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Evaluation of the New RapID-ANA II System for the Identification of Clinical Anaerobic Isolates

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The RapID-ANA II System (Innovative Diagnostic Systems, Inc., Atlanta, Ga.) is a recently revised and marketed 4-h system for the identification of anaerobic bacteria. The system was compared with conventional identification methods for its ability to identify 566 clinical anaerobic isolates. Overall, the system identified correctly to genus and species 68% of the total isolates (62% of 204 gram-negative bacilli, 70% of 69 nonsporeforming gram-positive bacilli, 74% of 130 *Clostridium* isolates, and 72% of 163 anaerobic cocci), without the use of additional tests. With the additional tests suggested by the manufacturer, 78% of the total isolates were identified correctly to species. The routine use of a few simple and practical tests (e.g., egg yolk agar for *Clostridium* spp.), in addition to the RapID-ANA II, would improve significantly the accuracy of the system in the identification of anaerobic bacteria. This second-generation system offers a number of improvements over the original system, including an updated data base and the option of overnight refrigeration of the system before the addition of reagents.

Establishment of the potential significance of many species of anaerobic bacteria in infectious diseases has stimulated the commercial development of several new methods for the identification of these organisms. The macrotube system described in the *Anaerobe Laboratory Manual* of the Virginia Polytechnic Institute for characterization of anaerobes (2, 9, 15), with prereduced anaerobically sterilized biochemical test media and gas-liquid chromatography (GLC) for analysis of acid metabolic products, is still the most accurate method and is considered to be the "gold standard" against which alternative identification systems should be compared. These conventional methods are, however, costly, time-consuming, and beyond the capabilities of many smaller clinical laboratories.

In recent years, several new rapid systems have been developed commercially and marketed for the identification of clinical anaerobic isolates. Such systems as the AN-Ident (Analytab Products, Plainview, N.Y.), IDS RapID-ANA (Innovative Diagnostic Systems, Inc., Atlanta, Ga.), API ATB 32A (Analytab Products), MicroScan Rapid Anaerobe Identification System (American MicroScan, Sacramento, Calif.), and Vitek ANI Card (Vitek Systems, Hazelwood, Mo.) are based on the use of chromogenic and selected conventional nonchromogenic substrates that allow for the detection of preformed bacterial enzymes without the requirement of anaerobic incubation. The miniaturization of these systems, rapid identification within 4 h, and non-growth-dependent reactions have made these systems attractive alternatives for the identification of anaerobes in clinical laboratories. The AN-Ident, Vitek ANI Card, ATB 32A, MicroScan, and the first-generation RapID-ANA have been previously evaluated (3, 5, 7, 8, 10–12, 16, 17, 20–22). The identification accuracies for the systems alone have been generally in the 60 and 70% ranges.

The RapID-ANA II (Innovative Diagnostic Systems) is a second-generation, recently marketed micromethod for the identification of clinical anaerobic isolates. The system contains a revised test selection for the detection of 18 preformed enzyme reactions and an updated and expanded *Code Compendium*. The purpose of this study was to evaluate the accuracy of this newly revised system for the identification of clinical anaerobic isolates.

(This work was presented in part at the 84th Annual Meeting of the American Society for Microbiology [19]. In 1990, there were two additional poster presentations, with abstracts published on the RapID-ANA II [4, 6].)

MATERIALS AND METHODS

Bacterial strains. In this study, 566 different strains of anaerobic bacteria, representing 16 genera and 58 species, were included. All strains were fresh clinical isolates from the Anaerobe Section, Microbiology Division, Department of Pathology, Indiana University Medical Center. Isolates were grown on CDC-anaerobe blood agar (BA) (Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga.) for 48 h and checked for purity.

All strains were characterized and identified definitively by using conventional media and methods (2, 9, 15). This included relationship to oxygen, Gram reaction and cellular morphology, colonial characteristics, acid metabolic product analysis with GLC, growth on prereduced anaerobically sterilized biochemical media, reactions on egg yolk agar, growth in the presence of antimicrobial agents and other compounds, and, when indicated, toxin studies. Each isolate was blindly characterized, in duplicate, with the RapID-ANA II system.

RapID-ANA II system. Substrates included in the 10 wells of the test panel are urea, *p*-nitrophenyl-β,D-disaccharide, *p*-nitrophenyl-α,L-arabinoside, *o*-nitrophenyl-β,D-galactoside, *p*-nitrophenyl-α,D-glucoside, *p*-nitrophenyl-β,D-glucoside, *p*-nitrophenyl-α,D-galactoside, *p*-nitrophenyl-α,L-fucoside, *p*-nitrophenyl-*N*-acetyl-β,D-glucosaminide, *p*-nitrophenylphosphate, leucyl-glycine-β-naphthylamide, glycine-β-naphthylamide, proline-β-naphthylamide, phenylalanine-β-naphthylamide, arginine-β-naphthylamide, serine-β-naphthylamide, pyrrolidonyl-β-naphthylamide, and tryptophane.

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Eight wells are bifunctional. Reactions involving hydrolysis of the colorless aryl-substituted glycosides or phosphoester-releasing yellow *o*- or *p*-nitrophenol (wells 2 through 10) are interpreted before the addition of reagents. The determination of urease production (well 1) is also made before the addition of reagents. Reactions involving the hydrolysis of aryl-amide substrates resulting in the release of free β-naphthylamide (wells 3 through 9) are detected by the addition of the RapID-ANA reagent. Formation of indole from tryptophane is determined by using *p*-dimethylaminocinnamaldehyde.

Test procedure. Colonial growth was removed from the surface of a 48- to 72-h BA plate and suspended in RapID inoculation fluid (Innovative Diagnostic Systems) to achieve a turbid suspension (minimum turbidity equivalent to McFarland no. 3 standard). The entire bacterial suspension was transferred to the upper right quadrant of the panel and evenly distributed along the back section. The panel was then tilted forward to allow filling of the front reaction wells with the bacterial suspension. Inoculated panels were incubated aerobically for 4 h in the absence of CO_2 in a 35°C incubator.

All reactions were interpreted as described in the manufacturer's test interpretation guide. Tests requiring the addition of reagents (wells 3 through 10) were allowed to react for 30 s to 2 min, as recommended in the package instructions. Identifications were made with the RapID-ANA II *Code Compendium*. Codes not found in the *Code Compendium* were referred to the company for updated interpretations. All isolates were characterized in duplicate with the RapID-ANA II. Isolates with conflicting identifications between the conventional and RapID-ANA II systems were characterized and identified again with the conventional system.

RESULTS

Tables 1 through 4 illustrate the results obtained in the identification of the 566 isolates with RapID-ANA II. Isolates identified correctly at confidence levels, as determined by the manufacturer, of "adequate," "satisfactory," or "implicit" are indicated on the tables as correct identifications. Isolates identified correctly at lower confidence levels of "inadequate" or "questionable" are indicated on the tables as inadequate identifications. Additional tests were suggested in the *Code Compendium* to further identify these isolates.

Table 1 summarizes the identifications for the 204 gram-negative bacilli. The system, without the use of additional tests, correctly identified to genus and species 126 (62%) of the gram-negative bacilli. Of the 29 *Bacteroides ovatus* isolates tested, 12 were given correct genus but incorrect species identifications. Fourteen isolates were given inadequate identifications with *B. ovatus* as one of the choices in each case. The alternate choice in each case was either *Bacteroides thetaiotaomicron*, *B. uniformis*, or *B. eggerthii*. Similar identifications were observed with *B. uniformis*. Seven of 14 isolates were given inadequate identifications with *B. uniformis* listed as one of the choices. Other choices listed for *B. uniformis* isolates with inadequate identifications were: *B. thetaiotaomicron*, *B. ovatus*, or *B. eggerthii*. Of the eight isolates of *Bacteroides vulgatus* that were given incorrect species identifications, six were identified as *B. fragilis* and two were identified as *Prevotella buccae*. Three of 4 *Fusobacterium necrophorum* and 10 of 11 *F. nucleatum* isolates produced "inadequate" identifications. In each of the cases, *F. necrophorum* and *F. nucleatum* were listed as the most likely choices.

TABLE 1. Gram-negative bacilli: comparison of identifications with RapID-ANA II and conventional reference methods

IUMC[a] identification (no. of isolates)	No. of identifications classified as:					
	Correct		Inadequate (needed additional tests)		Incorrect	No code
	Genus and species	Genus only	Genus and species	Genus only		
Bacteroides caccae (3)	2	1				
B. distasonis (19)	19					
B. fragilis (28)	26	2				
B. ovatus (29)	1	12	16			
B. splanchnicus (2)	1					1
B. thetaiotaomicron (24)	22	1		1		
B. uniformis (14)	4	1	7	2		
B. ureolyticus (12)	12					
B. vulgatus (27)	19	8				
Capnocytophaga spp. (2)	2					
Fusobacterium mortiferum (3)	2					1
F. nechrophorum (4)	1		3			
F. nucleatum (11)	1		10			
F. varium (3)	3					
Prevotella bivia (7)	7					
P. buccae (1)				1		
P. intermedia (2)	2					
P. melaninogenica (1)			1			
P. melaninogenica group (2)						2
P. mel/P. denticola (2)	1	1				
P. oralis group (7)	1	2	1	2		1
Porphyromonas asaccharolytica (1)		1				

[a] IUMC, Indiana University Medical Center.

Table 2 summarizes the results obtained for the 69 anaerobic nonsporeforming gram-positive bacilli tested. The system identified correctly, without the use of additional tests, 48 (70%) of the isolates. Seven of nine *Actinomyces* isolates were identified to the correct genus, but identification to species was not achieved with the rapid system. Various identifications were obtained for 5 of 10 *Lactobacillus* spp. that were not identified correctly to genus and species.

Table 3 summarizes the identifications of the 130 *Clostridium* isolates. Ninety six isolates (74%) were identified correctly, without the use of additional tests, to genus and species. Six of 16 *C. clostridiiforme* isolates had similar codes that were not in the current data base. The system misidentified two *C. difficile* isolates as *C. subterminale* and gave inadequate identifications for two additional isolates with *C. difficile* and *C. subterminale* being the given choices. Thirteen of 26 *C. innocuum* misidentifications were characterized by this system as *C. subterminale*. Three additional isolates were inadequately identified, and *C. subterminale* and *C. limosum* were listed as the most probable choices.

Table 4 illustrates the identification results for the 163 isolates of anaerobic cocci; 117 isolates (72%) were identified

TABLE 2. Gram-positive bacilli: comparison of identifications with RapID-ANA II and conventional reference methods

IUMC[a] identification (no. of isolates)	No. of identifications classified as:					
	Correct		Inadequate (needed additional tests)		Incorrect	No code
	Genus and species	Genus only	Genus and species	Genus only		
Actinomyces spp. (4)		2		2		
A. meyeri (2)		1	1			
A. naeslundii (1)		1				
A. odontolyticus (2)	2					
Bifidobacterium spp. (3)	2					1
Eubacterium lentum (13)	9		2		2	
E. limosum (3)	1		1		1	
Lactobacillus spp. (10)	4		2		4	
L. acidophilus (1)	1					
L. casei subsp. *rhamnosus* (3)	3					
Propionibacterium acnes (25)	24		1			
P. granulosum (2)	2					

[a] IUMC, Indiana University Medical Center.

TABLE 4. Cocci: comparison of identifications with RapID-ANA II and conventional reference methods

IUMC[a] identification (no. of isolates)	No. of identifications classified as:					
	Correct		Inadequate (needed additional tests)		Incorrect	No code
	Genus and species	Genus only	Genus and species	Genus only		
Gemella morbillorum (3)	1				2	
Peptostreptococcus anaerobius (24)	15		2	3	4	
P. asaccharolyticus (29)	25	3				1
P. magnus (27)	26					1
P. micros (20)	20					
P. prevotii (25)	4	12	2	4	1	2
P. tetradius (1)						1
Staphylococcus saccharolyticus (3)	3					
Streptococcus intermedius (17)	14	1		1		1
Veillonella spp. (14)	9				5	

[a] IUMC, Indiana University Medical Center.

correctly, without the use of additional tests, by the RapID-ANA II. Two of three *Gemella morbillorium* isolates were misidentified as *Peptostreptococcus* spp., with *P. micros* as the most likely identification. A variety of misidentifications was noted with *Peptostreptococcus anaerobius*, e.g., *G. morbillorum*, *P. tetradius*, and *P. prevotii*. Three of 29 *P. asaccharolyticus* isolates were misidentified as *P. indolicus*. *P. prevotii* presented with several misidentifications. Twelve of 25 strains gave acceptable (or better) identifications for a variety of other *Peptostreptococcus* species (*P. magnus*, *P. tetradius*, *P. indolicus*). Other choices included *Veillonella* spp. and *Streptococcus constellatus*. All of the *Veillonella* spp. that were not correctly identified were called *Staphylococcus saccharolyticus* at a satisfactory level.

TABLE 3. Clostridia: comparison of identifications with RapID-ANA II and conventional reference methods

IUMC[a] identification (no. of isolates)	No. of identifications classified as:					
	Correct		Inadequate (needed additional tests)		Incorrect	No code
	Genus and species	Genus only	Genus and species	Genus only		
Clostridium bifermentans (3)	3					
C. botulinum type A (1)	1					
C. butyricum (10)	8	1		1		
C. cadaveris (7)	7					
C. clostridiiforme (25)	16		1		2	6
C. difficile (8)	4	2	2			
C. innocuum (26)	9	13	1	3		
C. paraputrificum (2)	1	1				
C. perfringens (19)	19					
C. ramosum (20)	20					
C. septicum (3)	3					
C. sordellii (1)	1					
C. sporogenes (1)			1			
C. tertium (4)	4					

[a] IUMC, Indiana University Medical Center.

DISCUSSION

This study involving 566 strains of anaerobic bacteria represents an extensive evaluation of the RapID-ANA II system. The system performed best with some of the most commonly encountered anaerobic species (e.g., *Bacteroides fragilis*, *B. ureolyticus*, *Prevotella bivia*, *Peptostreptococcus magnus*, *P. micros*, *P. asaccharolyticus*, *Clostridium perfringens*, *C. ramosum*, and *Propionibacterium acnes*). The system, when used without the addition of tests other than Gram reaction, identified 68% of the 566 total isolates to the correct genus and species. An additional 10% of the isolates were correctly identified to genus and species by using the additional tests as suggested in the *Code Compendium*. This percentage, however, increased significantly when the system was used in conjunction with morphologic observations and selected simple and practical key differential tests.

The percent correct identification for gram-negative bacilli could be improved with the implementation of a few practical recommendations. Because of the difficulty in differentiating *B. ovatus* and *B. uniformis* with any system, we feel that an identification of "indole-positive *B. fragilis* group" or "*B. ovatus-B. uniformis*" would be appropriate. The determination of growth in 20% bile could eliminate the misidentification of organisms from the "indole-positive *B. fragilis* group" (called *B. oralis* or *B. melaninogenicus* by the system). Several species, formerly of the genus *Bacteroides*, were recently placed in the new genus *Prevotella* (18) (Table 1). This taxonomic change occurred after the most recent publication of the *Code Compendium*. In the case of *F. necrophorum* and *F. nucleatum* differentiations, the ad-

dition of simple Gram stain and colonial morphology descriptions (13) to the identification process would improve the level of identification for both species.

Identifications of nonsporeforming gram-positive bacilli could be improved. Since most of the *Actinomyces* isolates were not identified to the correct species, we would recommend an identification of simply "*Actinomyces* spp." Species level identifications of these organisms, even by conventional prereduced anaerobic sterilization methodology, is difficult because of the overlapping of fermentation patterns exhibited by members of this genus (1). A genus-level identification would be preferred over an incorrect species identification. Further studies would be warranted for the *Lactobacillus* species, since a variety of misidentifications were noted.

The addition of a few simple recommended tests would greatly aid in correct identification of the clostridia to species. The use of a test for determining acid production from glucose would aid in the differentiation between *C. difficile* (positive) and *C. subterminale* (negative). Glucose and lecithinase are two simple tests that, if used with the RapID-ANA II results, would improve on the identification of *C. innocuum* (glucose positive, lecithinase negative) versus *C. subterminale* (glucose negative, lecithinase variable) or *C. limosum* (glucose negative, lecithinase positive).

The difficulty encountered with *C. clostridiiforme* was unique. Six of 16 *C. clostridiiforme* isolates were relatively unreactive in the RapID-ANA II. According to IDS representatives, their *C. clostridiiforme* isolates were considerably more reactive. Staff at Innovative Diagnostic Systems identify their isolates after growth on BA from Remel (Lenexa, Kans.). When these isolates were retested on the RapID-ANA II system after growth on Remel BA all six were reactive and identified correctly. It appears that a variable exists depending on the manufacturer and the medium used. Further studies are warranted in this area.

The gram-positive cocci often present identification difficulties even with the best of systems. Again, the addition of a few simple tests would improve the identifications of many species. The difficulty in separating *G. morbillorum* from *Peptostreptococcus* spp. can be alleviated with the use of GLC to demonstrate the large lactic acid peak of the former. The misidentifications of *P. anaerobius* could be avoided with the addition of GLC or the sodium polyanethol sulfonate disk test. The large peak of isocaproic and the positive sodium polyanethol sulfonate disk tests for *P. anaerobius* are unique to the species. GLC and indole, glucose, and urease tests are recommended to better differentiate the peptostreptococci. These tests would be particularly helpful for the identification of *P. prevotii*, since misidentifications ranged from several other *Peptostreptococcus* spp. to *Veillonella* spp. and *Streptococcus constellatus*. The characterization of *P. asaccharolyticus* isolates as *P. indolicus* may need to be reevaluated by Innovative Diagnostic Systems. *P. indolicus* is rarely encountered in clinical specimens. A coagulase test perhaps could be included when the identification of *P. indolicus* is observed. (*P. indolicus* is coagulase positive, *P. asaccharolyticus* is coagulase negative.) Since 5 of 14 *Veillonella* spp. isolates presented with "satisfactory" identifications as *S. saccharolyticus*, perhaps *Veillonella* spp. should be listed as an additional identification choice for this nonreactive code. It would be our recommendation to perform a metabolic product analysis with GLC to further aid in the differentiation of these nonreactive isolates.

In previous studies evaluating other rapid identification systems against similar reference methods, results appear to be comparable. An evaluation of the MicroScan System by Stoakes et al. (21) indicated a 66 to 70% accuracy of identification to genus and species. Studies of the AN-Ident by Stenson et al. (20) indicated a 67% correlation with reference methods. The first-generation RapID-ANA was evaluated by Burlage and Ellner (5), Downes et al. (7), and Summanen et al. (22), and accuracies for identifications ranged from 72 to 75% without the use of additional tests. The slightly lower accuracy observed in our evaluation of the RapID-ANA II system could have resulted, in part, from the numbers and diversity of strains included in the different studies and from the differences in the systems. Our experience with both the RapID-ANA (14) and the RapID-ANA II has demonstrated some distinct improvements in the second-generation system. Misidentifications with the RapID-ANA II seemed to be less serious than those with the previous system. For example, the misidentification of *C. difficile* as *Eubacterium aerofaciens*, as we observed with the first-generation RapID-ANA, is a more serious problem than the misidentification of *B. uniformis* as *B. thetaiotaomicron* by the second-generation RapID-ANA II.

In summary, the RapID-ANA II provides a battery of characteristics that can be used most effectively along with selected simple and practical tests to aid in the differentiation of commonly encountered anaerobes. Some recommended key tests to use along with the RapID-ANA II include growth in the presence of 20% bile (for gram-negative bacilli), reactions on egg yolk agar (for *Clostridium* spp.), a sodium polyanethol sulfonate disk test (for anaerobic cocci), use of lactobacillus selective medium (for suspected *Lactobacillus* spp.), and a few carbohydrate fermentation tests as previously suggested. Metabolic product analysis by GLC is highly recommended to aid in the identification of anaerobic nonsporeforming gram-positive bacilli, anaerobic cocci, and *Clostridium* spp. other than *C. perfringens* and *C. ramosum*. In laboratories unable to perform analysis by GLC, definitive identification of certain organisms (*F. necrophorum*, *P. prevotii*, *G. morbillorum*, various gram-positive bacilli, etc.) may not be possible. When clinically relevant, these laboratories may need to refer such isolates to reference laboratories. Gram reaction, cellular morphology, and colonial characteristics should always be used in conjunction with this system, as with all identification systems. The *Code Compendium* includes tables containing additional characteristics and should be consulted to improve the likelihood of correct identifications.

Further studies are recommended to evaluate the accuracy of identifications after the panels are inoculated, incubated, and refrigerated but before the addition of reagents (as recommended by the manufacturer). The option of refrigeration could of a potential advantage for this system by allowing more efficient incorporation of a 4-h system in the work flow of some laboratories. The RapID-ANA II system appears to be a promising, rapid system for the identification of the more commonly isolated anaerobic bacteria. The system offers a convenient and flexible alternative for laboratories unable to use conventional identification methods. Optimal use of the system depends upon a basic knowledge of key characteristics of clinical anaerobic bacteria and the use of a few simple additional tests.

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