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Evaluation of the MicroScan Rapid Neg ID3 Panel for Identification of *Enterobacteriaceae* and Some Common Gram-Negative Nonfermenters

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The MicroScan Rapid Neg ID3 panel (Dade Behring, Inc., West Sacramento, Calif.) is designed for the identification of gram-negative bacilli. We evaluated its ability to accurately identify *Enterobacteriaceae* that are routinely encountered in a clinical laboratory and glucose nonfermenting gram-negative bacilli. Using 511 stock cultures that were maintained at −70°C and passaged three times before use, we inoculated panels according to the manufacturer's instructions and processed them in a Walk/Away instrument using version 22.01 software. The time to identification was 2 h and 30 min. All panel identifications were compared to reference identifications previously determined by conventional tube biochemicals. At the end of the initial 2.5-h incubation period, 405 (79.3%) identifications were correct. An additional 49 (9.6%) isolates were correctly identified after required additional off-line biochemical tests were performed. Thus, at 24 h, 88.8% of the 511 strains tested were correctly identified. Twenty-two (4.3%) were identified to the genus level only. Twenty-six (5.1%) strains were misidentified. Because the system is based on fluorogenics, there are no conventional tests readily available with which to compare possibly incorrect reactions. Of the 28 *Salmonella* strains that were tested, 5 were incorrectly reported. The 21 remaining errors were scattered among the genera tested. Testing on nine strains gave a result of "no identification" (very rare biotype). The Rapid Neg ID3 panel in this study approached 89% accuracy for the identification of gram-negative organisms encountered in the hospital laboratory.

Automated identification and susceptibility testing methods have become the mainstay of clinical microbiology laboratories and have provided laboratory staff with an efficient means of organism classification, thereby giving physicians the availability of rapid and generally accurate results that is necessary to guide therapy. Errors do occur, however, and as competing resources put more demands on shrinking laboratory staffs, expertise in recognizing errors and evaluating questionable results from these instruments is diminishing. This places a greater demand on the manufacturer to provide an instrument and a database that are as free from error as possible. However, the laboratory is no less accountable for undetected errors that may or may not result in an adverse outcome for patients. As new versions of databases become available, it is critical that appropriate evaluations be conducted in order to provide laboratory staff with unbiased data from which to make purchasing decisions. The update that resulted in the MicroScan Rapid Neg ID3 (RNID3) panel was designed to improve accuracy in the identification of common human clinical taxa by modifying the biochemical tests in the panel by replacing 10 tests on the RNID2 panel with new substrates. The new panel also eliminated the need for a mineral oil overlay on the decarboxylase tests and increased the shelf life from 6 months to 1 year when stored at 2 to 8°C. In this study, the MicroScan RNID3 panel was evaluated using members of the *Enterobacteriaceae* and some common gram-negative nonfermenters. (Part of this research was presented at the 99th General Meeting of the American Society for Microbiology, Chicago, Ill., 30 May to 3 June 1999.)

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MATERIALS AND METHODS

Bacterial strains. Table 1 lists the 475 biochemically typical and atypical *Enterobacteriaceae* and Table 2 lists the 36 commonly isolated biochemically typical glucose nonfermenting gram-negative bacilli that were used in this study. All strains were of human origin. This set also contained larger numbers of genera and species not likely to be found in routine work but which were contained in the database, thereby presenting a true challenge to the system.

Stock cultures from the collection of the Centers for Disease Control and Prevention were maintained in defibrinated sheep blood at −70°C and were passed two times on trypticase soy agar with 5% sheep blood (TSA II; BD, Sparks, Md.) before inoculation onto MacConkey's agar for testing in the panels.

Media and biochemical tests. Biochemical tests for identification were performed using conventional media and the methods described by Edwards and Ewing (2), with some modifications by Hickman and Farmer et al. (3, 4). Commercial media were used wherever possible.

Identification panel. The RNID3 panel is manufactured by Dade Behring, Inc., MicroScan Inc., West Sacramento, Calif., and is designed for use with the Walk/Away automated instrument. The 36 substrates contained on the panel include arabitol, adonitol, lysine, and ornithine with the decarboxylase base, glucuronic acid, arabinose, urea, inositol, mannitol, mannose, melibiose, raffinose, salicin, sorbitol, sucrose, glucose, and tryptophan for indole production; 4-methylumbelliferyl (MeU)-β-D-xyloside, MeU-α-D-galactopyranoside, MeU-α-D-glucopyranoside, MeU-*N*-acetyl-β-D-glucosaminide, MeU-β-D-glucopyranoside, MeU-β-D-mannopyranoside, MeU-β-D-glucuronide, MeU-β-D-*N*,*N*′-diacetylchitobioside, MeU-β-D-galactopyranoside, MeU-phosphate (both acid and alkaline pH); L-arginine-7-amido-4-methylcoumarin (AMC), α-L-glutamate-AMC, *N*-glutaryl-glycyl-arginine-AMC, γ-L-glutamic acid-AMC, L-pyroglutamic acid-AMC, L-proline-AMC, and L-tyrosine-AMC. These substrates work by one of the following mechanisms: hydrolysis of fluorogenic substrates, pH changes following substrate utilization, production of specific metabolic byproducts, or the rate of production of specific metabolic byproducts after 2.5 h of incubation in the Walk/Away. While this newer panel does require the automated addition of a rapid indole reagent to the tryptophan well, it no longer requires mineral oil overlays on the decarboxylases.

The data management software used in this study was version 22.01. The current version of software is 22.26 (May 1999), but this version does not include changes in the identification database that would invalidate the results of this evaluation.

Definitions. The category of "correct" implies that the organism identification was correct to the genus and species levels at the end of the 2.5-h incubation time. The category of "correct with additional tests" implies that the organism identification was correct to the genus and species levels after the performance

TABLE 1. Accuracy of identification of *Enterobacteriaceae* using RNID3[a]

Reference ID	No. tested	No. correct at 2.5 h	No. correct with additional tests	No. correct to genus	Error (no.)	No ID (no.)
Citrobacter amalonaticus	10	6		4		
Citrobacter braakii	6	5	1			
Citrobacter farmeri	5	4		1		
Citrobacter freundii	2	2				
Citrobacter koseri	10	10				
Citrobacter werkmanii	3	2	1			
Citrobacter youngae	4	3	1			
Cedecea davisae	6	4			2	
Cedecea lapagei	4			3	1	
Enterobacter aerogenes	10	7	1			2
Enterobacter asburiae	10	4	3	3		
Enterobacter cancerogenus	10	9		1		
Enterobacter cloacae	10	8		1	1	
Enterobacter gergoviae	10	7	1	1	1	
Enterobacter hormaechei	6	6				
Enterobacter sakazakii	10	10				
Edwardsiella tarda	10	9			1	
Escherichia coli	30	24	3		3	
Escherichia fergusonii	10	10				
Escherichia hermannii	10	8	1		1	
Escherichia vulneris	10	9	1			
Ewingella americana	9	7			2	
Hafnia alvei	10	10				
Klebsiella ornithinolytica	10	9				1
Klebsiella oxytoca	10	9		1		
Klebsiella ozaenae	10	9			1	
Klebsiella pneumoniae	10	10				
Klebsiella rhinoscleromatis	10	9				1
Kluyvera sp.	10	6	3		1	
Morganella morganii	10	9	1			
Pantoea agglomerans	9	6	2			1
Proteus mirabilis	10	10				
Proteus penneri	10	7	3			
Proteus vulgaris	8	4	4			
Providencia alcalifaciens	7	4	2	1		
Providencia rettgeri	8	8				
Providencia rustigianii	2	2				
Providencia stuartii	14	12	2			
Salmonella enterica subsp. *arizonae*	10	8	1		1	
Salmonella enterica serotype						
Choleraesuis	2	0		1	1	
Paratyphi A	2	1			1	
Typhi	2	2				
Salmonella spp.	12	5	2	3	2	
Serratia fonticola	10	5		1	2	2
Serratia liquefaciens	10	8	1			1
Serratia marcescens	10	10				
Serratia odorifera	10	10				
Serratia plymuthica	10	8	2			
Serratia rubidaea	10	8	1		1	
Shigella boydii	2	1				1
Shigella dysenteriae	2	1			1	
Shigella flexneri	2	1			1	
Shigella sonnei	4	3	1			
Yersinia enterocolitica	12	10	2			
Yersinia frederiksenii	2	2				
Yersinia intermedia	2	1	1			
Yersinia kristensenii	2	2				
Yersinia pseudotuberculosis	6	4	1		1	
Yokenella regensburgei	10	8	2			
Total no. (%)	475	376 (79.2)	44 (9.3)	21 (4.4)	25 (5.3)	9 (1.9)

[a] See Materials and Methods for an explanation of categories. ID, identification.

TABLE 2. Accuracy of identification of commonly occurring glucose-fermenting and glucose-nonfermenting organisms using RNID3[a]

Reference ID	No. tested	No. correct at 2.5 h	No. correct with additional tests	No. correct to genus	Error (no.)	No ID (no.)
Acinetobacter lwoffii	5	2	3			
Acinetobacter baumannii	7	6			1	
Burkholderia cepacia	7	6	1			
Pseudomonas aeruginosa	10	8	1	1		
Stenotrophomonas maltophilia	7	7				
Total no. (%)	36	29 (80.6)	5 (13.9)	1 (2.8)	1 (2.8)	0

[a] See Materials and Methods for an explanation of categories. ID, identification.

of additional off-line biochemical tests that are suggested by the manufacturer. These tests are suggested when the probability of accuracy of identifications of the first organisms listed is below 85% (low probability). There are 48 possible additional tests that would extend the time of final identification to 24 h. The category of "correct to genus" indicates that the genus identification was correct but the species was incorrect when the database indicated that the organism could be identified to the species level. The category of "error" implies that both the genus and species identifications are incorrect, and "no ID" indicates that no identification was given by the system or that the system gave a choice of multiple answers that were not resolved by additional testing.

Tests were repeated in duplicate when a response other than "correct" or "correct with additional tests" occurred.

Statistical analysis. Results were evaluated by chi-square analysis with Yates' corrected coefficient to arrive at a *P* value.

RESULTS AND DISCUSSION

Table 1 shows the results of testing 475 strains of *Enterobacteriaceae* in the RNID3. Table 2 shows the accuracy of identification of five glucose-fermenting and nonfermenting organisms that are commonly isolated in clinical laboratories. At 24 h, after additional testing, the RNID3 accurately identified 88.8% of 511 strains evaluated in this study.

Table 3 lists the errors that occurred in identification. Twenty-six (5.1%) strains were misidentified. Of these 26 errors, 21 were at probability levels of 88% or greater; 14 were at probability levels of 95% or higher. Of the five *Salmonella* misidentifications, one was at a probability level of 76%, two were at a probability level of 90%, one was at a probability level of 95%, and one was at a probability level of 99%, suggesting that an inaccurate result might be submitted to the physician. Both of the *Shigella* misidentifications were at probability levels of 98% or greater. Because the system is based on fluorogenics, there are no conventional tests readily available with which to compare possibly incorrect reactions. Testing on nine strains yielded a result of "no identification" (very rare biotype).

Table 4 shows the results of testing 130 strains that more closely resemble the assortment of strains that might be routinely found in local area hospital laboratories. After additional testing was completed, 91.5% of the strains were correctly identified.

Bascomb et al. reported 97.1% accuracy with 405 fresh clinical isolates and 90.7% accuracy with 247 stock isolates (1). Schreckenberger et al. reported an accuracy of 96.2% after additional testing was completed and an error rate of 3.8% using 317 fresh clinical isolates (P. C. Schreckenberger, S. Connell, J. Skinner, B. L. Zimmer, D. Glenn, D. A. Bruckner, J. M. Janda, and S. L. Abbott, 98th Gen. Meet. Am. Soc. Microbiol. 1998, poster C-153, 1998). One might expect that the accuracy of the system when testing fresh clinical isolates might be higher than that observed with stock strains.

In an effort to determine the cause of the errors in identification, the strains for which incorrect answers were given were retested in duplicate. One panel was processed without oil overlays on the decarboxylases; a matching panel had the de-

TABLE 3. Misidentification of strains by RNID3

Reference identification	No. of strains	RID3 identification (probability level [%])
Cedecea davisae	2	*Tatumella* sp. (15); *Leminorella* sp. (99)
Cedecea lapagei	1	*Ewingella americana* (93)
Enterobacter cloacae	1	*Enterobacter hormaechei* (99)
Enterobacter gergoviae	1	*Shigella boydii*/*S. dysenteriae*/*S. flexneri* (95)
Edwardsiella tarda, biogroup 1	1	*Pasteurella multocida* (89)
Escherichia coli	3	*Citrobacter braakii*/*C. freundii*/*C. sedlakii* (89–95); called one strain O157, which it was not
Escherichia hermannii	1	*Citrobacter braakii*/*C. freundii*/*C. sedlakii* (93)
Ewingella americana	2	CDC Group EO-2 (46; 98)
Klebsiella ozaenae	1	*Pantoea agglomerans* (97)
Kluyvera ascorbata	1	*Enterobacter amnigenus*-1 (96)
Salmonella enterica subsp. *arizonae*	1	*Escherichia coli* (90)
Salmonella enterica serotype Choleraesuis	1	*Shigella boydii*/*S. dysenteriae*/*S. flexneri* (76)
Salmonella enterica serotype Paratyphi A	1	*Leminorella* sp. (99)
Salmonella spp.[a]	2	*Citrobacter braakii*/*C. freundii*/*C. sedlakii* (90); *Escherichia coli* (95)
Serratia fonticola	2	*Yersinia enterocolitica* group (99); *Leclercia adecarboxylata* (99)
Serratia rubidaea	1	*Leclercia adecarboxylata* (98)
Shigella dysenteriae	1	*Pasteurella multocida* (98)
Shigella flexneri	1	*Escherichia coli* (99)
Yersinia pseudotuberculosis	1	*Acinetobacter lwoffii*/*alcaligenes* (30)
Acinetobacter baumannii	1	*Oligella urethralis* (88)

[a] One strain was lysine negative.

TABLE 4. Accuracy of identification of a weighted assortment of clinical isolates[a]

Reference ID (no. of isolates)	No. correct	No. correct with additional tests	No. correct to genus	Error (no.)	No ID (no.)
Acinetobacter baumannii (6)	6				
Acinetobacter lwoffii (1)	1				
Citrobacter freundii (2)	2				
Citrobacter koseri (4)	4				
Enterobacter aerogenes (6)	4				2
Enterobacter cloacae (10)	8		2		
Escherichia coli (30)	24	3		3	
Klebsiella pneumoniae (10)	10				
Klebsiella oxytoca (6)	5		1		
Morganella morganii (4)	3	1			
Proteus mirabilis (10)	10				
Proteus vulgaris (8)	4	4			
Providencia stuartii (2)	2				
Pseudomonas aeruginosa (10)	8	1	1		
Salmonella spp. (4)	2			2	
Serratia marcescens (10)	10				
Shigella spp. (2)	1	1			
Stenotrophomonas maltophilia (5)	5				
Total no. (%)[b]	109 (83.8)	10 (7.7)	4 (3.1)	5 (3.8)	2 (1.5)

[a] See Materials and Methods for an explanation of categories. ID, identification.
[b] The total number of isolates identified by the reference method was 130.

carboxylases overlaid with mineral, as was the procedure with the RNID2 panels. One strain each of *Escherichia coli* and *Escherichia hermannii* gave correct identifications on the panels that were overlaid with oil, while the answers from the panels with no oil overlays remained incorrect.

In this study, the RNID3 panel performed as well for the identification of bacteria routinely isolated in hospital laboratories as it does for the identification of a challenge set of strains ($P > 0.05$). In addition, the accuracy of the new panel in this study was not significantly different ($P > 0.05$ [Yates' correction]) from the RNID2 results previously published (5), for which the same study set was used.

An in-depth study of the accuracy of this panel to correctly identify less commonly encountered glucose nonfermenting gram-negative organisms will be the subject of a future publication.

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