinations matched a number in the code book, database, or analytical profile, the identification was considered incorrect. Identifications that matched those provided by ATCC and RADIL and that were rated good likelihood, acceptable, very good, or excellent according to the API 20E system were considered positive identifications of the organisms. For the BBL Crystal E/NF system, an identification was indicated by matching the 10-digit number obtained exactly to a number in the BBL Crystal code book or database. A confidence rating of greater than 0.6000, with 1.0000 being the highest level of confidence, was considered a positive identification with the BBL Crystal E/NF kit. Although this Crystal rating and an API rating of good likelihood are not acceptable in most clinical applications, considerable leniency was allowed for each system in this evaluation since additional supplemental testing which would be used to completely identify clinical isolates in routine practice was omitted. Identifications

were considered correct only when the API or Crystal identifications matched the known identifications of the bacteria provided by the supplier. Results were determined to be either positive for a correct identification or negative for an unacceptable identification. Identifications to the genus or group level were also noted and evaluated as positive or negative.

RESULTS

API 20E. A total of 105 organisms (15 isolates) were inoculated for identification by the API 20E identification system. Of these, 66 organisms were of animal origin and 39 were human source organisms, as described in Materials and Methods. By application of the stringent criteria discussed earlier under "Data interpretation," 53% of the animal source bacteria were correctly identified to the genus and species level with the API 20E system. Taking into account the fact that identification of the genus *Salmonella* with the API 20E or BBL Crystal E/NF systems requires serology for positive species classification and that each system therefore yields only an identification of a *Salmonella* sp., 76% of the animal source bacteria were identified to the correct genus or group by API 20E. In comparison, 40% of the human source isolates were correctly identified by API 20E to the genus and species level and 83% to the genus or group. There were no incorrect identifications (both genus and species) by the API 20E for either the human or animal source bacteria. Human source *K. pneumoniae* was identified to the genus and species level two of the five times tested and identified only to the genus level three of the five times tested. In contrast, 100% of the *K. pneumoniae* isolates of animal origin (beaver) were identified to the genus and species. There were 24% unidentified organisms of animal source and 17% unidentified organisms of human source with the API 20E. All unidentified bacteria belonged to the group *Pasteurella*, with the exception of *C. freundii* 4280 from a dog (Table 1). Correct identification confidence ratings for API 20E are summarized in Table 2. Only 4% of the total identifications gave a confidence rating of good likelihood or acceptable; 96% gave a rating of very good or excellent. In the clinical

TABLE 2. Correct identification confidence ratings produced
by BBL Crystal E/NF and API 20E systems

Confidence rating	No. of IDs[a] with rating (%)
Crystal E/NF	
Below 0.600	10 (9)
0.601–0.850	10 (11)
0.851–1.000	90 (80)
Total	110
API 20E	
Good likelihood	4 (3)
Acceptable	1 (1)
Very good or excellent	100 (96)
Total	105

[a] IDs, identifications.

setting, unidentified bacteria, or those with very low confidence ratings, would be identified by other methods such as tube media or supplemental testing. This additional step was intentionally omitted from the study in order to evaluate the ability of API 20E and BBL Crystal E/NF to produce a correct identification.

Crystal. For identification by the BBL Crystal identification system, a total of 110 organisms (15 isolates) were inoculated. The evaluation included 70 animal source and 40 human source bacteria. Again, by application of the criteria outlined in "Data interpretation," 68% of the animal source bacteria were correctly identified to the genus and species level and 90% of the organisms were correctly identified to the genus or group level (Table 1). Included in the 90% was *P. pneumotropica*, which was identified with a confidence rating of greater than 0.900 as *Pasteurella aerogenes* 10 of the 10 times tested with Crystal E/NF. Also included in the 90% was *K. pneumoniae*, which was correctly identified four out of five times with a confidence rating less than 0.650 (Table 2). The data for the identification of human source bacteria using the BBL Crystal identification system showed that 47% were correctly identified to the genus and species level and that 90% were correctly identified to the genus or group (Table 1). Included in the 90% was *K. pneumoniae*, which was identified as *Klebsiella ozaenae* twice and *Klebsiella oxytoca* once with confidence ratings below 0.700 (Table 2). There was one incorrect identification (both genus and species) of human source *K. pneumoniae* by the BBL Crystal E/NF kit. In addition, there were 10% unidentified animal isolates, 7% unidentified human isolates, and 3% incorrectly identified human isolates (Table 1). In the clinical setting, the unidentified isolates would be tested by tube media or some other commercial method.

Questionable reactions occurred more frequently with the BBL Crystal E/NF identification system than with the API 20E system. The Crystal system produced 34 10-digit identification numbers that contained at least one questionable result out of a total of 110 (31%). In comparison, API 20E generated six seven-digit identification numbers with at least one questionable result out of 106 (6%).

P. pneumotropica from animal and human sources yielded an insufficient number of valid reactions (zero to three coding numbers out of seven possible) for identification by API 20E. However, growth of the organism was confirmed on TSA with 5% sheep blood agar. By using BBL Crystal E/NF, significantly more reactions (6 out of 10 possible coding numbers) were obtained, yet none provided reaction combinations required for positive identification with the Crystal code book and da-

tabase. Identification of the organism *P. multocida* of animal source was 100% correct (genus and species) with the BBL Crystal E/NF system. In contrast, API 20E identification of this organism was only 60% correct (genus and species). There was considerable variability in reactions with sorbitol and mannitol on the API 20E strip, which accounted for the incorrect combination or insufficient number of reactions required for identification by the analytical profile index. Human source *P. multocida* was not tested in this study.

Cost comparison results were calculated on the basis of the commercial cost for each kit. The API 20E identification system has a cost of $117.00 for a kit containing 25 test strips. BBL Crystal E/NF commercially costs $92.40 for a kit containing 20 tests. Inoculum is supplied with the BBL Crystal kit and not with the API kit. The cost for making 0.85% NaCl to use as inoculum in the API test was calculated to be $0.01 per test. Calculating these costs on a per-test basis gives values of $4.69 for API 20E and $4.62 for BBL Crystal E/NF.

Also considered in our study was the amount of required technician time for each system. The API 20E strip took slightly longer to inoculate since each microtube must be filled with inoculum using a Pasteur pipette, and then anaerobic conditions must be satisfied by placing mineral oil on some microtubes. In contrast, BBL Crystal E/NF was easily inoculated by pouring the entire inoculum into the test chamber and rotating until all wells were filled. Excess inoculum was absorbed by placing the base, containing an absorbent pad, onto the lid. There was no addition of mineral oil to the Crystal test chamber. Following incubation, the results of both kits were read. Again, API 20E took slightly longer to process because of the addition of reagents to the TDA, Voges-Proskauer, and IND microtubes. In general, color changes in the API biochemicals were easily detected and numbers were rapidly generated for the seven-digit code number. The color changes in the Crystal E/NF biochemicals, however, were more difficult to interpret, and therefore more time was required to reach an acceptable 10-digit code number. In the cases where multiple identification codes were possible because of uncertain reactions, additional time was needed to look up all the codes.

DISCUSSION

A study similar to the present comparison which reported an accuracy of 74.4% for all isolates tested at 18 to 24 h by API 20E and roughly 92% for all isolates tested at 18 to 24 h by BBL Crystal E/NF without additional testing with human clinical samples was published recently by Robinson et al. (3). Supplemental testing with API and Crystal systems was eliminated from the current study in order to evaluate the abilities of the test strip (API) and test chamber (Crystal) to identify bacteria solely on the basis of the biochemicals incorporated in the strip or chamber. The bacteria chosen for this study were clinical isolates, extensively characterized by ATCC and RADIL, and were used to ensure that the identifications produced by the kits were correct. Although these bacteria were representative of common isolates in human and animal bacteriology, any variation due to differences among strains could affect the results of this study. However, strain variation can be considered negligible since the accuracy results from this study corresponded well with those reported earlier and since there was good correspondence between human and animal source bacterium accuracy results in the current study. With the API 20E system, 76% of the animal source bacteria and 83% of the human source bacteria were correctly identified to at least the genus level (including genus level identification for *Salmonella*) without supplemental testing. The accuracy of the BBL

Crystal E/NF system was slightly higher than that of the API 20E system for human isolates, yielding correct genus level identification in 90% of the animal source bacteria and 90% of the human source bacteria. It was not apparent that either API 20E or Crystal E/NF performed differently when animal source bacteria were tested. Both systems were unable to identify human and animal source *P. pneumotropica*, although the Crystal system did identify the organism as *P. aerogenes* 10 of the 10 times tested for both animal and human source *P. pneumotropica*. Crystal E/NF correctly identified *P. multocida* from a dog to the genus and species level 10 of the 10 times it was tested. In contrast, API 20E correctly identified this organism only six of the ten times tested. Of all the bacterial organisms tested in this study, those from the group *Pasteurella* were the only isolates identified more accurately by Crystal E/NF than by API 20E. This observation may be explained by the design of the API 20E kit to primarily identify gram-negative enteric bacteria; the Crystal E/NF kit incorporates features for the identification of non-fermentative gram-negative bacteria with enteric bacteria. Overall, the API 20E identification system and the Crystal E/NF system performed well in testing the human and animal isolates in this study. Table 2 summarizes the correct-identification confidence ratings produced by each test kit. Although the API 20E kit had slightly lower percentages of correct identifications, those that were correct had higher confidence ratings than those of the Crystal E/NF kit. Only 4% total of the correct identifications by API 20E were below very good or excellent. In contrast, Crystal E/NF produced a total of 20% below the 0.850 confidence rating, with 9% being below 0.600 (Table 2). The primary problem encountered with the Crystal system was the frequency of questionable reactions. For example, the set of reactions located between 2A (*p*-nitrophenyl phosphorycholine) and 2J (*p*-nitrophenyl-*N*-acetylglucosaminide) produces a yellow color when there is a positive reaction with one of these biochemicals. It was our experience in interpreting these subtle reactions that the change from clear or white (negative) to yellow (positive) was sometimes too difficult to distinguish. Moreover, with the Crystal E/NF system, some biochemicals' reaction colors for positive and negative reactions overlap. For example, a color reaction of green for urea, glycine, citrate, and malonate can be either positive or negative. The technician performing the test must decide if the reaction is actually blue-green or yellow-green. Aside from these technical differences and difficulties between the API 20E and the BBL Crystal E/NF kits, both tests performed well. There was no significant price difference between the two systems, and the technical time required was only slightly different.

In summary, both the API 20E and the BBL Crystal E/NF kits are acceptable systems for the clinical identification of animal source bacteria. The significant characteristics that distinguish the two systems in our opinion include increased safety with the BBL Crystal E/NF system because there is no pipetting of the inoculum, greater ease in interpreting the color reactions of the API 20E system, and lower occurrence of questionable results with the API 20E system.

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