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Comparison of API and Minitek to Center for Disease Control Methods for the Biochemical Characterization of Anaerobes

SHARON L. HANSEN* AND BARBARA J. STEWART

Laboratory Service, Veterans Administration Hospital, Baltimore, Maryland 21218

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Two commercially available micromethod multitest systems (API, Analytab Products, Inc., Minitek-Bioquest) were compared with conventional tests suggested by the Center for Disease Control for the identification of anaerobes. Anaerobiosis for the microsystems was achieved using the GasPak system (BBL). A total of 175 anaerobes, including 158 clinical isolates and 17 reference strains, were used. Gram morphology, gas-liquid chromatography data, and biochemical reactions from the Center for Disease Control and Virginia Polytechnic Institute anaerobic manuals were used to identify the organisms. The Minitek system included a new anaerobe inoculum broth and two new disks, dextrose without nitrate and nitrate reductase disks. The percentage of correlation of 12 biochemicals using Minitek and 11 biochemicals using the API were compared with the Center for Disease Control reactions. The percentage of correlation of both positive and negative reactions with the API anaerobic strip ranged from 70.8 to 99.4% and with the Minitek from 97.1 to 100%. The microsystems were also evaluated as to the ease of use, adaptability to a clinical laboratory, time, and cost.

There are currently many microsystems available commercially for the identification of *Enterobacteriaceae*. Recently, two such systems have become available to determine the biochemical reactions of anaerobes. Starr et al. (8) and Moore et al. (6) compared the API system (Analytab Products, Inc.) to the Center for Disease Control (CDC) methods and Virginia Polytechnic Institute (VPI) and CDC methods, respectively. Using the Minitek system, Stargell et al. (7) and McCarthy et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C15, p. 28) compared it to the CDC and VPI methods.

To clinical laboratories faced with increased work loads, rising costs, and inadequate storage space, microsystems that perform accurately, yield results that are reproducible, are time and space saving, and are inexpensive offer an excellent alternative to the conventional methods used.

Anaerobic isolation and identification is an essential part of a clinical microbiology laboratory. The infectious role these organisms play is well documented (3, 4). Several anaerobic systems are currently available, though the quality of anaerobic techniques varies in different laboratories. Excellent reference manuals are available detailing the necessary procedures for isolation and identification of anaerobes (2, 5, 10).

In an effort to determine the efficacy of changing to a more rapid system, we compared the API and Minitek systems with the CDC methods used in this laboratory.

MATERIALS AND METHODS

Bacterial strains. The 158 clinical isolates used in this study represent all anaerobes isolated in this laboratory during a 2-month period. The 17 reference strains represent organisms seldom encountered during this study and were tested at the end of the study.

Conventional system. Conventional fermentation media included dextrose, mannitol, lactose, sucrose, maltose, salicin, glycerol, xylose, and arabinose. Indol, nitrate, and esculin were also used. All substrates were prepared according to Dowell and Hawkins (2).

Microsystems. The API anaerobe system consists of a strip of 20 microtubules containing dehydrated substrates, ampoules of anaerobe suspension medium, inoculation trays, and covers. Eleven of the 21 tests were used; nitrate is not available on the strip.

The Minitek anaerobe system (BBL) includes a substrate dispenser, disposable plate and lid, pipette, anaerobe inoculum broth, and a choice of 35 available paper disk substrates. The 12 substrates used were the same as the conventional substrates; included were new dextrose without nitrate and nitrate reductase disks.

Procedure. Clinical specimens appropriate for anaerobic culture were plated on sheep blood agar, phenylethyl alcohol blood agar, Schaedler agar with vitamin K and laked sheep blood, and Schaedler broth with vitamin K. Plates were incubated for a

minimum of 48 h in the GasPak jar. With the aid of a stereoscopic microscope, colony morphology was determined, and each colony type was Gram stained and plated to a blood agar plate incubated under CO_2, and a Schaedler plate was incubated anaerobically. After appropriate anaerobic growth, pure cultures of the anaerobic organisms were inoculated into conventional media following CDC recommendations. The microsystems were inoculated as suggested by the manufacturers' instruction sheets. The turbidity of the inoculum broth for the API system was increased after 20 tests to approximate a McFarland no. 5 turbidity standard. Colony morphology, Gram stain, and slide catalase were performed on each isolate at the time of biochemical inoculation. A Schaedler broth tube supplemented with vitamin K was inoculated and used for gas-liquid chromatography (GLC) determination of the end products of metabolism by the procedure outlined in the VPI manual (5).

Conventional tests for indole, nitrate, and esculin were performed after appropriate growth, but no sooner than 48 h. Fermentation readings were made after incubation for 48 h and 7 days, and at 14 days on tubes that were negative at 7 days. Spot plates using bromothymol blue were done on each fermentation tube for each of the fermentation readings. Identification of the organisms was arrived at by using the reaction data from the conventional media, GLC end products of metabolism, Gram reaction, catalase, and colony morphology. Results of these procedures were compared to the expected reactions listed by Dowell and Hawkins (2), Holdeman and Moore (5), and Sutter et al. (10).

Anaerobiosis was obtained for both microsystems using the GasPak jar. The GasPak 100 jar was used to incubate up to 14 Minitek plates and the GasPak 150 jar to incubate up to 16 API strips at a time.

The Minitek plates were read after 48 h of incubation and occasionally after 72 h of incubation. The indole reaction was determined in a well containing three portions (0.15 ml) of the inoculum broth. Kovac reagent was added directly to the well. Xylene extraction was not done. A pink to red color indicated a positive test. Nitrate reagents were added to the nitrate disk and, if negative, zinc dust was added to the well for confirmation. Fermentation reactions were recorded before and after a drop of 0.25% (wt/vol) phenol red, pH 7.2, was added. Only a clearly yellow color was considered positive; any shade of orange or red was negative. The esculin reaction was considered positive if a brown or brown-black color developed. The esculin disk remained off-white if negative. If no positive reactions were present, a viability check was done from the well that contained the inoculum broth (before performing the indole reaction) by transferring a loopful of the broth to a Schaedler plate and incubating it anaerobically.

The API strips were read after 48 h of incubation and after 72 h of incubation when there were no reactions observed after 48 h. The fermentation reactions were considered positive if a yellow/yellow-green color was present. Reactions were recorded before and after a drop of 0.02% bromocresol purple aqueous solution was added to each fermentation microtube. Esculin hydrolysis was determined by using an ultraviolet light (366 nm) and checking for fluorescence. If there was no fluorescence, esculin hydrolysis was positive, and, if there was fluorescence, esculin hydrolysis was negative. The indole test was performed last; extraction with xylene was not performed. Kovac reagent was added to the indole tube and a pink to red color was positive. Catalase was performed after exposure to air for at least 30 min by adding two drops of 3% H_2O_2 to one of the microtubules.

Results of the three methods were recorded on separate sheets, and then the microsystems reactions were compared to the conventional method.

RESULTS

Table 1 lists the clinical isolates identified during this study and includes the 17 reference strains. The reference strains represent gram-positive sporulating and nonsporulating bacilli not, or seldom, encountered during the study. The high incidence of gram-negative nonsporulating rods and anaerobic cocci compared with the low incidence of gram-positive sporulating organisms no doubt reflects our patient population and the type of lesion. *Clostridium chauvoei* listed in Table 1 was a clinical isolate; however, it may well be a lecithinase-negative, glycerol-negative *Clostridium perfringens*.

Table 2 summarizes the percentage of correlation for total reactions with the 175 organisms tested. The overall agreement for the 11 substrates with the API system was 88.0%, and with the Minitek the overall percentage of agreement for the 12 substrates was 98.9%.

The majority of discrepancies with either microsystem were false-negative reactions when compared with the conventional methods. Table 3 shows the number of positive reactions of the 175 isolates for each substrate using conventional media and the results obtained with the microsystems.

An inoculum adjustment was made after the first 20 tests with API as suggested by Moore et al. (6). Allowing for adaptation to the micromethods as well as the need to increase the inoculum size using the API, Table 4 shows the results of positive reaction correlation eliminating the first 20 organisms tested. There were no discrepancies with the Minitek system on the last 155 organisms tested. The majority of discrepancies with the API were the result of false-negative reactions.

DISCUSSION

The overall agreement of the API system to the conventional method was 88.0%. Lactose, sucrose, maltose, and xylose gave less than 85%

TABLE 1. *Anaerobes tested representing 158 fresh clinical isolates and 17 reference strains*

Organism	No. of isolates
Bacteroides	
B. fragilis	22
B. melaninogenicus	10
B. oralis	10
B. capillosus	7
B. furcosus	2
B. biacutus	2
B. clostridiiformis	3
Bacteroides sp.	6
Fusobacterium	
F. mortiferum	3
F. nucleatum	1
F. russii	1
F. bullosum	1
Fusobacterium sp.	2
Cocci	
Peptostreptococcus anaerobius	14
P. productus	15
Peptococcus asacchyrolyticus	3
P. sacchyrolyticus	5
P. constellatus	2
P. morbillorum	1
Peptococcus sp.	11
Sarcina ventriculi	3
Gaffkya anaerobia	2
Unspeciated positive cocci	17
Veillonella alcalescens	1
Gram-positive nonsporulating rods	
Actinomyces israelii[a]	1
Eubacterium lentum[b]	2
Eubacterium limosum[a]	1
Bifidobacterium eriksonii[a]	1
Proprionibacterium acnes[b]	9
Clostridia	
C. sphenoides[b]	4
C. tetani[a]	5
C. ramosum[a]	2
C. perfringens[b]	2
C. sordelli[a]	1
C. acetobutylicum[a]	1
C. bifermentans[a]	1
C. chauvoei	1

[a] Represents American Type Culture Collection or CDC reference strains.
[b] One reference strain included.

agreement for both positive and negative reactions. The very low correlation with positive reactions using the API system for 175 organisms (Table 3), as well as the low correlation of positive reactions on the last 155 organisms (Table 4) for lactose, sucrose, maltose, salicin, and xylose, suggests that the results were not dependent on procedural manipulation, but rather on these particular substrates. It was our experience that these substrates consistently did not agree with the conventional method. The discrepancies did not occur with a particular genus, but were observed with organisms representing many genera that gave positive reactions in the conventional media. Discrepancies with these same substrates also occurred with the reference strains and should have been positive as outlined in the API reaction chart supplied with the instructions. The agreement for dextrose, mannitol, glycerol, and arabinose improved after the inoculum was adjusted to a McFarland no. 5 standard. Esculin

TABLE 2. *Tests in agreement and percentage of correlation with conventional reactions for 175 organisms*

Substrate	System			
	API		Minitek	
	No. in agreement	%	No. in agreement	%
Dextrose	159	90.8	173	98.8
Mannitol	163	95.4	173	98.8
Lactose	129	73.7	174	99.4
Sucrose	124	70.8	171	97.7
Maltose	138	78.9	174	99.4
Salicin	155	88.6	172	98.3
Glycerol	168	96.0	174	99.4
Xylose	148	84.6	170	97.1
Arabinose	162	92.6	170	97.1
Esculin	173	98.8	175	100.0
Nitrate	NA[a]		175	100.0
Indole	174	99.4	175	100.0
Overall agreement	1,693 (1,925)[b]	88.0	2,076 (2,100)[b]	98.9

[a] NA, Not available.
[b] Total number of tests.

TABLE 3. *Tests in agreement and percentage of correlation with positive reactions in conventional media for 175 organisms*

Substrate	No. positive in conventional media	API		Minitek	
		No. in agreement	%	No. in agreement	%
Dextrose	162	146	90.1	161	99.4
Mannitol	38	29	78.4	37	97.4
Lactose	100	55	55.0	100	100.0
Sucrose	103	53	51.4	102	99.1
Maltose	107	73	68.2	107	100.0
Salicin	41	21	51.2	39	95.1
Glycerol	43	36	83.7	42	97.7
Xylose	62	38	61.3	59	95.2
Arabinose	52	39	75.0	48	92.3
Esculin	102	101	99.0	102	100.0
Nitrate	50	NA[a]		50	100.0
Indole	42	41	97.6	42	100.0
Overall agreement		632 (852)[b]	74.2	889 (902)[b]	98.6

[a] NA, Not available.
[b] Total number of positives.

TABLE 4. *Tests in agreement and percent correlation with positive reactions in conventional media with last 155 organisms tested*

Substrate	No. positive in conventional media	API		Minitek	
		No. in agreement	%	No. in agreement	%
Dextrose	143	139	97.2	143	100.0
Mannitol	31	27	87.1	31	100.0
Lactose	88	43	48.9	88	100.0
Sucrose	95	52	59.7	95	100.0
Maltose	93	67	72.0	93	100.0
Salicin	34	22	64.7	34	100.0
Glycerol	40	36	90.0	40	100.0
Xylose	54	37	68.5	54	100.0
Arabinose	44	37	84.1	44	100.0
Esculin	91	90	98.9	91	100.0
Nitrate	46	NA[a]		46	100.0
Indole	38	37	97.4	38	100.0
Overall agreement		587 (751)[b]	78.2	797 (797)[b]	100.0

[a] NA, Not available.
[b] Total number of positives.

and indole agreement essentially remained the same before and after the adjustment.

With the API system, we chose not to use the small GasPak jar because it required cutting off nearly one-third of the GasPak. The larger jar could be adapted to hold 16 API strips, and the GasPak envelope could be used as recommended by the manufacturer. Though the instructions suggested observing and recording the reactions after 24 h, we found little advantage in doing so since most of the organisms studied required 48 h of incubation or longer for the reactions to occur. Reduction of the bromocresol purple indicator was not observed with any genera except the clostridia. Addition of a drop of 0.02% bromocresol purple to all fermentation microtubes greatly facilitated the interpretation of the reactions. We did not extract the indole with xylene. The 99.4% correlation with the conventional method would suggest the efficacy of using Kovac reagent alone.

We agree with the suggestions made by Moore et al. (6) that the inoculum for the API system must be heavy and should approximate a McFarland no. 4 or 5 and that readings after incubation for 3 to 4 days will give more definitive results with slow-growing organisms. A further suggestion is that viability checks be performed on all API strips that are nonreactive after a 48-h incubation.

The overall agreement to conventional methods with the Minitek anaerobe system was 98.9%. Stargell et al. (7) achieved an overall agreement with these 12 substrates of 95.1%, using the Lombard-Dowell medium as the inoculum broth. Our study used the same inoculum broth (Anaerobe inoculum broth, BBL) with two new disks—dextrose without nitrate and nitrate reductase. Correlation with these disks was 98.8 and 100%, respectively. Indole in their study was done on the H_2S/indole disk and in our study from a well that contained three portions of the inoculum broth. Correlation by our method with the conventional was 100%. The turbidity of the inoculum broth should approximate a McFarland no. 5 as stated in the instructions. For laboratories that do not have such standards prepared, a density that is turbid to the degree that printed material could not be read through it approximates the correct density. Fermentation reactions were easy to read. The addition of a drop of 0.025% phenol red, pH 7.2, facilitates interpretation of the reactions. Fermentation and the esculin reactions should be read first and then nitrate reagents should be added to the nitrate reductase disk for the nitrate test. Zinc dust should be added if the nitrate reaction is negative. Kovac reagent was added to the inoculum control well. Extraction with xylene was not done. Incubation for 72 h was not necessary with Minitek. Reactions were complete after 48 h of incubation with all organisms tested. Some reactions were apparent after 24 h of incubation, but final readings should be recorded after 48 h of incubation.

Both microsystems are more rapid, less time consuming, and easier to work with than the conventional media. With conventional methods, the time involved in preparation of the media, preboiling before use, inoculation, and time of incubation, is lengthy. Though many conventional reactions were complete within 48 h, arabinose and xylose occasionally required more than 7 days of incubation. Slower-growing anaerobes seldom gave positive reactions in 48 h with the conventional methods. All reactions in the Minitek were complete in 48 h. By using the Minitek considerable time is saved not only in manipulations but also in reporting results.

Some anaerobes grow eugonically, whereas others are dysgonic. We chose to use a Schaedler plate to obtain pure colony growth of the anaerobes because the growth was more luxuriant than on a blood agar plate. The inoculum size to achieve proper density for the Minitek is approximately one-fourth that needed for the API because the total volume of the inoculum broth in the Minitek is less than that in the API. The screw cap on the Minitek broth permits vortexing, which assures proper suspen-

sion of organisms. A uniform suspension in the API broth was difficult to achieve with a capillary pipette.

Each API strip contains 20 microtubules and 21 biochemical tests. The Minitek plate contains 12 wells. The user has a choice of 35 available substrates. Lecithinase, lipase, and 20% bile are not available in either system, and nitrate is not available in the API. The flexibility of the Minitek system offers a more economical approach to the biochemical characterization of anaerobes than the API. Many of the clinically significant anaerobes do not require 21 biochemical parameters for identification. Selective use of biochemicals can lower the cost of processing clinical specimens likely to contain multiple anaerobic organisms.

Gram morphology, catalase, lipase, lecithinase, GLC end products, and the 12 biochemicals used in this study permitted speciation of 86.3% of the organisms (Table 1). Of the clinical isolates the most difficult genera to speciate were the *Peptostreptococcus* and the *Peptococcus*. Seventeen of the 73 gram-positive cocci could not be placed in a genus using the expected reaction data from the reference manuals. We chose to use the VPI classification scheme for the anaerobic cocci. With the exception of two isolates of *Peptococcus constellatus* and one *Peptococcus morbillorum*, no other positive cocci producing lactic acid were included in this study. The 11 *Peptococcus* species were all catalase positive; the GLC and biochemical data were not conclusive enough to speciate these isolates. Long-chain fatty acid analysis as outlined by Wells and Field (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C106, p. 43) perhaps would have permitted grouping of the 28 unspeciated cocci.

Holdeman and Moore (5) recommend the use of the Gram stain and GLC analysis for rapid genus identification. GLC has been shown to be an important tool in anaerobic bacteriology and is essential for identification of certain fusobacteria and anaerobic gram-positive, nonsporulating bacilli. Without GLC it is still possible to identify *Bacteroides fragilis*, *Bacteroides melaninogenicus*, gram-positive and -negative cocci, *C. perfringens*, other clostridia, *Proprionibacteria acnes*, and *Fusobacterium nucleatum* (1).

The Gram reaction, colony morphology, selective use of biochemical reactions, and the use of antimicrobial disks as outlined by Sutter and Finegold (9, 10) offer methods for identification to the laboratory with no GLC. We do not suggest that GLC data are not useful, but if they are not available, identification of many anaerobes is still possible.

We conclude that the Minitek system is a rapid, useful, and reliable substitute for the more time-consuming conventional methods for biochemical characterization of anaerobes. The Minitek system in concert with other available procedures should permit the average clinical laboratory to accurately identify clinically significant anaerobes.

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