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Evaluation of the AnaeroPack System for Growth of Clinically Significant Anaerobes

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The AnaeroPack (Mitsubishi Gas Chemical America, Inc., New York, N.Y.) system was compared with the GasPak (Becton Dickinson Microbiology Systems, Cockeysville, Md.) system and a conventional anaerobe chamber to evaluate the ability of the AnaeroPack system to support the growth of clinically significant anaerobes. The AnaeroPack system requires no catalyst or water, produces no hydrogen, and is oxygen absorbing and carbon dioxide generating. It is simple to use and reduces preparation time to a minimum. One hundred forty clinical isolates obtained from various anatomic sites and 10 American Type Culture Collection type strains were evaluated. Isolates were plated on various media, and bacterial growth was examined after 24, 48, 72, and 168 h of incubation. Criteria for evaluation and comparison of systems included rate and quality of growth, colonial morphology, hemolytic reactions, and pigment production. Results indicate that the AnaeroPack system is highly effective in creating an anaerobic atmosphere. The AnaeroPack system never failed to reduce the methylene blue indicator, while the GasPak system failed 15% of the time. The rate or quality of growth achieved by the AnaeroPack system compared with that of established anaerobic culturing techniques was similar and significantly better for several genera including the *Bacteroides fragilis* group, *Fusobacterium*, *Clostridium*, and *Peptostreptococcus*. The AnaeroPack system appears to be an excellent alternative to established methods for generating an environment for anaerobic incubation.

Anaerobic bacteria are the causative agents in a wide variety of human infections of the skin and soft tissues and the respiratory, gastrointestinal, and female genital tracts. Because many anaerobes grow more slowly than facultative or aerobic bacteria, it is critical to optimize growth conditions to obtain prompt and reliable culture results. While anaerobic chambers are the ideal tool for isolating anaerobes from clinical specimens, anaerobic jars and anaerobic pouch systems are acceptable alternatives. By the latter method, an atmosphere-generating chemical reaction has traditionally been used to generate appropriate anaerobic conditions to support growth. The AnaeroPack, a novel anaerobic atmosphere-generating system, is a disposable oxygen-absorbing and carbon dioxide-generating, sealed, porous sachet for use in anaerobe jars or pouches. The AnaeroPack does not require the addition of water or the use of palladium catalyst. The sachet's contents become activated on contact with oxygen. Once the AnaeroPack is placed into a sealed container, the atmospheric oxygen in the container is absorbed and the oxygen level is reduced to $<1\%$ in 30 min. Simultaneously, carbon dioxide is generated to a concentration of 18% in approximately 12 min (7).

The AnaeroPack system for both jar and pouch use was evaluated against the GasPak jar and pouch system for growth of 33 anaerobes by Van Horn et al. (8). The results of that study indicated that the AnaeroPack system compared favorably with the other systems tested (8). Susceptibility testing in which the AnaeroPack system was used as a source for the establishment of anaerobiosis demonstrated that the higher carbon dioxide concentration produced by the AnaeroPack system resulted in better overall growth of the isolates tested, which enabled easier interpretation of the MICs (3).

The BBL GasPak Anaerobic System consists of the GasPak hydrogen and carbon dioxide generator envelope and a room temperature palladium catalyst in the jar. The system requires the addition of water to produce hydrogen, which combines, in the presence of fresh palladium catalyst, with atmospheric oxygen to form water and produce anaerobic conditions. Within 2 h of incubation at 35°C, the oxygen concentration is $<1\%$ and the carbon dioxide concentration is approximately 4 to 10% (5).

In the present study, the AnaeroPack and the GasPak systems' ability to support the growth of clinically significant anaerobic isolates was compared against growth in an anaerobic chamber. Some of these results have been presented earlier (1).

MATERIALS AND METHODS

Bacterial isolates. A total of 150 anaerobic strains were obtained from the frozen stock culture collection of the Anaerobe Research Laboratory (Channing Laboratory, Boston, Mass.). Of the 150 strains, 140 were isolated from clinical specimens and were selected to represent those species commonly isolated from human clinical infections, including fastidious anaerobes such as *Fusobacterium necrophorum* and pigmented *Prevotella* species. Strains were identified by established criteria, including the Microbial Identification System (Microbial ID Inc., Newark, Del.) (4, 6). Ten reference strains from the American Type Culture Collection (ATCC) were included. Table 1 lists the strains used in the study.

Coy anaerobic chamber. The anaerobic chamber used is a flexible glove box (Coy Laboratory Products, Grass Lake, Mich.) kept at 35°C and filled with an atmosphere of 10% carbon dioxide, 10% hydrogen, and 80% nitrogen. The anaerobic conditions in the chamber were monitored with an indicating solution of methylene blue-glucose and a solution of resazurin in cysteine hydrochloride (2). Both solutions become colorless under anaerobic conditions.

BBL GasPak system. The GasPak system included the GasPak jar (2.5 liters) with a catalyst chamber containing new palladium pellets and the GasPak Anaerobe envelope. Before each use the pellets were conditioned in a hot-air oven at 120°C for 2 h. A GasPak Anaerobe envelope was placed in each jar, and 10 ml of water was added to the envelope. Freshly conditioned palladium catalyst was added to the jar, and the atmospheric conditions in the jar system were monitored with a disposable BBL Dry Anaerobic Indicator Strip (Becton Dickinson).

AnaeroPack system. The AnaeroPack (Misubishi Gas Chemical America, Inc., New York, N.Y.) system included the medium-sized AnaeroPack rectangular container (9.5 by 6.75 by 3.25 in.; 3.2 liters) and two AnaeroPack sachets. The sachets were removed from their foil packs and placed in the container along with a disposable BBL Dry Anaerobic Indicator Strip to monitor the atmospheric conditions. In addition, the AnaeroPack sachets were tested in the GasPak jar.

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TABLE 1. Bacterial strains used to evaluate growth with the AnaeroPack system

Organism group and organism	No. of isolates tested
Gram-negative bacilli	
Bacteroides fragilis	10
Bacteroides distasonis	3
Bacteroides ovatus	4
Bacteroides thetaiotaomicron	4
Bacteroides uniformis	3
Bacteroides ureolyticus	1
Bacteroides vulgatus	4
Capnocytophaga sp.	1
Fusobacterium mortiferum	3
Fusobacterium necrogenes	1
Fusobacterium necrophorum	1
Fusobacterium nucleatum	4
Porphyromonas asaccharolytica	1
Prevotella bivia	3
Prevotella buccae	1
Prevotella intermedia	3
Prevotella loeschii	1
Prevotella melaninogenica	3
Gram-negative cocci	
Acidaminococcus fermentans	1
Veillonella sp.	4
Gram-positive bacilli	
Actinomyces israelii	2
Actinomyces odontolyticus	4
Actinomyces pyogenes	1
Bifidobacterium bifidum	2
Bifidobacterium breve	1
Clostridium bifermentans	2
Clostridium cadaveris	1
Clostridium difficile	3
Clostridium innocuum	3
Clostridium paraputrificum	3
Clostridium perfringens	3
Clostridium putrificum	2
Clostridium septicum	3
Clostridium sordellii	2
Clostridium tertium	2
Eubacterium contortum	2
Eubacterium lentum	5
Lactobacillus sp. strain D12	1
Lactobacillus fermentum	1
Lactobacillus jensenii	1
Lactobacillus minutus	1
Lactobacillus sp.	1
Propionibacterium acnes	10
Gram-positive cocci	
Gemella morbillorum	3
Peptostreptococcus anaerobius	4
Peptostreptococcus asaccharolyticus	3
Peptostreptococcus magnus	3
Peptostreptococcus micros	3
Peptostreptococcus prevotii	3
Peptostreptococcus saccharolyticus	3
Peptostreptococcus tetradius	3
Streptococcus intermedius	3
ATCC strains	
Bacteroides fragilis ATCC 25285	1
Bacteroides thetaiotaomicron ATCC 29741	1
Bacteroides ureolyticus ATCC 33387	1
Clostridium perfringens ATCC 13124	1
Clostridium sporogenes ATCC 19404	1
Eubacterium lentum ATCC 43055	1
Fusobacterium mortiferum ATCC 25557	1
Fusobacterium nucleatum ATCC 25586	1
Prevotella melaninogenica ATCC 25845	1
Porphyromonas asaccharolytica ATCC 29743	1

One AnaeroPack sachet was placed in a GasPak jar (2.5 liters) without catalyst along with a disposable BBL Dry Anaerobic Indicator Strip.

Media. The plate media used included brucella base agar with 5% sheep blood containing 0.01 g of both hemin and vitamin K$_1$ (BMB) per liter, brucella base agar with 5% laked sheep blood, 100 μg of kanamycin and 7.5 μg of vancomycin per ml, and 0.01 g of both hemin and vitamin K$_1$ (LKV) per liter, and phenylethyl alcohol anaerobic agar with 5% sheep blood (PEA). All plate media was purchased from PML Microbiologicals, Tualatin, Oreg.

Inoculation. Isolates were taken from frozen stock cultures and passed twice on BMB within the anaerobic chamber before use. The colonies were Gram stained, and the plates were checked for purity. Colonies from pure cultures of each strain were suspended in phosphate-buffered saline to a McFarland standard of 0.5, and a 1-μl aliquot was streaked for isolation onto agar plates. All 150 isolates were plated onto BMB for evaluation. In addition, depending on the Gram stain reaction, 58 strains were tested for growth with LKV and 87 strains were plated onto PEA. For each strain tested, multiple sets of plates were inoculated to be read after 24, 48, 72, and 168 h of uninterrupted incubation. One set of plates was placed in the anaerobic chamber, four sets (one set for each incubation period) were placed in four separate AnaeroPack containers, four sets were placed in four separate GasPak jars, and four sets were placed in four separate GasPak jars with an AnaeroPack sachet. After addition of the appropriate atmosphere-generating envelope or sachets, jars or containers were sealed and incubated at 35°C. A numerical coding system combining degree of growth and colony size was used to evaluate each isolate, as follows: 0, no growth; 1, <30 colonies and <1 mm in diameter; 2, <30 colonies and >1 mm in diameter; 3, 30 to 300 colonies and <1 mm in diameter; 4, 30 to 300 colonies and >1 mm in diameter; 5, >300 colonies and <1 mm in diameter; 6, >300 colonies and 1 to 3 mm in diameter; 7, >300 colonies and >3 mm in diameter. Plate evaluations were performed in a blinded manner so that the evaluator did not know the conditions of incubation.

Statistical evaluation. Statistical evaluations of the data were performed with commercially available software (INSTAT; GraphPad Software, San Diego, Calif.).

RESULTS

Both the rate and quality of growth of the 150 anaerobic bacterial strains grown in a Coy anaerobe chamber were compared with those of the same strains grown in three anaerobic atmosphere-generating systems: the AnaeroPack system, the GasPak system, and the AnaeroPack sachet in the GasPak jar. A comparison of these systems to determine the number of bacterial strains for which growth was the same, better, or less than the chamber's at 24 and 48 h indicated that the systems

TABLE 2. Mean growth scores[a] for strains by four anaerobic methods on BMB

Test strain (no. of isolates)	Mean (SD) growth score							
	24 h				48 h			
	Chamber	AnaeroPack	GasPak	AnaeroPack 2[b]	Chamber	AnaeroPack	GasPak	AnaeroPack 2
Bacteroides fragilis (11)	5 (0.00)	5 (0.52), 0.0162[c]	5 (0.50), 0.0380[c]	5 (0.52), 0.0162[c]	6 (0.75)	6 (0.47), 0.0107[d]	6 (0.67)	6 (0.53), 0.0162[d]
Bacteroides fragilis group (16)	5 (1.31)	5 (1.50)	5 (1.71)	5 (1.44)	5 (1.03)	6 (0.88)	5 (1.35)	6 (1.13)
Bacteroides spp. (5)	4 (1.09)	4 (1.09)	5 (0.89)	4 (1.09)	7 (0.89)	7 (0.55)	7 (0.55)	6 (0.89)
Capnocytophaga sp. (1)	3	7	7	7	7	7	7	7
Fusobacterium spp. (11)	4 (1.63)	4 (1.17)	4 (1.17)	4 (1.17)	5 (1.12)	5 (0.47)	5 (0.65)	5 (0.65)
Porphyromonas asaccharolytica (1)	0	0	0	0	0	5	3	3
Prevotella spp. (5)	5 (0.89)	5 (0.00)	5 (0.00)	5 (0.00)	6 (0.45)	6 (0.45)	6 (0.55)	6 (0.45)
Pigmented *Prevotella* spp. (7)	3 (0.00)	4 (1.07)	4 (1.07)	4 (1.07)	5 (1.25)	5 (0.53)	5 (1.00)	5 (0.49)
Actinomyces spp. (7)	2 (2.29)	4 (1.89)	3 (1.81)	3 (2.24)	4 (1.91)	4 (0.97)	4 (0.97)	4 (0.97)
Bifidobacterium spp. (3)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	6 (0.58)	6 (0.58)	6 (0.58)
Clostridium spp. (26)	6 (1.13)	6 (0.82)	6 (1.02)	6 (1.08)	6 (1.07)	6 (0.76)	6 (0.76)	6 (0.78)
Eubacterium spp. (8)	3 (1.55)	4 (1.07)	4 (1.03)	4 (1.07)	5 (0.46)	5 (0.00)	5 (0.46)	5 (0.00)
Lactobacillus spp. (5)	4 (2.24)	5 (0.89)	4 (2.24)	5 (0.89)	5 (1.22)	5 (0.45)	5 (0.55)	5 (0.55)
Propionibacterium acnes (10)	3 (0.00)	3 (0.00)	3 (0.00)	3 (0.00)	3 (0.00)	3 (0.63)	3 (0.00)	3 (0.95)
Acidaminococcus fermentans (1)	3	5	5	5	5	5	5	5
Veillonella sp. (4)	4 (1.15)	5 (0.00)	4 (1.15)	5 (1.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)
Gemella morbillorum (3)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)
Peptostreptococcus spp. (23)	3 (1.45)	4 (1.76), 0.0003[c]	4 (1.78), 0.0005[c]	4 (1.80), <0.0001[c]	4 (1.78)	5 (0.71), 0.0438[c]	4 (1.76)	5 (0.77), 0.0182[c]
Streptococcus intermedius (3)	3 (2.89)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	6 (1.15)	6 (1.15)

[a] Numerical score rounded to the nearest whole number: 0, no growth; 1, <30 colonies and <1 mm in diameter; 2, <30 colonies and >1 mm in diameter; 3, 30 to 300 colonies and <1 mm in diameter; 4, 30 to 300 colonies and >1 mm in diameter; 5, >300 colonies and <1 mm in diameter; 6, >300 colonies and 1 to 3 mm in diameter; 7, >300 colonies and >3 mm in diameter.

[b] AnaeroPack sachet in the GasPak jar.

[c] P value for t test comparing growth in the atmosphere-generating systems to that in the chamber.

[d] P value for t test comparing growth in the AnaeroPack systems to that in the GasPak jar.

performed similarly ($P = 0.8967$; chi-square test). At 24 h, 95% of the isolates tested grew in the AnaeroPack and GasPak systems as well as or better than in the anaerobic chamber, and with the AnaeroPack sachet in the GasPak jars, 96% grew as well as in the anaerobic chamber. At 48 h, the two AnaeroPack systems performed slightly better than the GasPak system when compared with the performance of the anaerobic chamber (91 versus 87%, respectively). Of the isolates that grew less vigorously than the isolates in the anaerobic chamber, the differences were minimal and were restricted to the quality of growth, with the strains grown within the chamber exhibiting larger colonies than the same strains grown in the atmosphere-generating systems. An exception to this observation was a strain of *Bacteroides thetaiotaomicron*, which did not show visible growth in any of the jar systems but did show visible growth in the chamber at 24 h. This difference was eliminated for the AnaeroPack system at 48 h, but growth of this strain in the GasPak jar was different from that in the chamber (<30 colonies and >1 mm in diameter versus >300 colonies and 1 to 3 mm in diameter, respectively). Additionally, one strain of *Veillonella* did not grow as well in the GasPak system at 24 h as in the chamber (30 to 300 colonies and <1 mm in diameter versus >300 colonies and <1 mm in diameter, respectively).

A mean growth score, based on plate growth, was determined for each genus tested at the various incubation periods, and the results following 24 and 48 h of incubation are presented. The results for plate growth on BMB are presented in Table 2. The growth differences seen were not statistically significant ($P > 0.05$ by paired t test) for the various genera tested with the exception of *Bacteroides fragilis* and *Peptostreptococcus* spp. Following 24 h of incubation, *B. fragilis* grew significantly better in all the atmosphere-generating systems than in the chamber ($P = 0.0162$, $P = 0.0380$, and $P = 0.0162$ for the AnaeroPack system, GasPak system, and AnaeroPack sachet in the GasPak jar, respectively). By 48 h, *B. fragilis* isolates grown in the chamber exhibited growth similar to those of isolates grown in the atmosphere-generating systems; however, the AnaeroPack systems tested yielded significantly better growth compared to that in the GasPak jar ($P = 0.0107$ and $P = 0.0162$ for the AnaeroPack system and the AnaeroPack sachet in the GasPak jar, respectively). These growth differences were confined to the size of the colonies, with incubation in the AnaeroPack systems yielding larger colonies. *Peptostreptococcus* spp. grew significantly better in the atmosphere-generating systems than in the chamber following 24 h of incubation ($P = 0.0003$, $P = 0.0005$, and $P < 0.0001$ for the AnaeroPack system, GasPak system, and AnaeroPack sachet in the GasPak jar, respectively). At 48 h, *Peptostreptococcus* spp. grew equally in the chamber and in the GasPak jar, whereas growth in the AnaeroPack systems was significantly better compared to that in the chamber or the GasPak jar ($P = 0.0438$ for the AnaeroPack system and $P = 0.0182$ for the AnaeroPack sachet in the GasPak jar versus the chamber, and $P = 0.0024$ for the AnaeroPack system and $P = 0.0015$ for the AnaeroPack sachet in the GasPak jar versus the GasPak jar). At 72 and 168 h, growth of *Peptostreptococcus* spp. was consistently better in the atmosphere-generating systems than in the chamber ($P < 0.005$ for comparisons at 72 h and $P < 0.05$ for comparisons at 168 h; data not shown). Better initial growth at 24 h was seen for strains of *Capnocytophaga* and *Acidaminococcus* in the atmosphere-generating systems than in the anaerobic chamber (statistical analysis could not be performed due to the small number of strains used). These growth differences encompassed both the quantities of colonies present and the sizes of the colonies. By 48 h, these differences no longer persisted. *Porphyromonas asaccharolytica* was not detected on plates incubated in the chamber until 168 h of incubation, but grew well by the other incubation methods. Slight differences that existed between the chamber and the atmosphere-generating systems at 72 and 168 h were not significant and were

TABLE 3. Mean growth scores[a] for strains by four anaerobic methods on LKV

Test strain (no. of isolates)	Mean (SD) growth score							
	24 h				48 h			
	Chamber	AnaeroPack	GasPak	AnaeroPack 2[b]	Chamber	AnaeroPack	GasPak	AnaeroPack 2
Bacteroides fragilis (11)	5 (0.60)	5 (0.00)	5 (0.00)	5 (0.00)	6 (0.82)	5 (0.67)	5 (0.40)	5 (0.82)
Bacteroides fragilis group (16)	4 (1.03)	5 (0.81), 0.0197[c]	5 (0.81), 0.0090[c]	5 (1.00), 0.0057[c]	5 (0.83)	6 (0.93), 0.0057[c]	5 (1.02)	6 (1.15)
Bacteroides spp. (5)	4 (1.15)	4 (1.15)	4 (1.15)	4 (1.15)	6 (0.58)	6 (1.15)	6 (0.58)	6 (0.58)
Capnocytophaga sp. (1)	3	7	7	7	7	7	7	7
Fusobacterium spp. (11)	1 (2.50)	3 (2.37), 0.0214[d]	1 (2.24)	2 (2.29), 0.0311[d]	3 (2.60)	3 (2.53)	2 (2.62)	3 (2.50)
Porphyromonas asaccharolytica (1)	0	0	0	0	0	5	3	3
Prevotella spp. (5)	4 (1.79)	5 (0.89)	4 (1.34)	4 (1.34)	5 (1.30)	5 (0.84)	5 (1.30)	5 (1.30)
Pigmented *Prevotella* spp. (7)	3 (1.13)	3 (1.46)	3 (1.67)	3 (1.67)	4 (2.03)	4 (2.03)	4 (2.16)	4 (2.22)
Acidaminococcus fermentans (1)	3	5	5	5	5	5	5	5

[a] Numerical score rounded to the nearest whole number: 0, no growth; 1, <30 colonies and <1 mm in diameter; 2, <30 colonies and >1 mm in diameter; 3, 30 to 300 colonies and <1 mm in diameter; 4, 30 to 300 colonies and >1 mm in diameter; 5, >300 colonies and <1 mm in diameter; 6, >300 colonies and 1 to 3 mm in diameter; 7, >300 colonies and >3 mm in diameter.
[b] AnaeroPack sachet in the GasPak jar.
[c] *P* value for *t* test comparing growth in the atmosphere-generating systems to that in the chamber.
[d] *P* value for *t* test comparing growth in the AnaeroPack systems to that in the GasPak jar.

related to colony size, with the chamber isolates being slightly smaller, with the exception of *Acidaminococcus fermentans* at 168 h (data not shown). There was no difference in pigment production for strains of *Actinomyces odontolyticus* in the systems tested.

Table 3 presents the mean growth scores for the strains grown on LKV. *B. fragilis*, *Bacteroides* spp., *Prevotella* spp., and pigmented *Prevotella* strains grew equally well by all the methods tested at 24 and 48 h. The pigmented *Prevotella* strains demonstrated quicker pigment production in the AnaeroPack system than in the anaerobic chamber or in the GasPak system. At 24 h the *B. fragilis* group grew significantly better in the atmosphere-generating system tested than in the chamber ($P = 0.0197$, $P = 0.0090$, and $P = 0.0057$ for the AnaeroPack system, GasPak system, and AnaeroPack sachet in the GasPak jar, respectively). This difference persisted for the AnaeroPack system, which yielded significantly larger colonies than the chamber at 48 h ($P = 0.0057$). *Fusobacterium* spp. grew better in the AnaeroPack systems than in the GasPak jar at 24 h ($P = 0.0214$ and $P = 0.0311$ for the AnaeroPack system and the AnaeroPack sachet in the GasPak jar, respectively). The minor differences that existed among the genera tested at time points following 24 h were related to colony size and were not significant.

The mean growth scores for gram-positive isolates grown on PEA are presented in Table 4. After 24 h of incubation, the most dramatic differences that existed related to the rate, quantity, and quality of growth for strains of *Eubacterium*, which grew significantly better in all the atmosphere-generating systems than in the chamber ($P = 0.0017$, $P < 0.0001$, and $P < 0.0001$ for the AnaeroPack system, GasPak system, and AnaeroPack sachet in the GasPak jar, respectively). *Propionibacterium acnes* strains grew slightly better in the chamber at 24 and 48 h than in the other systems. Differences for other strains at the various time points were not significant and were confined to colony size, with no one particular incubation method performing consistently better. All 26 strains of *Clostridium* grew equally well in the four systems tested, with slighter larger colonies noted in the AnaeroPack systems at 24 h. For the *Clostridium perfringens* strains tested, double-zone hemolysis was similar for all incubation systems.

DISCUSSION

The present study indicates that the AnaeroPack sachet along with the AnaeroPack rectangular container is a highly effective system for creating an anaerobic atmosphere. This system never failed to reduce the methylene blue indicator;

TABLE 4. Mean growth scores[a] for strains by four anaerobic methods on PEA

Test strain (no. of isolates)	Mean (SD) growth score							
	24 h				48 h			
	Chamber	AnaeroPack	GasPak	AnaeroPack 2[b]	Chamber	AnaeroPack	GasPak	AnaeroPack 2
Actinomyces spp. (7)	2 (1.81)	2 (1.46)	2 (1.60)	2 (1.46)	4 (2.44)	4 (1.91)	4 (2.44)	4 (1.91)
Bifidobacterium spp. (3)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)	5 (0.00)
Clostridium spp. (26)	5 (1.38)	6 (0.86)	5 (1.01)	6 (0.90)	6 (1.12)	6 (0.80)	6 (0.82)	6 (1.13)
Eubacterium spp. (8)	1 (1.39)	4 (1.77), 0.0017[c]	4 (1.03), <0.0001[c]	4 (1.03), <0.0001[c]	5 (0.00)	5 (0.00)	5 (0.46)	5 (0.35)
Lactobacillus spp. (5)	4 (2.24)	4 (2.24)	4 (2.24)	4 (2.24)	5 (1.22)	4 (2.24)	4 (2.24)	4 (2.24)
Propionibacterium acnes (10)	3 (0.95)	2 (1.26)	2 (1.23)	2 (1.23)	3 (0.00)	2 (1.26)	2 (1.26)	2 (1.26)
Gemella morbillorum (2)	3 (3.54)	3 (3.54)	3 (3.54)	4 (1.41)	4 (1.41)	5 (0.00)	3 (3.54)	4 (1.41)
Peptostreptococcus spp. (23)	2 (1.69)	2 (1.88)	2 (1.78)	2 (1.88)	4 (1.96)	3 (2.09)	3 (2.09)	4 (1.88)
Streptococcus intermedius (3)	2 (2.65)	3 (2.52)	3 (2.52)	3 (2.00)	3 (1.53)	3 (2.89)	5 (0.58)	5 (0.58)

[a] Numerical score rounded to the nearest whole number: 0, no growth; 1, <30 colonies and <1 mm in diameter; 2, <30 colonies and >1 mm in diameter; 3, 30 to 300 colonies and <1 mm in diameter; 4, 30 to 300 colonies and >1 mm in diameter; 5, >300 colonies and <1 mm in diameter; 6, >300 colonies and 1 to 3 mm in diameter; 7, >300 colonies and >3 mm in diameter.
[b] AnaeroPack sachet in the GasPak jar.
[c] *P* value for *t* test comparing growth in the atmosphere-generating systems to that in the chamber.

however, the GasPak system failed 15% of the time, while the AnaeroPack sachets in the GasPak jars failed 7% of the time. It is possible to attribute this finding to the age of the containers used in this study: the AnaeroPack containers were brand new, whereas the GasPak jars had been used for several years. GasPak jars and lids were inspected for flaws, and cracked O rings were replaced prior to the start of the study. Because failed jars and lids could be switched for successful use during subsequent testing, it is possible to conclude that the AnaeroPack containers provide a more airtight environment that facilitates the generation and maintenance of the appropriate anaerobic atmosphere.

The AnaeroPack system is easier to use than the GasPak system, which requires the addition of water and the maintenance of the palladium catalyst, neither of which is required for the AnaeroPack system. The rectangular shape of the AnaeroPack container facilitates storage and allows for the stacking of multiple containers, and the various sizes of the containers available (0.4, 2.5, and 5.5 liters) accommodate the diverse needs of most laboratories. The handle-like closure on the AnaeroPack containers eliminates the guesswork associated with the hand-tightened screw closure of the GasPak system.

A cost analysis based on the list prices for the two atmosphere-generating systems shows that the capital cost outlay for the GasPak system is much greater than that for the AnaeroPack system. The list price for the 2.5-liter GasPak jar is $356.72, while the list price for the 2.5-liter AnaeroPack container is $55.00. Furthermore, the need to condition the palladium catalyst requires the use of a hot-air oven, which must be added to the capital cost outlay, if one is not available otherwise. The costs of the atmosphere-generating envelope and sachet are comparable; the GasPak envelope lists at $1.79 ($17.95/10 pack) and the AnaeroPack sachet lists at $2.10 ($42.00/20 pack). Although the cost of the AnaeroPack sachet is slightly higher, it is important to note that the GasPak system also requires the use of a pipet or syringe to add the water to the envelope as well as the maintenance and replacement of the palladium catalyst.

When performing anaerobic cultures, whether for research or clinical purposes, it is critical to optimize the environmental growth conditions to obtain reliable culture results. The initial growth in the AnaeroPack system after 24 and 48 h of incubation was equal to or, in many cases, better than growth after incubation in the chamber or in the GasPak system. The increased carbon dioxide concentration in the AnaeroPack system (18% versus 4 to 10% in the GasPak system and 10% in the chamber) may account for this improved initial growth. The AnaeroPack atmosphere-generating system proved to be highly effective in establishing and supporting the growth of clinically significant anaerobic bacteria. Furthermore, the ease of setup and maintenance of the AnaeroPack system makes it an excellent alternative to the GasPak system.

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