JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1985, p. 980–983
0095-1137/85/120980-04$02.00/0

Vol. 22, No. 6

Evaluation of the RapID-ANA System for Identification of Anaerobic Bacteria of Veterinary Origin

WILLIAM S. ADNEY AND ROBERT L. JONES*

Diagnostic Laboratory, Colorado State University, Fort Collins, Colorado 80523

Received 1 July 1985/Accepted 10 September 1985

This study evaluated the ability of the RapID-ANA system (Innovative Diagnostic Systems, Inc., Atlanta, Ga.) to accurately identify a spectrum of freshly isolated veterinary anaerobes. A total of 183 isolates were tested and included 7 *Actinomyces* spp., 53 *Bacteroides* spp., 32 *Clostridium* spp., 2 *Eubacterium* spp., 65 *Fusobacterium* spp., 1 *Peptococcus* spp., 22 *Peptostreptococcus* spp., and 1 *Propionibacterium* spp. All isolates were initially identified by conventional biochemical testing and gas-liquid chromatography of short-chain fatty acid metabolites. Additional tests were performed as required by the RapID-ANA system. Of these isolates, 81.4% were correctly identified to the genus level, including 59.6% to the species level, 14.2% were incorrectly identified at the genus level, and 4.4% were not identified. Initially, 20.2% of the strains were not identified because the microcodes were not in the code book. The majority of the incorrect identifications were caused by the misidentification of *Fusobacterium* spp. as *Bacteroides* spp. Errors also occurred when veterinary anaerobes not included in the data base were assigned an identification from the existing data base. The RapID-ANA system appears to be a promising new method for rapid identification of veterinary anaerobes; however, further evaluation with an extended data base is needed before the system can accurately identify all clinically significant anaerobes.

The isolation and identification of anaerobes from clinical samples is an important function of the clinical microbiology laboratory, since antimicrobial therapy for these infections can differ from therapy used to treat infections caused by aerobic or facultative anaerobic bacteria (3, 8, 12). Conventional test procedures, including biochemical tests and gas-liquid chromatography analysis of metabolic products, have been developed by the Centers for Disease Control, Virginia Polytechnic Institute, and Wadsworth anaerobe laboratories (1, 13). However, these systems have the disadvantages of being time-consuming, expensive, and beyond the capabilities of many clinical laboratories.

During the past decade, several commercially prepared microsystems, designed specifically for the identification of anaerobes, have been introduced (1, 13). Generally, these systems offer a number of benefits, including standardized selection of biochemical tests, improved identification accuracy compared with the limited conventional system used in many laboratories, and decreased time and cost for identification.

The accuracy and reproducibility of the RapID-ANA system (Innovative Diagnostic Systems, Inc., Atlanta, Ga.) for the identification of anaerobes of human origin has been evaluated (2, 11). However, the usefulness of the RapID-ANA method for the identification of clinically significant anaerobes of veterinary origin has not been established. The purpose of this study was to evaluate the accuracy of the RadID-ANA system for the identification of a spectrum of freshly isolated veterinary anaerobes.

MATERIALS AND METHODS

Test organisms. A total of 183 recent veterinary clinical isolates were tested in the RapID-ANA system. The strains represented members of the genera *Actinomyces*, *Bacteroides*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Peptococcus*, *Peptostreptococcus*, and *Propionibacterium*. The clinical isolates were obtained from samples submitted to the Diagnostic Laboratory, Colorado State University. During the study, no attempt was made to select specific bacterial species. Only isolates considered to be clinically significant were included. The following strains were used as quality control organisms: *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 27941, *Clostridium histolyticum* ATCC 19401, and *Clostridium sporogenes* ATCC 19404.

The organisms were isolated on Centers for Disease Control anaerobe blood agar (6) supplemented with 5% sheep or horse blood, in a flexible anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) with an atmosphere of 85% N_2–10% H_2–5% CO_2. Characteristics such as Gram stain reaction, cellular and colonial morphology, and aerotolerance were examined before additional biochemical testing. Metabolic products from all strains were analyzed by gas-liquid chromatography of Lombard-Dowell or peptone-yeast broth containing glucose, lactate, or threonine (9, 10). Biochemical testing was performed by using the following three quadrant plates of differential media: (i) anaerobic Presumpto plate (Pasco Laboratories, Wheatridge, Colo.), (ii) anaerobic gram-positive ID Quad plate (Remel, Lenexa, Kans.), and (iii) anaerobic CDC Quad #3 plate (Remel, Lenexa, Kans.). Characteristics that were determined by using these plates included glucose, lactose, rhamnose, and mannitol fermentation, esculin, starch, casein, and gelatin hydrolysis, catalase, lipase, lecithinase, and deoxyribonuclease activity, indole and H_2S production, and growth in the presence of 20% bile. These characteristics were used to establish the correct identification of strains (10, 13).

RapID-ANA system. The RapID-ANA system is a qualitative micromethod for anaerobe identification that has previously been described in detail (2, 11). Inoculation, 4 h of incubation in a 35°C air incubator, and interpretation of the RapID-ANA tests were performed according to the instructions of the manufacturer. A single subculture on an

* Corresponding author.

TABLE 1. RapID-ANA system identification test results for veterinary anaerobes

Organism	No. tested	No. correct to:		No. incorrect to genus	No. not identified
		Genus	Species		
Actinomyces spp.	7	7 (4)[a]	0	0	0
Bacteroides asaccharolyticus	10	10	10	0	0
B. fragilis	6	6	6	0	0
B. gingivalis	2	2	2	0	0
B. intermedius	1	1	1	0	0
B. melaninogenicus	4	4 (3)	2 (1)	0	0
B. oralis	6	6	4	0	0
B. uniformis	4	4	3	0	0
Bacteroides sp.	20	9 (4)	0	4 (4)	7 (7)
Clostridium bifermentens	2	2	2	0	0
C. sporogenes	2	2	1	0	0
C. novyi	2	0	0	2	0
C. perfringens	15	14 (1)	13	0	1 (1)
C. septicum	1	1	0	0	0
C. sordellii	6	6 (3)	5 (3)	0	0
Clostridium sp.	4	3 (2)	0	1 (1)	0
Eubacterium spp.	2	2	0	0	0
Fusobacterium necrophorum	38	24 (1)	16 (1)	14 (3)	0
F. nucleatum	21	21	21	0	0
F. russii	6	1 (1)	0	5 (2)	0
Peptococcus indolicus	1	1	1	0	0
Peptostreptococcus anaerobius	21	21	20	0	0
P. micros	1	1	1	0	0
Propionibacterium spp.	1	1	1	0	0
Total no.	183	149 (19)	109 (5)	26 (10)	8 (8)
Total %		81.4	59.6	14.2	4.4

[a] Numbers in parentheses represent strains for which an identification was not listed in the code book. Microcodes were telephoned in to the manufacturer for interpretation.

anaerobic blood agar plate from the primary isolation plate was required to obtain sufficient organisms.

Identification was made by assigning each test result a numerical value from which a numerical code (microcode) was generated, and the RapID-ANA *Code Compendium* (1983 ed.) was consulted for identification. Microcodes not found in the code book were telephoned in to the manufacturer for interpretation. When identifications with low confidence values (<95%) occurred, additional test results or characteristics were evaluated.

Interpretation of results. RapID-ANA test results were classified as (i) correct to the genus level, (ii) correct to the species level, (iii) incorrect genus, and (iv) not identified. Identifications tabulated as correct to the genus level included all identifications correct to the species level as well as isolates not identified beyond the genus level or when an incorrect species assignment was made within the correct genus.

RESULTS

Identification results of all organisms are presented in Table 1. The RapID-ANA system correctly identified 149 (81.4%) of 183 isolates to the genus level, including 109 (59.6%) to the species level. Identification was incorrectly assigned at the genus level for 26 (14.2%) of the isolates. The identification microcode obtained for 37 (20.2%) strains was not listed in the code book. After the manufacturer was telephoned for interpretation of the unlisted microcodes, the RapID-ANA system was unable to assign an identification to eight (4.4%) strains.

The RapID-ANA system correctly identified 28 (52.8%) strains of *Bacteroides* at the species level and an additional 14 at the genus level. Four strains of *Bacteroides* sp. were incorrectly identified at the genus level as *Clostridium clostridiiforme* (3) or *Fusobacterium mortiferum*.

The RapID-ANA system correctly identified 21 (65.6%) of the 32 *Clostridium* strains to the species level and an additional 7 (21.9%) strains to the genus level. Two strains of *Clostridium novyi* were incorrectly identified as *Lactobacillus minutis*, and *Clostridium* sp. was incorrectly identified as *Eubacterium* sp. One strain of *Clostridium septicum* required urease as a supplemental test for differentiation from *Clostridium bifermentans*.

The RapID-ANA system was able to identify 15 (42.1%) strains of *Fusobacterium necrophorum* correctly to the species level and an additional 8 strains to the genus level. In addition, the system incorrectly identified 14 (36.8%) strains as *Bacteroides asaccharolyticus* (13) or *Bacteroides bivius*. Additional testing was required for 10 *F. necrophorum* strains. The supplemental tests included lipase to differentiate eight strains from *F. nucleatum* and pigment production to differentiate two strains from *B. asaccharolyticus*. Of

those strains identified correctly to the species level, six were identified with an inadequate confidence level. However, "contraindicated test results" (atypical characteristics for the taxon listed as the identification) were not listed for these strains. Additional tests were recommended and performed. All 21 *Fusobacterium nucleatum* strains were correctly identified. Six *Fusobacterium russii* strains were tested. One strain was correctly identified at the genus level, while five strains were incorrectly identified as *Bacteroides capillosus* (2), *Bacteroides ureolyticus*, *C. clostridiiforme*, or *Bacteroides* sp. (2).

Strains representing the genera *Actinomyces*, *Eubacterium*, *Peptococcus*, *Peptostreptococcus*, and *Propionibacterium* were also tested. The *Actinomyces* spp. included only catalase-positive strains and were correctly identified at the genus level as such. Correct genus level identifications were obtained for all strains of non-spore-forming gram-positive anaerobes tested.

DISCUSSION

The introduction of commercially prepared microsystems, designed specifically for the identification of anaerobes, has decreased the time and cost required for the identification of anaerobes from clinical specimens. The RapID-ANA system offers the advantages of aerobic incubation and 4-h identifications based on enzymatic degradation of chromogenic substrates by preformed bacterial enzymes. The instructions for the RapID-ANA system indicate that it is intended for the identification of anaerobes of human origin and that its accuracy for identification of anaerobes of veterinary origin has not been established. In the present study, the identification of 183 anaerobes that represented the typical spectrum of clinically significant veterinary anaerobes (3, 4, 7, 8, 14) was tested in the RapID-ANA system. The overall accuracy of the identification system for veterinary anaerobes was found to be similar to the accuracy for identification of human anaerobes as reported in one study (11). The correct identification rate for veterinary isolates was 81.4 and 59.6% to the genus and species levels, respectively. Karachewski and co-workers (11) reported the correct identification rates for human isolates as 90 and 62% to genus and species levels, respectively. However, Appelbaum and co-workers (2) reported correct identification to the species level with additional tests to be 81.1 and 92.1% in two separate laboratories. The wide range of correct identification rates in these studies is not apparent but may depend upon the selection of strains to be tested or the interpretation of results by laboratory personnel. Additional studies of reproducibility and accuracy are needed to define this unsettled point.

In our study, 26 strains were misidentified at the genus level. Four *Bacteroides* strains were misidentified, and 18 strains were incorrectly identified as *Bacteroides* strains. Clinically significant antimicrobial resistance within veterinary anaerobes is limited to *Bacteroides* strains (8). Therefore, correct identification of these strains is more important than it may be for most anaerobes. Most of the incorrect identifications (19 of 26) occurred with *Fusobacterium* strains. Although the number of human origin *Fusobacterium* strains tested in the previous reports was quite small, misidentification at the genus level was not reported (2, 11). The high rate of incorrect identifications for *F. necrophorum* in this study may have been due to the occurrence of biotypes in animals that are not present in humans.

The reasons for misidentifications in this study were not determined for each strain. All tests in this study were set up and interpreted by one investigator (W.S.A.) to minimize variability due to personnel. Appelbaum and co-workers (2) reported test discrepancies when 21 strains were tested separately by two laboratories and a lack of reproducibility in triplicate tests at the same laboratory. They attributed these errors to either product deficiencies or problems in interpretation of test reactions. In the previously reported studies (2, 11), they did not indicate whether the discrepancies occurred with recent isolates or laboratory stock cultures. Organisms sequentially transferred and maintained for long periods in the laboratory may demonstrate aberrant reactions and reduce test selectivity. In this study, only recent clinical isolates were tested. A single subculture from the primary isolation plate was always required to obtain sufficient organisms for testing. Reproducibility of the identification system was not evaluated.

Previously published evaluations of the RapID-ANA system have reported that some of the color reactions were difficult to interpret, thereby giving equivocal results (2, 11). We also found that the clarity of the reactions varied with tests and the reactivity of a specific organism. Interpretation of the tests which detected hydrolysis of a colorless aryl-substituted glycoside or phosphate ester was easy. The release of the yellow-colored *o*- or *p*-nitrophenol resulted in a sufficiently strong yellow color change (positive) that could be distinguished from clear to very pale yellow (negative). Hydrolysis of the amide substrates was considerably more difficult to read. The positive reaction varied from purple to dark red to very dark pink, while the negative reactions varied from yellow to pale orange to pale pink. Dark pink (positive) and pale pink (negative) reactions were difficult to distinguish. *Clostridium* and *Peptostreptococcus* strains were generally more reactive and produced a rapid, almost immediate color change to purple or dark red when positive reactions were observed. Some of the more fastidious organisms such as *Fusobacterium* and *Bacteroides* spp. rarely produced strong positive reactions.

The RapID-ANA system uses a numerical approach for the identification of anaerobes. One of the limitations of this probability method is the fact that it cannot recognize a strain outside of its existing data base. Therefore, an unknown strain may be incorrectly assigned to the identification taxon that its microcode most closely resembles rather than being recognized as an unidentifiable strain. In this study, the misidentification of several veterinary anaerobes may have been due to deficiencies in the data base. The RapID-ANA system was unable to assign an identification for eight strains. *C. novyi* is not included in the RapID-ANA data base; as a result, two strains tested in this study were incorrectly identified as *Lactobacillus minutis*. Six strains of *F. russii*, another organism not in the data base, were tested, and five were incorrectly identified at the genus level (Table 1). The seven strains of *Actinomyces* tested were catalase positive and were correctly identified as such by the RapID-ANA system. However, they were not classified as correct identifications at the species level because the data base did not include *Actinomyces hordeovulneris* (5) and was unable to differentiate catalase-positive *Actinomyces* spp.

In summary, the RapID-ANA system appears to be a promising new method for the rapid identification of anaerobes, with overall accuracy for veterinary strains within the range of correct identification rates reported for human strains. However, further evaluation with an extended data base which includes strains of veterinary origin is needed before the system can accurately identify all clinically significant veterinary anaerobes.

LITERATURE CITED

1. **Allen, S. D., J. Å. Siders, and L. M. Marler.** 1985. Isolation and identification of anaerobic bacteria, p. 413–433. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
2. **Appelbaum, P. C., C. S. Kaufmann, and J. W. Depenbusch.** 1985. Accuracy and reproducibility of a four-hour method for anaerobe identification. J. Clin. Microbiol. **21**:894–898.
3. **Berg, J. N., W. H. Fales, and C. M. Scanlan.** 1979. Occurrence of anaerobic bacteria in diseases of the dog and cat. Am. J. Vet. Res. **40**:876–881.
4. **Berkhoff, G. A., and J. L. Redenbarger.** 1977. Isolation and identification of anaerobes in the veterinary diagnostic laboratory. Am. J. Vet. Res. **38**:1069–1074.
5. **Buchanan, A. M., J. L. Scott, M. A. Gerencser, B. L. Beaman, S. Jang, and E. L. Biberstein.** 1984. *Actinomyces hordeovulneris* sp. nov., an agent of canine actinomycosis. Int. J. Syst. Bacteriol. **34**:439–443.
6. **Dowell, V. R., Jr., G. L. Lombard, F. S. Thompson, and A. Y. Armfield.** 1977. Media for isolation, characterization, and identification of obligately anaerobic bacteria. Centers for Disease Control, Atlanta, Ga.
7. **Hirsh, D. C., E. L. Biberstein, and S. S. Jang.** 1979. Obligate anaerobes in clinical veterinary practice. J. Clin. Microbiol. **10**:188–191.
8. **Hirsh, D. C., M. C. Indiveri, S. S. Jang, and E. L. Biberstein.** 1985. Changes in prevalence and susceptibility of obligate anaerobes in clinical veterinary practice. J. Am. Vet. Med. Assoc. **186**:1086–1089.
9. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobic bacteriology manual, 4th ed. Anaerobic Laboratory, Virginia Polytechnic Institute and State University, Blacksburg.
10. **Holdeman, L. V., R. W. Kelley, and W. E. C. Moore.** 1984. Family I. *Bacteroidaceae* Pribram 1933, 10, p. 602–662. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
11. **Karachewski, N. O., E. L. Busch, and C. L. Wells.** 1985. Comparison of PRAS II, RapID ANA, and API 20A systems for identification of anaerobic bacteria. J. Clin. Microbiol. **21**:122–126.
12. **Kimsey, P. B., and D. C. Hirsh.** 1978. Obligate anaerobes in clinical veterinary medicine: susceptibility to antimicrobial agents. J. Vet. Pharmacol. Ther. **1**:63–68.
13. **Koneman, E. W., S. D. Allen, V. R. Dowell, Jr., and H. M. Sommers.** 1983. Color atlas and textbook of diagnostic microbiology, 2nd ed. J. B. Lippincott Co., Philadelphia.
14. **Prescott, J. F.** 1979. Identification of some anaerobic bacteria in nonspecific anaerobic infections in animals. Can. J. Comp. Med. **43**:194–199.