

Evaluation of Quantum II Microbiology System for Identification of Gram-Negative Bacteria of Veterinary Origin

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The ability of a rapid, semiautomated bacterial identification system, the Quantum II microbiology system (Abbott Laboratories, Irving, Tex.), to accurately identify gram-negative bacteria from veterinary sources was evaluated. A total of 378 isolates were tested, including 298 organisms in the family *Enterobacteriaceae* and strains representing *Acinetobacter* sp., *Aeromonas* sp., *Flavobacterium* sp., *Pasteurella multocida*, *Plesiomonas* sp., and *Pseudomonas* spp. Of these isolates, 333 (88.1%) were correctly identified, 20 (5.3%) were not identified, 10 (2.6%) were incorrectly identified at the genus level, and 15 (4.0%) were incorrectly identified at the species level. The Quantum II system correctly identified 268 (89.9%) of the isolates of *Enterobacteriaceae* and 65 (81.3%) of the nonenteric isolates. *P. multocida* was not identified correctly, and some nonenteric gram-negative bacteria of clinical significance in veterinary medicine are not included in the data base. The Quantum II system provided an accurate identification system for isolates of *Enterobacteriaceae* but had limited usefulness for the identification of other gram-negative bacteria of clinical significance in veterinary medicine.

A recent trend in clinical microbiology has been the development of commercially prepared systems for the rapid identification of bacteria (4). These systems offer a number of benefits, including standardized selection of biochemical tests and decreased time and cost required for identification, and some provide automated instruments to facilitate interpretation of test reactions. These systems are generally accepted as yielding improved identification accuracy (4).

The use of packaged identification systems has led to the development of methods to tabulate biochemical test results as biotype numbers (9). It then becomes possible to use a computer to assist in the identification of bacteria by comparing the biotype number with the established data base. The data bases are established by examining a large number of strains of bacteria to determine the possible biotype numbers for numerous bacterial species. The final identification of the organism being tested is usually qualified with a probability score. One of the limitations of this probability method is the possibility that the identification system may not contain the appropriate tests to recognize a strain outside its existing data base. Therefore, an unknown or rare biotype may be incorrectly assigned to an identification taxon that is in the data base rather than being recognized as a rare or unidentifiable strain. Biotype differences between human and veterinary strains of bacteria have been noted (3, 15). Therefore, packaged identification systems must be evaluated with veterinary strains of bacteria before they can be accepted for routine identification of veterinary pathogens. Evaluations of packaged identification systems have shown varied ability to accurately identify clinically significant bacteria from veterinary sources (1, 3, 5, 15). The purpose of this study was to evaluate the accuracy of the Quantum II microbiology system (Abbott Laboratories, Irving, Tex.) for the identification of a spectrum of gram-negative bacteria recently isolated from veterinary sources.

MATERIALS AND METHODS

Test organisms. A total of 378 recent isolates of gram-negative bacteria from veterinary sources were tested. Bacteria were isolated from clinical specimens on blood agar (BBL Microbiology Systems, Cockeysville, Md.), MacConkey agar (BBL), or Hektoen enteric agar plates (BBL). Organisms isolated on selective media were subcultured on blood agar before identification tests were performed. The identification of strains representing the family *Enterobacteriaceae* was established by using the Micro-ID system (General Diagnostics, Morris Plains, N.J.) (4). Identification of an isolate was considered to be complete when both the Micro-ID and Quantum II systems agreed and the Micro-ID result was classified as an acceptable identification or better. Identification of all isolates with low-probability Micro-ID identification results or with discrepant identification results was established with conventional biochemical tests (2, 9, 15). Serological typing of all *Salmonella* isolates was performed by National Veterinary Services Laboratories, Ames, Iowa. Other gram-negative bacteria were identified by conventional biochemical tests (8, 10, 13).

The selection of non-*Enterobacteriaceae* organisms was limited to those organisms that the manufacturer claimed could be identified (Quantum II microbiology system procedures manual, 1983) (see Table 2).

Test system. The Quantum II tests were performed according to instructions in the procedures manual of the manufacturer as previously described (11, 12). After the prescribed incubation period, the cartridges were removed from the incubator, 50 μ l of indole reagent (50 mg of *p*-dimethylaminobenzaldehyde [Mallinckrodt Chemical Works, St. Louis, Mo.] per ml of 1.2 M HCl) was added to the indole reaction chamber, and the chamber was immediately examined for a pink-red indicative of a positive indole test result (absence of any pink-red indicated a negative test result). Results of the indole reaction and a manual oxidase test

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TABLE 1. Identification of *Enterobacteriaceae* with the Quantum II system

Organism	No. tested	No. of isolates			
		Correctly identified ^a	Not identified	Identified to incorrect genus	Identified to incorrect species
<i>Citrobacter diversus</i>	3	3	0	0	0
<i>Citrobacter freundii</i>	9	9 (3)	0	0	0
<i>Enterobacter aerogenes</i>	31	24 (10)	1	0	6
<i>Enterobacter agglomerans</i>	9	7 (2)	2	0	0
<i>Enterobacter cloacae</i>	21	16	1	1	3
<i>Escherichia coli</i>	30	27 (1)	1	2	0
<i>Hafnia alvei</i>	2	2 (1)	0	0	0
<i>Klebsiella oxytoca</i>	11	11 (1)	0	0	0
<i>Klebsiella pneumoniae</i>	46	44	2	0	0
<i>Morganella morganii</i>	6	6	0	0	0
<i>Proteus mirabilis</i>	10	10 (1)	0	0	0
<i>Proteus vulgaris</i>	2	1	1	0	0
<i>Providencia rettgeri</i>	3	3	0	0	0
<i>Providencia stuartii</i>	3	3 (1)	0	0	0
<i>Salmonella arizonae</i>	6	6 (1)	0	0	0
<i>Salmonella choleraesuis</i>	2	2	0	0	0
<i>Salmonella enteritidis</i>	82	73 (4)	0	4	5
<i>Serratia liquefaciens</i>	2	1 (1)	0	1	0
<i>Serratia marcescens</i>	20	20 (2)	0	0	0
Total no.	298	268 (28)	8	8	14
Total %		89.9	2.7	2.7	4.7

^a Numbers in parentheses represent isolates for which additional tests were recommended and a probability score was not given for the final identification.

(Taxo N disks; BBL) performed with an isolated colony were entered into the microprocessor, and the cartridge was inserted into the reading chamber. The system then interpreted the individual reactions and compared the test results with the available data base. The test result for each biochemical test, a profile number, the most likely identification, and additional test information were automatically printed. Additional tests were performed as recommended on the printout when the probability of an identification was low (<80%).

Interpretation of results. Identification of an isolate by the Quantum II system was considered to be final when the likelihood of the first-choice identification was greater than 80% or when additional tests suggested on the printout were completed. The test identification results were classified as follows: (i) correct to the species level, (ii) not identified, (iii) incorrect genus, and (iv) incorrect species. Identifications tabulated as incorrect at the genus level were not included in the tabulation of identifications incorrect at the species level.

RESULTS

A total of 378 gram-negative bacteria, including 298 organisms in the family *Enterobacteriaceae* (Table 1) and 80 nonenteric gram-negative organisms (Table 2), were tested in the Quantum II system. Overall, 333 isolates (88.1%) were correctly identified. Of the 298 organisms belonging to the *Enterobacteriaceae*, 268 (89.9%) were correctly identified. Additional tests were required for 28 isolates which were identified without indication of the probability of the final identification. Additional incubation for a total of 5 h was required for 126 enteric bacteria (42.3%).

For nonenteric organisms, the data base of the Quantum II system is limited to the gram-negative organisms shown in Table 2. From this group, we tested 80 isolates of which 65 (81.3%) were correctly identified.

No identification was reported for 8 enteric (Table 1) and 12 nonenteric organisms (Table 2). This result was obtained more frequently for *Pasteurella multocida* (8 of 10 strains)

TABLE 2. Identification of non-*Enterobacteriaceae* gram-negative organisms with the Quantum II system

Organism	No. tested	No. (%) of isolates			
		Correctly identified	Not identified	Identified to incorrect genus	Identified to incorrect species
<i>Acinetobacter calcoaceticus</i> subsp. <i>anitratus</i>	11	11	0	0	0
<i>Acinetobacter calcoaceticus</i> subsp. <i>lwoffii</i>	5	4	1	0	0
<i>Aeromonas hydrophila</i>	6	6	0	0	0
<i>Flavobacterium meningosepticum</i> IIb	2	2	0	0	0
<i>Pasteurella multocida</i>	10	0	8	2	0
<i>Pleisomonas shigelloides</i>	1	1	0	0	0
<i>Pseudomonas aeruginosa</i>	38	34	3	0	1
<i>Pseudomonas cepacia</i>	1	1	0	0	0
<i>Pseudomonas fluorescens</i> or <i>Pseudomonas putida</i>	2	2	0	0	0
<i>Pseudomonas maltophilia</i>	4	4	0	0	0
Total no. (%)	80	65 (81.3)	12 (15.0)	2 (2.5)	1 (1.3)

TABLE 3. Incorrect identifications of gram-negative bacteria with the Quantum II system

Correct identification	No. of isolates	Test result
<i>Enterobacter aerogenes</i>	5	<i>Enterobacter cloacae</i>
	1	<i>Enterobacter agglomerans</i>
<i>Enterobacter cloacae</i>	1	<i>Citrobacter freundii</i>
	1	<i>Enterobacter aerogenes</i>
	2	<i>Enterobacter agglomerans</i>
<i>Escherichia coli</i>	2	<i>Klebsiella oxytoca</i>
<i>Pasteurella multocida</i>	2	<i>Pleisomonas shigelloides</i>
<i>Pseudomonas aeruginosa</i>	1	<i>Pseudomonas fluorescens</i> or <i>Pseudomonas putida</i>
<i>Salmonella enteritidis</i>	3	<i>Enterobacter aerogenes</i>
	5	<i>Salmonella choleraesuis</i>
	1	<i>Shigella</i> sp.
<i>Serratia liquefaciens</i>	1	<i>Enterobacter cloacae</i>

than for any other organism. For *P. multocida*, most reactions were interpreted as negative results rather than false-positives.

Incorrect identifications (Table 3) were obtained at the genus level for 10 isolates and the species level for 15 isolates. Incorrect identifications were usually the result of two to four discordant test results, except for *Salmonella enteritidis* serotype Dublin. Five strains of serotype Dublin were incorrectly identified as *Salmonella choleraesuis* because they did not ferment arabinose.

DISCUSSION

Our results demonstrated the importance of evaluating a new microbial identification system with representative strains of bacteria from veterinary sources if its accuracy of identification of these organisms has not been established. Then limitations of the system, such as the inability to identify *P. multocida* and misidentification of *Salmonella* serotype Dublin as *S. choleraesuis*, can be considered when results are evaluated. Evaluations of other packaged identification systems have demonstrated limitations in their application in veterinary microbiology laboratories, either due to incomplete data bases (1, 5) or biotype differences between isolates from human and veterinary sources (1, 3, 15).

Identification of isolates from the family *Enterobacteriaceae* was quite accurate (89.9%) and was comparable with results reported for isolates from humans (90 to 98.2%) (11, 12, 14, 16). The selection of organisms tested and reference identification methods account for some of the variability among these studies. Biochemical reactions are quite variable among the species and biogroups of the genus *Enterobacter* (2), a group which accounted for 10 of 25 (40%) misidentifications. A similar problem with identification of *Enterobacter* strains of human origin has been reported (14). Therefore, inclusion of more of these strains in an evaluation study tends to lower the overall accuracy.

Five isolates of *Salmonella* serotype Dublin were tested, and each was incorrectly identified as *S. choleraesuis* rather than *S. enteritidis* (6). Analysis of the biochemical reactions indicated that all strains failed to ferment arabinose. This

problem appears to be a deficiency of the data base rather than of the biochemical test system. Isolates of *Salmonella* serotype Dublin in one report were uniformly negative for fermentation of arabinose (7), while only 14.3% were reported to ferment arabinose in another study (17). All isolates identified as *Salmonella* sp. should be confirmed by serological testing rather than relying upon the biotype identification.

The nonenteric gram-negative bacteria data base of the Quantum II system was found to be too limited for recommendation as the only identification method in veterinary microbiology laboratories. Correct identifications were obtained for 81.3% of the strains tested which were listed in the data base. However, some common veterinary pathogens, such as *Pasteurella haemolytica*, *Actinobacillus* sp., and *Bordetella bronchiseptica*, were not included in the data base. In preliminary tests, three *P. haemolytica*, one *Actinobacillus* sp., and five *B. bronchiseptica* were in the category of organism not in data base. Although these results were correct, data from isolates not included in the data base were not included in this report, because the system does not claim to be able to identify them and nonidentification results have limited practical value. The rate of correct identifications (81.3%) for nonenteric bacteria was lower than that reported for tests of human isolates (83 to 94%) (11, 12, 14, 16). This difference was attributed to the number of *P. multocida* isolates tested. Of the 10 strains tested in this study, none were correctly identified. Other studies tested fewer strains (zero to four) (11, 12, 14, 16).

We found the Quantum II system to be simple, rapid, and convenient to use. The cartridge was easy to inoculate and required the addition of only one reagent after incubation. Identifications were completed after 5 h of incubation, except for those isolates requiring additional tests. Because many isolates required additional incubation after 4 h, we endorse the recommendations of others (11, 12) that the system be considered a 5-h test for all organisms. A major convenience of the system was standardized interpretation of test reactions rather than subjective evaluation of color changes by laboratory personnel. In addition, few additional tests (usually two or three) were required when low-probability identifications were obtained. However, for some low-probability identification results for oxidase-positive organisms, additional tests were recommended but the specific tests were not listed.

A serious limitation to the rapidness of the Quantum II system was the requirement that an isolate be obtained from nonselective medium for identification as specified in the procedures manual of the manufacturer. Because the system is primarily for identification of enteric bacilli, many of the organisms were isolated on primary plates of selective media. When they were subcultured onto a nonselective medium, an additional day was required for identification.

In summary, the Quantum II system provided a rapid, semiautomated procedure for simple and convenient identification of organisms from the family *Enterobacteriaceae* and selected nonenteric gram-negative bacteria. With the present limited data base, this system cannot identify some common nonenteric gram-negative bacteria of clinical significance in veterinary medicine.

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