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# Evaluation of the ATB 32 A System for Identification of Anaerobic Bacteria Isolated from Clinical Specimens

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**A new miniaturized 4-h method for the identification of anaerobic bacteria, ATB 32 A (API System SA, Montalieu Vercieu, France), was evaluated against conventional methods of identification. The evaluation was done by using 260 recent clinical isolates and 21 reference strains of anaerobic bacteria. All reference strains were correctly identified and did not figure in the detailed analysis. Of the 140 gram-negative bacilli, 90.6% of *Bacteroides* spp. and 95.5% of *Fusobacterium* spp. were correctly identified to the species level, with an additional 8.4% of the *Bacteroides* spp. being identified to the genus level. Clostridia were correctly identified in 85.9% of cases, with an additional 9.9% being identified to the genus level. Peptostreptococci were correctly identified in 91.6% of cases. The 4 strains of *Actinomyces* spp. were all identified correctly, as were 10 of the 11 strains of *Propionibacterium* spp. A total of 3.1% of strains were not identified by ATB 32 A, while for 1.9% of strains, completely false identifications were obtained. Estimation of the individual preformed enzyme results may pose problems, although these decrease with familiarity with the system. With certain enzyme profiles, additional testing was necessary to arrive at an identification; however, there was no requirement for gas-liquid chromatography. If certain additions are made to the data base and the difficulties of determination of organisms to the species level among the non-*Bacteroides fragilis* (sensu stricto) members of the *B. fragilis* group can be reduced, this system holds promise as a reliable standardized alternative for the identification of anaerobic bacteria in clinical laboratories.**

The accepted reference method for the identification of obligate anaerobic bacteria of medical importance (4) is both time-consuming and costly. It has been 14 years since the report by Guillermet et al. (2) indicated the usefulness of rapid enzyme detection (API ZYM; API System SA, Montalieu Vercieu, France) in the differentiation of bacteria of the genera *Bacteroides* and *Fusobacterium*. Further studies by this methodology followed (1, 3, 7, 8, 14). Enzyme activities demonstrated by the same procedure were included in the descriptions of new *Bacteroides* species proposed by Shah and Collins (11). A more recent report (9) assessed the potential usefulness of the API ZYM system for identification of clinically encountered anaerobes. The results were encouraging, although a later study (16) indicated the advantages of incorporating additional enzymes to achieve better differentiation among certain species. No data base was available from the manufacturers.

Then, two rapid enzyme systems for the identification of anaerobic bacteria of clinical relevance were commercialized (the RapID-ANA system [Innovative Diagnostic Systems Inc., Atlanta, Ga.] and the AN-Ident system [Analytab Products, Plainview, N.Y.]).

A new product for the identification of clinical isolates of anaerobic bacteria, based upon experience with the API ZYM system, began to be evaluated (10, 15; R. A. Nash, 5th Int. Symp. Rapid Methods Automation Microbiol. Immunol., Florence, Italy, 1987). The prototype system comprised 32 tests and required only 4 h of incubation in room air. The prototype was refined to produce the commercially available ATB 32 A system (API System SA), which consists of 29 tests. Twenty-four tests are used for the manual system, giving an eight-digit code. All 29 tests are used in the computerized system to give a 10-digit code. When the differentiation of two or more species remains uncertain, supplementary tests may be indicated; the manufacturer lists 19 of these. The data base consists of 80 taxa of anaerobic bacteria.

The purpose of this study was to evaluate the accuracy and ease of use of the ATB 32 A system in a routine diagnostic laboratory by using visual reading and interpretation of the results.

(A part of this study was presented at a poster session of the Third European Congress on Anaerobic Bacteria and Infections, Munich, Federal Republic of Germany, 8 to 10 March 1989.)

## MATERIALS AND METHODS

**Bacterial strains.** A total of 260 anaerobic bacteria of clinical significance, comprising 56 species of 10 genera, were tested. The strains were isolated in the 12 months preceding the study, most at the Lausanne University Hospital (Centre Hospitalier Universitaire Vaudois [CHUV]); the remainder were isolated at the Microbiology Institute of the Canton of Neuchâtel (at La Chaux-de-Fonds). The bacteria were maintained at −80°C in a peptone-yeast glycerol broth (85:15 [vol/vol]). A number of fresh isolates were included. The following 21 reference strains were tested at the beginning and at the end of the study: *Actinomyces israelii* ATCC 10048, *Actinomyces odontolyticus* ATCC 17929,* *Bacteroides asaccharolyticus* ATCC 25260, *Bacteroides bivius* ATCC 29303, *Bacteroides fragilis* ATCC 25285, *Bacteroides melaninogenicus* ATCC 25845, *Bacteroides ovatus* ATCC 8483, *Bacteroides thetaiotaomicron* ATCC 27941, *Bacteroides vulgatus* ATCC 8482,* *Clostridium barati* DMS 601,* *Clostridium difficile* ATCC 9689, *Clostridium histolyticum* ATCC 1940,* *Clostridium perfrin-*

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*gens* ATCC 13124, *Clostridium ramosum* ATCC 25582, *Clostridium sordellii* ATCC 9714,* *Fusobacterium nucleatum* ATCC 10953, *Fusobacterium mortiferum* ATCC 25557, *Lactobacillus acidophilus* ATCC 11506, *Peptostreptococcus anaerobius* ATCC 27337, *Peptostreptococcus magnus* ATCC 29328, and *Propionibacterium acnes* ATCC 6919. The five strains marked with an asterisk represent quality-control strains recommended by the manufacturer of the ATB 32 A system. All strains were subcultured a minimum of three times on supplemented (vitamin $K_1$, 10 μg/ml; hemin, 5 μg/ml) Columbia sheep blood (5%) agar before testing.

**Reference identification method.** The clinical isolates were identified by a combination of established methods (4, 13), including gas-liquid chromatography. *Bergey's Manual of Systematic Bacteriology* (5, 6) was considered to be the definitive taxonomic source for this study.

**ATB 32 A system.** The ATB 32 A system allows 29 biochemical reactions to be performed in a miniaturized disposable plastic strip. The dehydrated substrates for the tests are incorporated in the strip cupules. The following 24 tests are included in the strip and are used to produce an eight-digit code: urease (URE), arginine dihydrolase (ADH), α-galactosidase (α-Gal), β-galactosidase (β-Gal), β-galactosidase-6-phosphate (β-GP), α-glucosidase (α-Glu), β-glucosidase (β-Glu), α-arabinosidase (α-Ara), β-glucuronidase (β-Gur), β-*N*-acetyl-glucosaminidase (β-NAG), mannose acidification (MNE), raffinose acidification (RAF), nitrate reduction (NIT), indole production (IND), alkaline phosphatase (PAL), arginine arylamidase (Arg-A), proline arylamidase (Pro-A), leucyl-glycine arylamidase (LGA), phenylalanine arylamidase (Phe-A), leucine arylamidase (Leu-A), pyroglutamic acid arylamidase (Pyr-A), tyrosine arylamidase (Tyr-A), alanine arylamidase (Ala-A), and glycine arylamidase (Gly-A). The eight-digit profile can be looked up in the code book provided by the manufacturer. Five additional tests that serve to complete the 8-digit code to give a 10-digit code are also included: glutamic acid decarboxylase (GDC), α-fucosidase (α-Fuc), histidine arylamidase (His-A), glutamyl glutamic acid arylamidase (GGA), and serine arylamidase (Ser-A). The 10-digit code is for use with the computerized data base.

A fresh subculture (not more than 48 h old) of the anaerobe grown on Columbia sheep blood agar was used to prepare, in 2 ml of sterile distilled water, a suspension with a turbidity equal to that of a McFarland no. 4 standard. All cupules in the strip were inoculated with approximately 50 to 60 μl of suspension, which was accomplished with the aid of plastic disposable pipettes. The normal anaerobic and aerobic purity and aerotolerance subcultures were made from the same suspension. Following inoculation, a drop of mineral oil was added to the URE cupule. The plastic cover provided with the system was placed over the strip, and the whole strip was then incubated in room air at 37°C for 4 h.

Following incubation, reagents were added to the NIT, IND, and PAL through Ser-A wells listed above. After 5 min the resulting colors of each cupule were compared with the color chart provided with the system. Colors were read with the transparent strips placed on a well-lit, glossy white background. The color chart has a scale with four values, ranging from negative, 0, through doubtful negative, 1, and doubtful positive, 2, to positive, 3, for each reaction, which allows a relative quantification of enzyme activity. At this stage the results were coded into an eight-digit number which was matched with those in the Analytical Profile Index intended for use with the ATB 32 A system. Occasionally, additional tests (Gram stain; microscopic morphology; presence and location of spores; motility; coagulase, catalase, lecithinase, and lipase; hydrolysis of gelatin, esculin, starch, and hippurate; β-hemolysis; and acid from arabinose, fructose, glucose, maltose, ribose, and trehalose) were indicated to complete the identification.

Codes which were not found in the Analytical Profile Index were reconsidered with regard to tests giving uncertain, i.e., 1 or 2, values (see above). These values were reinterpreted, and the resulting code(s) was matched with those in the Analytical Profile Index. When more than one species was indicated by these codes, basic factors such as morphology and reaction to Gram stain were applied to eliminate nonvalid possibilities. Eight-digit codes which still did not give an identification were referred as 10-digit codes to the software provided by the manufacturer.

**Organization of the study.** The reference strains and frozen clinical isolates (previously identified by established methods) were recovered in the Anaerobe Laboratory (CHUV) and checked for purity. The bacteria were then subcultured onto Columbia sheep blood agar and sent in evacuation-replacement anaerobic jars by express post (journey time, not in excess of 4 h) to La Chaux-de-Fonds. All the Columbia sheep blood agar plates required for ATB 32 A testing were sent to La Chaux-de-Fonds at the same time to ensure the use of a uniform medium. Columbia sheep blood agar was used in the prereduced state. The additional tests required to complete certain of the ATB 32 A identifications were performed in the Anaerobe Laboratory (CHUV), with the exception of Gram staining, hemolysis, and catalase testing. Testing for catalase was performed by adding a drop of a 15% (vol/vol) hydrogen peroxide solution to a blank, inoculated cupule in the strip.

**Calculation of the separation index.** Calculation of the separation index (12) was performed as follows. For a given test the number of taxa that had a percentage of positive reactions greater than 85 was equal to $P$; less than 15 was equal to $N$. The separation index for a given test was equal to $P \times N$. The higher the value of the separation index, the more discriminatory the test.

## RESULTS

All 21 reference strains were correctly identified by the ATB 32 A system. These strains, which are commercially available, are no definitive test for the system.

The identification results of the clinical isolates are given in Table 1. For 17 of the strains, the genus was correctly identified but the wrong species was designated by the ATB 32 A system. The details of these identifications are given in Table 2. These identifications are not considered to be totally false, as it is often not possible to arrive at more than a genus identification when working with anaerobic bacteria in a medical microbiological context, i.e., with restrictions on time and facilities available to provide clinically useful information. A final group of 13 bacteria, for which there was either a false genus identification or no identification, are also included in Table 2.

In order to assess the ease of reading the results of any particular test, the enzyme activities of the 260 bacteria, as scored from the color chart, were grouped as clear-cut or doubtful and are presented in Table 3 in order of decreasing value of their separation indices. Those tests with the highest separation indices provide the greater part of the differentiating ability of the system. It is preferable that they give clear results.

TABLE 1. Comparative identification of 260 clinical isolates of anaerobic bacteria

| Strain | No. tested | No. of clinical isolates | | | | | |
|---|---|---|---|---|---|---|---|
| | | Identified to species level | | Identified to genus level | | Not identified | Incorrectly identified |
| | | Additional tests | | Additional tests | | | |
| | | Without | With | Without | With | | |
| *Actinomyces israelii* | 1 | 1 | | | | | |
| *Actinomyces odontolyticus* | 2 | 2 | | | | | |
| *Actinomyces meyeri* | 1 | 1 | | | | | |
| *Bacteroides asaccharolyticus* | 2 | 2 | | | | | |
| *Bacteroides bivius* | 6 | 6 | | | | | |
| *Bacteroides buccae* | 4 | 3 | 1 | | | | |
| *Bacteroides caccae* | 3 | 3 | | | | | |
| *Bacteroides denticola* | 1 | 1 | | | | | |
| *Bacteroides distasonis* | 4 | 1 | 2 | 1 | | | |
| *Bacteroides fragilis* | 51 | 49 | 1 | | 1 | | |
| *Bacteroides intermedius* | 6 | 6 | | | | | |
| *Bacteroides levii* | 1 | 1 | | | | | |
| *Bacteroides melaninogenicus* | 4 | 3 | 1 | | | | |
| *Bacteroides oris* | 1 | | | | 1 | | |
| *Bacteroides ovatus* | 5 | 1 | 4 | | | | |
| *Bacteroides splanchnicus* | 1 | | | | | 1 | |
| *Bacteroides stercoris* | 1 | 1 | | | | | |
| *Bacteroides thetaiotaomicron* | 14 | 10 | | 4 | | | |
| *Bacteroides uniformis* | 5 | | 3 | 1 | 1 | | |
| *Bacteroides ureolyticus* | 4 | 4 | | | | | |
| *Bacteroides vulgatus* | 5 | 4 | | 1 | | | |
| *Bifidobacterium adolescentis* | 1 | 1 | | | | | |
| *Clostridium absonum* | 1 | | | 1 | | | |
| *Clostridium beijerinckii/butyricum* | 6 | 6 | | | | | |
| *Clostridium bifermentans* | 2 | 2 | | | | | |
| *Clostridium botulinum* type A | 1 | | 1 | | | | |
| *Clostridium clostridiiforme* | 2 | | | | 1 | 1 | |
| *Clostridium coccoides* | 1 | | | 1 | | | |
| *Clostridium difficile* | 10 | | 10 | | | | |
| *Clostridium glycolicum* | 2 | 1 | | | | 1 | |
| *Clostridium histolyticum* | 2 | 2 | | | | | |
| *Clostridium innocuum* | 4 | 2 | 2 | | | | |
| *Clostridium paraputrificum* | 2 | | 2 | | | | |
| *Clostridium perfringens* | 16 | 16 | | | | | |
| *Clostridium ramosum* | 6 | 2 | 4 | | | | |
| *Clostridium scatologenes* | 1 | | | | 1 | | |
| *Clostridium sordellii* | 2 | 2 | | | | | |
| *Clostridium sporogenes* | 4 | 2 | | | 2 | | |
| *Clostridium subterminale* | 4 | 1 | 1 | | 1 | | 1 |
| *Clostridium tertium* | 4 | 3 | 1 | | | | |
| *Clostridium tetani* | 1 | 1 | | | | | |
| *Eubacterium lentum* | 3 | 1 | | | | | 2 |
| *Fusobacterium mortiferum* | 2 | 2 | | | | | |
| *Fusobacterium necrophorum* | 3 | | 3 | | | | |
| *Fusobacterium nucleatum* | 14 | | 14 | | | | |
| *Fusobacterium varium* | 3 | | 2 | | | | 1 |
| *Lactobacillus jensenii* | 3 | | | | | 3 | |
| *Peptostreptococcus anaerobius* | 7 | 6 | | | | 1 | |
| *Peptostreptococcus asaccharolyticus* | 7 | 6 | | | | 1 | |
| *Peptostreptococcus magnus* | 6 | 6 | | | | | |
| *Peptostreptococcus micros* | 4 | 4 | | | | | |
| *Propionibacterium acnes* | 10 | 10 | | | | | |
| *Propionibacterium* spp. | 1 | | | | | 1 | |
| *Veillonella* spp. | 3 | 2 | 1 | | | | |

TABLE 2. Strains giving discrepant results between the ATB 32 A and the conventional identification methods

| Conventional identification | No. of strains[a] | ATB 32 A identification[b] | Reasons for discrepancy[c] |
|---|---|---|---|
| *Bacteroides distasonis* | 1 | *Bacteroides uniformis* (70.1, 0.58) | False-negative Arg-A, Phe-A, Leu-A, Pyr-A, and Gly-A |
| *Bacteroides fragilis* | 1 | *Bacteroides ovatus* (76, 0.74) | False-positive α-ARA; false-negative Arg-A, Leu-A, and Tyr-A |
| *Bacteroides oris* | 1 | *Bacteroides uniformis* (70.1, 0.58) | *Bacteroides oris* not in data base |
| *Bacteroides splanchnicus* | 1 | | *Bacteroides splanchnicus* not in data base |
| *Bacteroides thetaiotaomicron* | 2 | *Bacteroides ovatus* (87.2, 0.85) | Weak Arg-A and Gly-A |
| *Bacteroides thetaiotaomicron* | 1 | *Bacteroides ovatus* (81.1, 0.56) | Weak Arg-A, Gly-A, and α-ARA |
| *Bacteroides thetaiotaomicron* | 1 | *Bacteroides ovatus* (94.5, 0.26) | False-negative Arg-A, Gly-A, Leu-A, and IND |
| *Bacteroides uniformis* | 1 | *Bacteroides fragilis* (99.2, 0.45) | False-positive Arg-A and Leu-A; false-negative α-GLU, β-GLU, α-ARA, and IND |
| *Bacteroides uniformis* | 1 | *Bacteroides buccae* (91.5, 0.62) | Weak α-FUC and GDC; false-negative IND |
| *Bacteroides vulgatus* | 1 | *Bacteroides fragilis* (90.3, 0.57) | False-negative α-ARA and Gly-A |
| *Clostridium absonum* | 1 | *Clostridium perfringens* (90.2, 0.34) | *Clostridium absonum* not in data base |
| *Clostridium clostridiiforme* | 1 | *Clostridium butyricum* (99.6, 0.66) | Weak β-GUR; false-negative LGA |
| *Clostridium clostridiiforme* | 1 | Unacceptable profile | False-positive IND; false-negative β-GUR, and LGA |
| *Clostridium coccoides* | 1 | *Clostridium sporogenes* (81.6, 0.92) | *Clostridium coccoides* not in data base |
| *Clostridium glycolicum* | 1 | Unacceptable profile | False-positive α-GAL and β-GLU |
| *Clostridium scatologenes* | 1 | *Clostridium histolyticum* (34.9, 0.49) | *Clostridium scatologenes* not in data base |
| *Clostridium sporogenes* | 1 | *Clostridium difficile* (71.5, 0.95) | Weak ADH; false-negative Pyr-A |
| *Clostridium sporogenes* | 1 | *Clostridium bifermentans* (22, 0.94) | False-negative ADH and Pyr-A |
| *Clostridium subterminale* | 1 | *Clostridium histolyticum* (72.6, 0.99) | False-negative LGA, Leu-A, Tyr-A, Ala-A, and Gly-A |
| *Clostridium subterminale* | 1 | *Fusobacterium varium* (22.9, 0.84) | False-negative LGA, Leu-A, Phe-A, Tyr-A, Ala-A, and Gly-A |
| *Eubacterium lentum* | 2 | *Actinomyces odontolyticus* (93.2, 0.43) | False-positive Pro-A, Tyr-A, Gly-A, Ala-A |
| *Fusobacterium varium* | 1 | *Clostridium tetani* (93.7, 0.99) | False-positive PAL; weak Pyr-A; and false-negative IND |
| *Lactobacillus jensenii* | 3 | | *Lactobacillus jensenii* not in data base |
| *Peptostreptococcus anaerobius* | 1 | Unreliable identification | False-positive β-NAG and MNE; false-negative Pro-A |
| *Peptostreptococcus asaccharolyticus* | 1 | Unacceptable profile | False-positive URE and MNE; weak Arg-A; false-negative Leu-A and Tyr-A |
| *Propionibacterium* spp. | 1 | Unacceptable profile | False-positive α-GLU; weak β-NAG; false-negative NIT |

[a] Number of strains having a specific ATB 32 A index profile.
[b] Values in parentheses are percentages of identification, *T*.
[c] False positive, 2 or 3 color intensity value for a normally negative reaction; weak, color intensity value for a normally positive reaction; false negative, 0 color intensity value for a normally positive reaction. See text for abbreviation definitions.

Attempts were made to use the percentage of identification and the *T* index (a value included with the corresponding identification of each ATB 32 A code which is a measure of a profile's proximity to the most typical profile in each of the taxa) to differentiate between those identifications which were correct and those which were incorrect. The most satisfactory results were obtained by considering the product of the percentage of identification and the *T* index, i.e., percentage of identification × *T*. For those bacteria which were correctly identified to the species level, the mean of the values of the product of percentage of identification × *T* was 80.39 with a standard deviation of 19.58. For those bacteria which were only correctly identified to the genus level or which were incorrectly identified, the mean of the product of percentage of identification × *T* was 50.48 with a standard deviation of 21.20.

## DISCUSSION

As stated by Allen (S. D. Allen, Clin. Microbiol. Newsl. 6:147–149, 1984), diagnostic laboratories are increasingly being scrutinized to determine whether they are cost effective. However, he also states the opinion that the most economic treatment of patients is that which is based upon a firmly established diagnosis. The current direction of medical anaerobic bacteriology is the production of more rapid and less expensive results.

The ATB 32 A system offers a number of advantages at the price of certain restrictions. A major advantage is that it allows identifications to be made without requiring gas-liquid chromatography; however, a specific medium (Columbia sheep blood agar enriched with hemin and vitamin K) must be used to grow the bacteria that are used to inoculate the test strips.

The strip takes approximately the same amount of time to inoculate as most other commercially available identification systems do. Furthermore, with the exception of *B. ureolyticus*, all the strips could be inoculated from only one plate of bacterial growth incubated for not more than 48 h. Those identifications not requiring additional tests are obtained in less than 5 h, once a suitable inoculum is available, and incubation is in room air. Even though most of the more commonly encountered bacteria required few, if any, additional tests for their identifications to be complete by the ATB 32 A system, each of the additional tests, with the

TABLE 3. Interpretation of enzyme activities for the 260 clinically isolated bacteria

| Enzyme[a] | Separation index | % Clear-cut interpretation[b] | % Doubtful interpretation[c] |
|---|---|---|---|
| PAL | 1,440 | 93.7 | 6.3 |
| Ala-A | 1,326 | 90.3 | 9.7 |
| β-Gal | 1,248 | 92.1 | 7.9 |
| β-NAG | 1,215 | 92.9 | 7.1 |
| α-Gal | 1,200 | 95.6 | 4.4 |
| α-Glu | 1,156 | 87.3 | 12.7 |
| LGA | 1,148 | 63.2 | 36.8 |
| Pro-A | 1,083 | 88.1 | 11.9 |
| Arg-A | 1,081 | 57.7 | 42.3 |
| β-Glu | 989 | 73.6 | 26.4 |
| IND | 976 | 91.0 | 9.0 |
| GGA | 954 | 57.6 | 42.4 |
| α-Fuc | 944 | 88.6 | 11.4 |
| Gly-A | 884 | 73.2 | 26.8 |
| RAF | 850 | 86.6 | 13.4 |
| Leu-A | 828 | 58.6 | 41.4 |
| MNE | 828 | 88.2 | 11.8 |
| Tyr-A | 767 | 64.3 | 35.7 |
| His-A | 741 | 57.5 | 42.5 |
| Phe-A | 704 | 67.4 | 32.6 |
| β-GP | 660 | 92.2 | 7.8 |
| α-Ara | 650 | 90.2 | 9.8 |
| Pyr-A | 605 | 81.3 | 18.7 |
| GDC | 406 | 55.5 | 44.5 |
| NIT | 350 | 85.6 | 14.4 |
| Ser-A | 330 | 83.1 | 16.9 |
| URE | 296 | 100 | |
| ADH | 252 | 94.4 | 5.6 |
| β-Gur | 216 | 96.5 | 3.5 |

[a] Tests are arranged in decreasing order of separation index values. See text for abbreviation definitions.
[b] Color intensity grade 0 or 3.
[c] Color intensity grade 1 or 2.

exception of hippurate hydrolysis, had to be used on at least one occasion. These factors, together with the standardization made possible by using a specific medium to grow the inoculum, combine to give an easy-to-use, efficacious identification method.

A feature of the system borrowed from its predecessor, the API ZYM system, is the quantification of enzyme detected. The colors of each test end product are compared with a reference chart. Some previous systems had only written descriptions of the colors of positive and negative tests, and interpretation of these tests was not always very clear. Reading of the ATB 32 A system visually is not entirely without difficulty, however. Table 3 indicates the relative number of occurrences of doubtful reactions. According to the manufacturer, by using the on-line data base, one can key in the results for doubtful tests as "?," leaving the software to work out the identification without taking the reaction(s) into account.

One way in which the interpretation of the tests can be made easier is to use internal comparison; i.e., for the glycolytic group and then for the proteolytic group of tests, choose a clear positive 3 and a clear negative 0 within each group and then compare the tests remaining in each group with these standards. Thus, any tendencies to over- or underestimate enzyme activities as a result of variation in inoculum, for example, are reduced. By considering the mean of percent doubtful interpretations (Table 3) for the glycolytic (13.4%) and proteolytic (29.8%) groups of enzymes, it can be seen that the proteolytic group is more difficult to interpret.

False-positive or false-negative enzyme activities, resulting in incorrect identifications, were equally divided between those enzymes that gave clear-cut results and those with a relatively high proportion of doubtful reactions. The information in Table 3 may aid in the correction of errors in the reading of test colors. Because of the large overlap of the standard deviations obtained from the percentage of identification $\times$ $T$ calculation, this estimation was not always helpful as an indication of the correctness of an identification.

Among the most commonly isolated anaerobic bacteria of clinical importance are those *Bacteroides* spp. belonging to the *B. fragilis* group, *C. perfringens*, and *C. difficile*.

Of the 88 strains belonging to the *B. fragilis* group, 88.7% were correctly identified to the species level; the remaining 11.3% were identified to the genus level (Table 2). However, of this 11.3%, all but one of the species identifications (*B. uniformis* versus *B. buccae*) were within the *B. fragilis* group. On four occasions, the ATB 32 A system identified *B. thetaiotaomicron* as *B. ovatus*. These bacteria are not too easily distinguished by conventional methods, but the ATB 32 A system has three enzymes which serve to differentiate them. The recurrent nature of this error, largely because of false-negative Gly-A and Arg-A tests, is thought provoking; this "group" (*B. thetaiotaomicron-B. ovatus*) may be more heterogeneous than previously thought. It is of interest that the percentage of identification $\times$ $T$ values for these four strains (45, 24, 74, and 74) were not particularly high. Although the ATB 32 A system can identify organisms to the group *B. fragilis* level well, its ability to identify organisms within the group to the species level is not yet as good as it could be.

All 16 strains of *C. perfringens* were correctly identified without the need for additional tests. In contrast, although the 10 strains of *C. difficile* were all identified correctly, additional testing (lecithinase) was required for each strain; this extended by 48 h the time required to identify these bacteria.

Because of the relatively frequent association of propionibacteria with contamination in certain specimens, but also bearing in mind that these bacteria may cause or play a role in genuine disease processes, it is important to be able to identify them. Of 11 *Propionibacterium* strains, the ATB 32 A system correctly identified 10 (90.9%) of them to the species level.

From the information presented in Table 2, it can be seen that of the four strains incorrectly identified to the genus level, two of the false identifications could have been detected with knowledge of the composition (Gram stain reaction, 3% KOH string test, vancomycin-colistin susceptibility) of the bacterial cell wall. The importance of a certain familiarity with anaerobic bacteria when using the ATB 32 A system is shown by the *F. varium* strain that gave an enzymatic profile typical of that of *C. tetani*.

The overall performance of the ATB 32 A system in this study indicates that, as a new system, initial results are very promising and already compare well with those of alternative commercialized systems (P. C. Appelbaum, Clin. Microbiol. Newsl. **11**:89–93, 1989) which have been available for many years. Expansion of the data base with regard to certain species not represented and others for which identification is not yet entirely satisfactory seems warranted. With such improvements the ATB 32 A system could become a fast, reliable alternative method, as it is standardized and comparatively inexpensive, for the identification of the anaerobic bacteria normally associated with clinical specimens.

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