

Methods for Detecting Carbohydrate Fermentation by Gram-Negative Nonsporeforming Anaerobes

GUNNAR D. FAY AND ARTHUR L. BARRY

Clinical Microbiology Laboratory, University of California, Davis, Sacramento Medical Center, Sacramento, California 95817

Received for publication 18 October 1973

Fermentation of carbohydrates by gram-negative anaerobes may be detected rapidly and conveniently in small volumes of thioglycollate broth, incubated in Gas-Pak jars.

Most schemes for the identification of gram-negative anaerobic bacilli include tests for the fermentation of various carbohydrates. For the past 2 years, we have utilized a system in which 1.0 to 1.5 ml of thioglycollate broth with the appropriate carbohydrate is inoculated, incubated in a Gas-Pak jar (Bioquest, Baltimore, Md.), and examined for acid production. Although negative tests are held for a total of 7 days, most isolates will be positive within 1 to 2 days. The present report summarizes our experience in comparing tests in small volumes of broth to more conventional methods.

Fermentation of dextrose, xylose, and arabinose was tested by the four methods outlined in Table 1. The pre-reduced, anaerobically sterilized (PRAS) media (4), purchased from Robbins Laboratories, New York, N.Y., and the thioglycollate medium without dextrose or indicator (BBL no. 11722), were both prepared with the appropriate carbohydrate (approximately 0.6%), according to Dowell and Hawkins (1). Volumes (1 ml) of both media were also dispensed into tubes (13 by 100 mm) without special precautions to avoid aeration of the media. Each test medium was inoculated with 0.05 ml of an overnight chopped meat or thioglycollate broth culture. For the small volumes of broth, four tubes of each type of medium were inoculated with each organism and one set of test was removed from the anaerobic jars after 1, 2, 3, and 7 days. At each time interval the pH of each medium was measured with a model 10 pH meter (Corning Glass Works, Corning, N.Y.), by using a semimicro combination electrode (Corning Glass Works). For measuring the pH of the large volume tests, 0.5-ml samples were removed by using nitrogen-gassed syringes and needles to sample the PRAS media.

A total of 50 clinical isolates were tested by all four methods (Table 1). These include four *Fusobacterium* species and 46 *Bacteroides*

fragilis (16 ss *thetaiotaomicron*, 10 ss *fragilis*, 2 ss *distasonis*, 1 ss *vulgatus*, 1 ss *ovatus*, and 16 unidentified subspecies). Once acid began to accumulate, the pH dropped rather rapidly, but the lag time before this drop in pH was quite variable. For this study, a positive response was recorded when the medium reached a pH of 5.5 or less. Although not used in this study, bromthymol blue changes color in this pH range and may be used as an internal or external pH indicator. For routine purposes, we prefer to use an internal pH indicator, adding additional reagent when decolorization occurs.

After 7 days of incubation, all four methods gave nearly identical results, but preliminary readings made after 1 to 2 days demonstrated marked differences. Fermentation was much slower in large volumes of aerobically incubated thioglycollate as compared to the small volumes of thioglycollate incubated anaerobically. The small volumes of peptone yeast broth base gave results a little faster than those obtained when larger volumes of the same broth were prepared in PRAS tubes. The lag time before acid products accumulate is significantly reduced by using smaller volumes of broth. Apparently, the kind of medium used is not as important as the method for obtaining anaerobiosis (3), because the two media gave similar results when tested in 1-ml volumes in Gas-Pak jars but not with the larger volume conventional methods. We have arbitrarily selected thioglycollate for routine use because it is simple to prepare and gives reliable results.

The present study should be extended to include tests with species of anaerobes which grow less readily. Additional carbohydrates should also be studied. We can only conclude that with the less fastidious anaerobes which are most commonly found as pathogens in clinical specimens, rapid and accurate results can be obtained conveniently when small vol-

TABLE 1. Carbohydrate fermentations tests with 50 gram-negative, nonsporeforming, anaerobic bacilli

Method	Cumulative % positive after 1, 2, 3, and 7 days											
	Dextrose ^a				Xylose ^b				Arabinose ^c			
	1	2	3	7	1	2	3	7	1	2	3	7
8.5 ml of thioglycollate base incubated aerobically in 15- by 125-mm tubes	41	65	88	96	26	55	81	90	20	44	80	88
5.0 ml of peptone yeast base, prereduced, anaerobically sterilized	65	90	94	96	19	81	95	98	52	76	92	96
1.0 ml of thioglycollate base incubated anaerobically in 13- by 100-mm tubes	76	94	98	100	71	88	93	95	80	92	96	100
1.0 ml of peptone yeast base incubated anaerobically in 13- by 100-mm tubes	73	90	96	100	69	83	90	98	76	88	92	100

^a Percent based on 49 strains positive by one or more methods after 7 days.

^b Percent based on 42 strains positive by one or more methods after 7 days.

^c Percent based on 25 strains positive by one or more methods after 7 days.

umes of broth are inoculated and incubated in anaerobic jars, thus eliminating technical difficulties encountered with PRAS media. The system described in this report is much less expensive and is more rapid, but still gives results comparable to those obtained by the conventional methods.

LITERATURE CITED

1. Dowell, V. R., and T. M. Hawkins. 1968. Laboratory

methods in anaerobic bacteriology. Public Health Service Publication, no. 1803. U.S. Government Printing Office, Washington D.C.

2. Finegold, S. M., N. E. Harada, and L. G. Miller. 1967. Antibiotic susceptibility patterns as aids in classification and characterization of gram-negative anaerobic bacilli. *J. Bacteriol.* **94**:1443-1450.
3. Smith, L. D. S., and L. V. Holdeman. 1968. The pathogenic anaerobic bacteria. Charles Thomas Co., Springfield, Ill.
4. Virginia Polytechnic Institute and State University Anaerobe Laboratory. 1972. Anaerobic laboratory manual. Virginia Polytechnic Institute, Blacksburg, Va.