JOURNAL OF CLINICAL MICROBIOLOGY, July 1985, p. 32–35
0095-1137/85/070032-04$02.00/0

Vol. 22, No. 1

Comparison of the PRAS II, AN-Ident, and RapID-ANA Systems for Identification of Anaerobic Bacteria

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Received 2 November 1984/Accepted 18 March 1985

Two rapid systems for the identification of anaerobes were compared to a conventional growth system aided by a computer. The rapid systems (AN-Ident and RapID-ANA) are non-growth-dependent micromethods that identify anaerobes in 4 h by the action of various constitutive enzymes on chromogenic substrates. The organisms tested were 98 anaerobes, most of which were clinical isolates. The AN-Ident system identified 76 of these to species level and 86 to genus level; the RapID-ANA system correctly identified 74 of the organisms to species level and identified 93 to genus level. The PRAS II system correctly identified 77 to species level and 96 to genus level. In most instances, adequate identification could be obtained with either of the two rapid systems, but the conventional PRAS II system remains the most accurate.

Obligate anaerobic bacteria tend to grow more slowly than the aerobic or facultative organisms commonly encountered in clinical material, and as a result bacteriology reports are often delayed pending the identification of anaerobes. Identification procedures commonly used for anaerobes are growth dependent and involve a variety of fermentation reactions, inhibition of growth by bile and certain antibiotics, and analysis of metabolic acid end products by gas-liquid chromatography (GLC) (2, 4, 8). A commercially available system using a variety of prereduced anaerobically sterilized biochemical substrates (PRAS II) has been shown to provide accurate identification of obligate anaerobes (1). This system has been used as a standard in the evaluation of nonconventional identification systems (5).

Recently, two non-growth-dependent systems for the rapid identification of anaerobes have become commercially available. These micromethods detect a variety of constitutive enzymes on specific substrates, many of which have chromogenic reaction products.

These two rapid systems were evaluated here by comparing them with the PRAS II system. Both rapid systems were able to identify the majority of clinically significant anaerobes with sufficient accuracy to permit the adoption of these systems in the clinical laboratory.

MATERIALS AND METHODS

Organisms tested. The majority of the organisms tested were fresh clinical isolates obtained from patients at the Columbia-Presbyterian Medical Center or the Montefiore Hospital in New York. Stock cultures obtained from the manufacturers of the AN-Ident system (Analytab Products, Plainview, N.Y.) and the RapID ANA system (Innovative Diagnostic Systems, Decatur, Ga.) were also tested. Specimens appropriate for anaerobic culture were collected and transported in the syringe used for aspiration or in an anaerobic specimen collector (BBL Microbiology Systems, Cockeysville, Md.).

Primary plating. Primary media for anaerobes were as follows: reducible anaerobic Columbia agar with 5% sheep blood, cysteine, dithiothreitol, and palladium chloride (anBAP) (3); kanamycin-vancomycin laked blood agar with menadione and hemin (KV); colistin-nalidixic acid blood agar (CNA), neomycin blood agar (NEO); and prereduced, anaerobically sterilized, chopped-meat glucose broth (CMG) (Scott Laboratories, Inc., Fiskeville, R.I.). All plates were prereduced in a GasPak jar at room temperature for 24 h before inoculation. After inoculation, plates were incubated at 35°C in plastic bags equipped with hydrogen generators and redox indicators (Bio-Bags; Marion Scientific, Kansas City, Mo.).

Initial procedures. Colonies from primary plates were subcultured both aerobically and anaerobically to determine purity and aerotolerance. Gram stains were performed on strict anaerobes. Colonies on KV were examined under long-wave UV light; those showing red fluorescence were presumptively identified as species of black-pigmenting bacteroides. All organisms were inoculated into the PRAS II (Scott Laboratories), AN-Ident, and RapID ANA systems as described below. GLC was performed when necessary to help resolve discrepancies in identification.

Pure cultures of the organisms to be tested were inoculated into 5 ml of CMG and onto two anBAP plates, which were then incubated in an anaerobic jar for 24 h at 35°C. The CMG culture was used to inoculate the PRAS II system, and the blood agar cultures were used to inoculate the AN-Ident and RapID ANA systems as described below.

AN-Ident system. The AN-Ident system should not be confused with a previous product (API 20) that requires 24 h of growth for the identification of anaerobes. The AN-Ident strip consisted of two rows of 10 microcupules containing dehydrated substrates designed to test for indole production, *N*-acetylglucosaminidase, α-glucosidase, α-arabinosidase, β-glucosidase, α-fucosidase, phosphatase, α-galactosidase, β-galactosidase, indoxyl acetate, arginine utilization, leucine aminopeptidase, proline aminopeptidase, pyroglutamic acid arylamidase, tyrosine aminopeptidase, arginine aminopeptidase, alanine aminopeptidase, histidine aminopeptidase, phenylalanine aminopeptidase, glycine aminopeptidase, and catalase.

The colonies grown on anBAP were suspended in 2.5 ml of sterile distilled water in a test tube. The final turbidity was adjusted to match a McFarland no. 5 turbidity standard. Each cupule was inoculated with 2 drops (approximately 85

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μl) of the suspension by using a special disposable pipette provided by the manufacturer. The strip was placed in a covered plastic tray and incubated at 35°C for 4 h in air.

After incubation, the reactions not requiring the addition of reagents were read visually for the appropriate color change. The indole reaction was read after the addition of Kovacs reagent, 3% hydrogen peroxide was added to the glucosidase substrate to test for catalase production, and cinnamaldehyde reagent was added to the aminopeptidase substrates, which were then observed for color changes. The reactions were used to generate a seven-digit numerical profile, which was then referred to the manufacturer by telephone together with the morphology and Gram stain reaction of the isolate for the final identification.

RapID-ANA system. This system consists of a plastic tray with 10 wells containing dehydrated substrates including *p*-nitrophenylphosphate, *o*-nitrophenyl-β-D-galactoside, *p*-nitrophenyl-α-D-galactoside, *p*-nitrophenyl-β-D-glucoside, *p*-nitrophenyl-α-D-galactoside, *p*-nitrophenyl-α-D-fucoside, *p*-nitrophenyl-*N*-acetylglucosaminide, leucylglycyl-β-naphthylamide, glycyl-β-naphthylamide, prolyl-β-naphthylamide, phenylalanyl-β-naphthylamide, arginyl-β-naphthylamide, seryl-β-naphthylamide, pyrrolidonyl-β-naphthylamide, triphenyltetrazolium, arginine, trehalose, and tryptophan.

The pure culture from anBAP was suspended in 1 ml of inoculator fluid (provided by the manufacturer) which contained 0.75% KCl and 0.05% $CaCl_2$ and poured into the tray. The tray was slowly tilted, permitting an even distribution of inoculum into all of the wells. The trays were incubated at 35°C for 4 h in air.

The first 10 reactions as listed above were read visually for a color change before the addition of reagents. Cinnamaldehyde reagent was added to the tryptophan well. The "RapID-ANA Reagent" (0.1% sodium dodecyl sulfate, 0.01% D-phenyl-4-dimethylamino acrolein, 1% methyl cellosolve, 1% acetic acid, 0.1% hydrochloric acid, 1% surfactant) was added to the first seven wells, which were observed for a subsequent color change. A six-digit biotype number was generated and referred to a data base manual for identification. Biotypes not found in the manual were referred to the manufacturer for identification.

PRAS II system. This system included various prereduced anaerobically sterilized biochemical substrates in tubes containing 5 ml and sealed with a rubber septum.

The CMG culture was used to inoculate anBAP plates on which were placed disks containing vancomycin (5 μg/ml), colistin (10 μg/ml), kanamycin (1,000 μg/ml), and rifampin (15 μg/ml) (8). The appropriate biochemical substrates were inoculated with 0.05 ml of the CMG culture by using a special inoculator provided by the manufacturer. Tubes and plates were incubated for 48 h. Each anBAP plate was examined for zones of inhibition (zones ≥10 mm in diameter) around the disks. Growth from the anBAP plate was used to perform a test for catalase. Lecithinase and lipase were determined on egg yolk agar plates (Scott Laboratories).

The manufacturer also provided a Commodore VIC-20 computer with special software that included a group selection program and two additional programs (AS-1 and AS-2) for species identification.

The group selection program assigned the isolates to one of five groups based upon Gram stain reaction and morphology, susceptibility to vancomycin, colistin, and kanamycin, the presence of spores, growth in 2% bile, and the presence of catalase, lecithinase, or lipase. This program also permitted the use of GLC peaks if necessary. The five groups were anaerobic cocci, *Fusobacterium*, *Bacteroides*, and *Clostridium* spp., and gram-positive nonsporeforming rods. The program indicated the battery of biochemicals to be inoculated for each group.

Fermentation of the carbohydrates was determined by removing the rubber septum from each tube and measuring the pH with a pencil electrode. pH values of 6.0 or less were interpreted as acidic. A black precipitate in semisolid indole motility medium (SIM) indicated H_2S production, and a failure of the peptone-yeast gelatin culture to gel after 30 min at 5°C indicated hydrolysis.

The results of the various tests were entered into the computer using the AS-1 program to provide the identification. The AS-2 program is available for unusual isolates.

GLC. For volatile fatty acids, GLC was performed by acidifying a portion of the CMG culture with H_2SO_4 and adding one-half volume of ether. After the sample was vortexed, centrifuged, and then frozen in an alcohol-dry ice bath, 10 μl of the ether extract was injected into the port of a Dohrman Envirotech Anabac unit. Nonvolatile fatty acids were assayed on the same unit by using a methylated extract of the CMG.

TABLE 1. The 98 organisms tested by the PRAS II, AN-Ident, and RapID-ANA systems

Organism	No. of isolates
Bacteroides spp.	
B. fragilis	18
B. thetaiotaomicron	6
B. ovatus	4
B. uniformis	4
B. melaninogenicus	3
B. distasonis	2
B. oralis	2
Clostridium spp.	
C. perfringens	6
C. difficile	3
C. ramosum	2
C. sordelli	2
C. tetani	2
C. bifermentans	1
C. butyricum	1
C. cadaveris	1
C. histolyticum	1
C. sporogenes	1
C. paraputrificum	1
Fusobacterium spp.	
F. varium	8
F. mortiferum	5
F. nucleatum	5
F. necrophorum	1
F. prausnitzii	1
Streptococci	
Peptostreptococcus magnus	3
P. asaccharolyticus	2
P. anaerobius	2
P. micros	1
Streptomyces intermedius	1
Gram-positive nonsporeforming bacilli	
Propionibacterium acnes	5
Eubacterium lentum	2
Propionibacterium granulosum	1
Lactobacillus minutus	1

TABLE 2. The 30 organisms giving varied results with the three systems and GLC[a]

AN-Ident	RapID-ANA	PRAS II	GLC consistent with:
No match[b]	*B. thetaiotaomicron*	*B. thetaiotaomicron*	*B. fragilis* group
B. oralis	*B. vulgatus*	*B. vulgatus*	*B. oralis*
B. oralis	*B. distasonis*	*B. capillosus*	*B. oralis*
B. uniformis	*B. uniformis*	*B. eggerthii*	*B. fragilis* group
B. disiens	*B. disiens*	*B. ovatus*	*Bacteroides* sp.
B. ovatus	*B. thetaiotaomicron*	*B. ovatus*	*B. fragilis* group.
B. fragilis	*B. fragilis*	*B. distasonis*	*B. fragilis* group
No match	*B. fragilis*	*B. thetaiotaomicron*	*B. fragilis* group
No match	*B. thetaiotaomicron*	*B. thetaiotaomiron*	*B. fragilis* group
B. denticola	*B. thetaiotaomicron*	*B. capillosus*	*Bacteroides* sp.
C. subterminale	*C. tetani*	*C. subterminale*	*C. tetani*
C. innocuum	*C. tetani*	*C. glycolicum*	*C. tetani*
C. innocuum	*C. cadaveris*	*C. cadaveris*	*C. cadaveris*
C. limosum	*C. paraputrificum*	*C. ramosum*	*C. ramosum*
C. ramosum	*C. paraputrificum*	*C. ramosum*	*C. ramosum*
C. ramosum	*C. ramosum*	*F. mortiferum*	*F. mortiferum*
C. difficile	*C. sporogenes*	*C. difficile*	*C. difficile*
No match	*E. lentum*	*L. minutus*	*Lactobacillus* sp.
Actinomyces sp.	*P. acnes*	*E. lentum*	*Propionibacterium* sp.
P. granulosum	*Actinomyces meyeri*	*P. granulosum*	*Propionibacterium* sp.
Peptostreptococcus anaerobius	*Gaffkya anaerobia*	*P. anaerobius*	*P. anaerobius*
Peptostreptococcus magnus	*Peptostreptococcus magnus*	*S. intermedius*	*Peptostreptococcus magnus*
S. intermedius	*S. constellatus*	*S. intermedius*	*S. intermedius*
No match	*F. varium*	*F. prausnitzii*	*Fusobacterium* sp.
F. nucleatum	*F. necrophorum*	*F. necrophorum*	*Fusobacterium* sp.
No match	*F. varium*	*F. varium*	*Fusobacterium* sp.
No match	*F. varium*	*F. mortiferum*	*F. mortiferum*
No match	*F. mortiferum*	*F. mortiferum*	*Fusobacterium* sp.
No match	*F. varium*	*F. nucleatum*	*Fusobacterium* sp.
No match	*C. ramosum*	*F. mortiferum*	*F. mortiferum*

[a] Genera: *Bacteroides, Clostridium, Propionibacterium, Fusobacterium, Eubacterium, Lactobacillus,* and *Streptomyces* except where otherwise noted.
[b] No match indicates that profile number obtained was not found in the data base.

RESULTS

A total of 98 organisms were tested with the three systems (Table 1). The identification of 68 of these organisms concurred in all three systems. Organisms giving discrepant results were analyzed by GLC. Organisms were considered to be correctly identified if all three systems agreed with regard to genus and species or if the result agreed with that obtained by GLC. Thirty organisms gave varied results with the three systems and GLC (Table 2).

The AN-Ident system correctly identified 76 of the organisms to species level and 86 to genus level. Of these species, 68 agreed with the results as determined by other two systems, and 8 agreed with the species as determined by GLC. An additional 10 agreed with the genus as determined by GLC. Two isolates were considered to be incorrectly identified: a *Fusobacterium mortiferum* isolate misidentified as *Clostridium ramosum* and a *Propionibacterium* sp. misidentified as an *Actinomyces* sp. In 10 instances, a profile number was obtained which did not correspond to any identification (no match). The manufacturer acknowledged that its data base was incomplete at the time of our testing. These 10 organisms were returned to the manufacturer for retesting. Our identification was confirmed with one of these; two organisms were not found in the manufacturer data base; three *Fusobacterium* spp. were identified as *Clostridium* spp.; and four of the cultures were said to be mixed.

The RapID-ANA system correctly identified 74 of the organisms to species level and 93 to genus level. Of these, 68 species agreed with those determined by the other two systems and 6 agreed with the species determined by GLC. An additional 19 agreed with the genus determined by GLC. Five organisms were considered to be missed; two *F. mortiferum* isolates were misidentified as *C. ramosum*; a *Lactobacillus* sp., *Propionibacterium* sp., and a *P. anaerobius* isolate were misidentified as *Eubacterium lentum*, *A. meyeri*, and *Gaffkya anaerobia*, respectively.

The PRAS II system correctly identified 77 of the organisms to species level and 96 to genus level. Of these, 68 species agreed with those determined by the other two systems and 9 agreed with those determined by GLC. An additional 19 agreed with the genus as determined by GLC. Two organisms were considered missed, a *Propionibacterium* sp. was misidentified as *E. lentum*, and a *Peptostreptococcus magnus* isolate was misidentified as *Streptomyces intermedius*.

DISCUSSION

The ability to identify an anaerobic isolate within 4 h of the time a pure culture inoculum becomes available could shorten the turnaround time of some bacteriology laboratory reports by 24 to 48 h.

Rapid fermentation testing of anaerobic bacteria has been described by Schreckenberger and Blazevic (6), and the use of micromethods for the identification of anaerobes was reported by Starr et al. (7) and Tharagonet et al. (9).

This study compared two rapid non-growth-dependent micromethods that utilized the constitutive enzymes of anaerobic bacteria to identify isolates within 4 h to a conventional growth-dependent system.

The AN-Ident system correctly identified 78% of the organisms to species level and identified 88% to genus level without the need for additional tests. This system alone correctly identified all of the anaerobic cocci, but it was only able to identify slightly more than half of the fusobacteria tested. Although the actual test only took 4 h, the need for a heavy inoculum generally required a blood agar plate with confluent growth of the pure culture, often adding 24 h to the total identification time required. This heavy inoculum was sometimes difficult to achieve with some slow-growing organisms and may explain the poor performance of this system with fusobacteria. Assembly, inoculation, and reading of the tests were straightforward, although occasionally some wells gave borderline reactions. The chart supplied along with the test did not always provide an accurate presentation of the actual test colors; in particular, the IND well was never the solid red depicted on the chart, and the NPG well was often much lighter than the chart color.

The RapID-ANA system correctly identified 76% of the organisms to species level and identified 95% to genus level without the need for additional tests. It was somewhat easier to obtain the proper inoculum for the RapID-ANA system since only a density equivalent to a McFarland no. 3 was required, whereas the AN-Ident required an inoculum density equivalent to a McFarland no. 5. Inoculation was a simple procedure. Borderline color reactions were sometimes obtained, and the difference between positive and negative reactions was slight, requiring a practiced eye. The color in the wells was obscured by the addition of reagents, precluding the possibility of rechecking. However, most borderline wells were correctly judged.

The PRAS II system correctly identified 79% of the organisms to species level and identified 98% to genus level without the need for additional tests. A previous study with the PRAS II system obtained 73 to 81% correct identification to species level and 88-to-94% correct identification to genus level (5). Inoculation and reading of the results was somewhat time-consuming. Results were usually available by 48 h after inoculation of the system. The manufacturer conveniently packages the tubes in batteries appropriate for each of the anaerobe groups. The individual tubes of media were stable for at least 18 months. The computer was extremely simple to operate, and the programs made it possible for even an inexperienced technologist to quickly determine the group, select the appropriate battery of biochemicals to be inoculated, and obtain the correct identification of the organism. The computer was very helpful in resolving conflicting data and was often able to provide identification based upon limited test results.

Of the three systems tested, the PRAS II system was the most accurate and remains the standard by which other systems are evaluated. In most instances, adequate identification of clinically significant anaerobes could be obtained with either of the two rapid systems.

ACKNOWLEDGMENT

We thank John McKitrick, Director of Microbiology, Montefiore Hospital, New York, for supplying some of the anaerobic isolates.

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