JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1998, p. 2775–2777 Vol. 36, No. 9
0095-1137/98/$04.00+0

Comparison of Vitek GNI and GNI+ Cards for Identification of Gram-Negative Bacteria

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Received 6 April 1998/Returned for modification 15 May 1998/Accepted 16 June 1998

The GNI+ card has been developed by bioMerieux Vitek as an improvement over the GNI card for the identification of certain species of aerobic and facultative anaerobic bacteria. In this study, we tested 304 organisms from 30 different species on both the GNI and GNI+ cards. The GNI card correctly identified 285 (93.8%) of the isolates tested, and the GNI+ card correctly identified 287 (94.4%) of the isolates tested. The average time to reporting was 4.1 h for the GNI+ card compared to 5.7 h for the GNI card ($P < 0.001$). Overall, the GNI and GNI+ cards were comparable in identifying the organisms in this study while the GNI+ card gave substantially faster final test results.

The GNI+ card (bioMerieux Vitek, Hazelwood, Mo.) was developed as an improvement over the GNI card (bioMerieux Vitek) for the identification of certain species of aerobic and facultative anaerobic bacteria. Changes have been made to shorten the time to reporting, increase the number of organism profiles in the database, and improve the accuracy of identification of the organisms in the expanded GNI+ database (5). The database for the GNI+ card includes 18 new species as well as taxonomic changes not found in the GNI database. The purpose of this study was to compare the performance characteristics of the two cards.

(This study was presented, in part, at the 97th General Meeting of the American Society for Microbiology [2a]).

All organisms tested in this study were recent patient isolates which had been previously tested by using GNI cards. To increase the number of species included in this study, we attempted to limit each species to approximately 25 consecutive isolates (based on the initial GNI result). The study also included all isolates from the same period for which no identification was obtained with GNI cards. Collected isolates were stored on nutrient agar slants (Remel, Lenexa, Kans.).

The test cards were inoculated with organisms which were subcultured from the nutrient agar slants onto tryptic soy agar plates with 5% sheep blood (Remel) and then subcultured one additional time. Organisms were no more than 24 h old at the time of inoculation. Oxidase tests (Remel) were performed on all organisms prior to card inoculation.

In order to inoculate both cards from the same inoculum, a 1.0 McFarland suspension of each organism was prepared in a sufficient volume of 0.45% sterile saline (Remel) to inoculate both a GNI and a GNI+ card. The saline was then divided into separate tubes for the inoculation of the individual cards. All cards were inoculated within 20 min of inoculum preparation, and a portion of each bacterial suspension was streaked on a blood agar plate to check for inoculum purity. The use of the transfer tubes, filling module, sealing module, and loading of the GNI and GNI+ cards into the reader/incubator tray were performed according to the Vitek operator's manual. bioMerieux Vitek software version 5.01 was used.

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All isolates with initial results that were not in agreement and those with unacceptable results (<90% probability) were retested with both cards to rule out technical error. If the discrepant results repeated or the results agreed but were not acceptable (<90% probability), additional testing was performed. Additional testing included the API 20E system (bioMerieux Vitek) and BBL Crystal Identification System Enteric/Nonfermenter (Becton Dickinson Microbiology Systems, Cockeysville, Md.) for members of the family *Enterobacteriaceae* and other fermentative organisms. Nonfermentative organisms were tested with the RapID NF Plus System (Innovative Diagnostic Systems, L.P., Norcross, Ga.). Standard biochemical tests were also performed for some isolates. Some isolates were sent to the Centers for Disease Control and Prevention, Atlanta, Ga., for identification.

In comparing the results of testing of the GNI and GNI+ cards, the results were accepted as correct if both results gave a probability of ≥90%, both results were the same, and no supplemental or confirmatory tests were recommended. We did not consider spot indole tests, serological confirmation of *Salmonella* isolates, and hanging-drop motility tests required for a definitive identification to be supplemental tests, since final reporting was not delayed by these tests.

A total of 304 isolates representing 30 species of gram-negative bacilli were included in this study. As noted in Table 1, the GNI card and the GNI+ card correctly identified 285 (93.8%) and 287 (94.4%) of the isolates tested, respectively, with no supplemental testing required except for spot tests, as previously mentioned.

All results reported as *Acinetobacter lwoffii/junii* in this work are reported by Vitek as presumptive with confirmation recommended, even though the probability reported for the test result was ≥90%. Of the three isolates of *A. lwoffii* identified in this study, presumptive reports of *A. lwoffii/junii* were given for three results with the GNI card and two results with the GNI+ card.

Eleven tests (3.6%) with the GNI card and five tests (1.6%) with the GNI+ card gave a result of "good confidence marginal separation." The technical bulletins for the GNI and GNI+ cards state that this classification is used when the biochemical results for two organisms have an acceptable absolute likelihood for identification but resemble the biopatterns for both species in the database. Additional testing is required for identification (1, 2). Among the results with this

TABLE 1. Results of testing gram-negative bacilli with GNI and GNI+ cards[a]

Organism	Total tested	Correct identification		Presumptive		Incorrect identification		"Good confidence marginal separation"		"Questionable biopattern"		"Unidentified"	
		GNI	GNI+	GNI	GNI+	GNI	GNI+	GNI	GNI+	GNI	GNI+	GNI	GNI+
Acinetobacter calcoaceticus-baumanii complex	16	16	16										
Acinetobacter lwoffii	3			3	2		1						
Aeromonas sp.	1					1							1
Alcaligenes xylosoxidans	4	1	3					3			1		
Burkholderia cepacia	1		1									1	
Citrobacter amalonaticus	1	1	1										
Citrobacter freundii	23	23	22						1				
Citrobacter koseri	5	5	5										
Enterobacter aerogenes	20	20	20										
Enterobacter asburiae	1	1	1										
Enterobacter cloacae	25	24	24							1	1		
Escherichia coli	26	26	25						1				
Escherichia coli I.A.	2	0	1				1	2					
Flavobacterium odoratum	2	0	1					2			1		
Klebsiella oxytoca	18	18	18										
Klebsiella pneumoniae	27	25	26					1		1	1		
Morganella morganii	19	19	19										
Pantoea agglomerans	5	5	4										1
Proteus mirabilis	27	26	25				1	1	1				
Proteus vulgaris	5	5	4										1
Providencia rettgeri	3	3	3										
Providencia stuartii	3	3	3										
Pseudomonas aeruginosa	25	25	25										
Pseudomonas fluorescens group	1	0	0					1	1				
Pseudomonas stutzeri	1	0	0					1	1				
Salmonella sp.	5	5	5										
Serratia liquifaciens	1	1	1										
Serratia marcescens	25	25	25										
Stenotrophomonas maltophilia	8	8	8										
Yokenella regensburgei	1	0	1									1	
Total	304	285	287	3	2	1	3	11	5	2	4	2	3

[a] All results are given as numbers of isolates.

classification, 10 of the 11 from the GNI card and three of the five from the GNI+ card listed the correct answer as the first choice. Although supplemental tests are recommended to confirm the identification of isolates with a "good confidence marginal separation" result, in practice, we often choose to retest the isolates by an alternative kit method. That was the approach we took with these isolates.

A "questionable biotype" is assigned to results with several possible identifications, all of low probability, with biochemical results atypical compared to all organisms in the database. Additional testing is required. For practical purposes, "questionable biotype" is equivalent to the category "unidentified." For the GNI and GNI+ cards, there were two and four "questionable biotype" results and one and three "unidentified" results, respectively.

One isolate of *Aeromonas* sp. was misidentified as *Vibrio fluvialis* by the GNI card. The GNI+ card misidentified three isolates. The first was an *A. lwoffii* isolate misidentified as *Bordetella bronchiseptica*. The second was an *Escherichia coli* inactive isolate (I.A.) misidentified as *Salmonella* sp. The last was a *Proteus mirabilis* isolate misidentified as *Providencia stuartii*.

As insightfully stated by Miller (4), the most difficult question to answer in performing comparative biochemical identification studies, such as this, may be the definition of a correct versus an incorrect response. In this study, 280 (92.1%) of 304 isolates tested gave a result of ≥90% probability with both the GNI and GNI+ cards. These were accepted as correct answers, although no attempt was made to verify this accuracy by reference methods.

We classified three GNI+ results and one GNI result as incorrect. These results again highlight the issues raised by Miller about correct versus incorrect results (4). The one incorrect GNI result was an *Aeromonas* sp. which was reported as *V. fluvialis* with a 92% probability; however, growth in 6.5% salt was recommended as a confirmatory test. If this supplemental test had been performed, the result would likely have suggested that this identification was incorrect and additional testing was merited. Similarly, one of the three incorrect identifications with the GNI+ cards was an *E. coli* I.A. misidentified as *Salmonella* sp. All identifications of *Salmonella* are accompanied by the comment that serological confirmation is recommended. If such confirmation had been performed on this isolate, the results would likely have prompted additional testing.

It seems reasonable to us to classify the test results from this study into three categories: correct, as we have previously defined; incorrect; and "requires additional testing." Included in this last category are organisms fitting into the bioMerieux Vitek categories of "presumptive *Acinetobacter lwoffii/junii*," "good confidence marginal separation," "questionable biopattern," and "unidentified." With this classification system, the following results were obtained (with the GNI and GNI+

cards, respectively): correct, 93.8 and 94.4%; incorrect, 0.3 and 1.0%; and "requires additional testing," 5.9 and 4.6%. Thus, the results of testing with the two cards are essentially equivalent.

Clearly, the selection of organisms included in a study such as this may affect the outcome. As noted by Miller, it is important that investigators state how isolates were chosen for a particular study (4). For this study, we utilized recent, consecutive patient isolates but attempted to limit any one species to approximately 25 isolates, in order to broaden the number of species included in the study. Taking this approach, 30 different species were included in this study, with no more than 27 isolates of any one species tested. While there was no attempt to include stock organisms which historically may have been difficult to identify, we believe that our approach gave a somewhat more rigorous challenge than could have been anticipated had we tested 304 consecutive, clinical isolates.

The results of this study are similar to those reported by Colosante et al. (3) in their comparison of GNI and GNI+ cards. In another study which included an evaluation of the GNI+ card, O'Hara et al. reported an accuracy of 87.6% for the GNI+ cards; however, the authors acknowledged that the isolates included in their study represented a vigorous challenge to the products evaluated (6). The results of our study for the GNI card are very similar to results reported by Robinson et al. (8) and Rhoads et al. (7) for the GNI card.

The average time to reporting was 5.7 h for the GNI card and 4.1 h for the GNI+ card ($P < 0.001$ [paired t test]).

The GNI+ card is marketed as an improvement over the GNI card; however, the only significant performance difference between the two cards was the shorter time to reporting for the GNI+ card. Organism identification results were comparable for the isolates which we tested. Perhaps laboratories with a different organism mix would find additional benefits with the GNI+ card. More-rapid test results do not automatically result in more-rapid result reporting or better patient care. In our laboratory, we hold the organism identification until the antimicrobial susceptibility test results are completed (or vice versa if the susceptibility testing results are completed before the organism identification is completed). Thus, the benefits of more rapid identification must be evaluated in the context of the work flow for individual laboratories. The list price for the GNI and GNI+ cards is the same, so cost differences between the cards should not influence card selection.

We thank bioMerieux Vitek for providing support for this study.

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