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Evaluation of AnaeroGen System for Growth of Anaerobic Bacteria

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The Oxoid AnaeroGen system was compared with the BBL GasPak for the production of an anaerobic atmosphere and was evaluated for its ability to support the growth of 135 clinically significant anaerobic bacteria. An anaerobe chamber was used as the "gold standard" for supporting the growth of anaerobes. The AnaeroGen requires no catalyst, produces no hydrogen, requires no water, and reduces preparation time to a minimum. The water-activated BBL GasPak generates hydrogen. For 132 of the 135 strains tested, better initial growth at 48 h was noted for the jar methods than for the anaerobe chamber. At 72 h, 113 of the 135 strains showed equal growth, and at 7 days, only marginal differences in growth patterns were noted. The AnaeroGen never failed to reduce the anaerobic indicator, while the BBL GasPak occasionally failed to do so. The AnaeroGen performed at least as well as, and sometimes better than, the established methods. The AnaeroGen is a good alternative for use in anaerobic jars.

In order to obtain reliable culture results from an anaerobic jar, there must be adequate replacement of the oxygenated environment with an anaerobic atmosphere. The Oxoid AnaeroGen (Unipath Inc., Nepean, Ontario, Canada) is a new anaerobic atmosphere-generating system for use in anaerobic jars with both primary plates and subcultures. The AnaeroGen packet is unique in that it enables oxygen in the air to be absorbed without the production of hydrogen and without the addition of water. According to the manufacturer, when an AnaeroGen sachet is placed in a sealed jar, the atmospheric oxygen in the jar is rapidly absorbed without the production of hydrogen. There is a simultaneous generation of 9 to 13% carbon dioxide. The oxygen level is reduced in the jar to <1% within 30 min.

Another product used to produce an anaerobic atmosphere in a jar is the BBL GasPak Anaerobic System (Beckon Dickinson Microbiology Systems, Cockeysville, Md.). The two basic components of the GasPak anaerobic system are the GasPak hydrogen and carbon dioxide generator envelope and a room temperature palladium catalyst in the jar. Water is added to the GasPak envelope and hydrogen is produced. The hydrogen reacts with the atmospheric oxygen on the surface of the catalyst to form water and produce anaerobic conditions. According to the manufacturer, in the GasPak anaerobic conditions are achieved within 2 h with the oxygen concentration at <1% and the carbon dioxide concentration at 4 to 10% at 35°C.

We compared the AnaeroGen and the GasPak for their ability to support the growth of a wide variety of anaerobic isolates and evaluated the results against growth in an anaerobic chamber.

MATERIALS AND METHODS

A total of 135 anaerobic and facultatively anaerobic strains from the anaerobic reference laboratory of the Centers for Disease Control and Prevention (Atlanta, Ga.) were used to compare the effectiveness of the AnaeroGen and the GasPak with that of the anaerobic chamber (Coy Laboratory Products, Inc., Grass Lake, Mich.).

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Test organisms used in the growth comparison study included 6 species of *Actinomyces* (9 strains), 9 species of *Bacteroides* (13 strains), 6 species of *Bifidobacterium* (8 strains), 15 species of *Clostridium* (19 strains), 4 species of *Eubacterium* (8 strains), 7 species of *Fusobacterium*, 10 species of *Prevotella* (13 strains), 2 species of *Porphyromonas* (3 strains), 7 species of *Peptostreptococcus* (8 strains), 2 species of gram-negative cocci, 5 species of *Propionibacterium*, and 2 species of anaerobic *Lactobacillus* (4 strains). Motile gram-negative rods studied included *Anaerobiospirillum succiniciproducens* (three strains), three species of *Campylobacter* (seven strains), three species of *Mobiluncus* (eight strains), three species of *Selenomonas*, *Wolinella succinogenes* (one strain), and *Desulfomonas pigra* and *Desulfovibrio desulfuricans* (five strains), and nonmotile *Bilophila wadsworthia* (two strains) was also studied.

The anaerobe chamber is a flexible glove box kept at 35 to 37°C and filled with a gas mixture of 5% carbon dioxide, 10% hydrogen, and 85% nitrogen. Before the chamber is entered, the port is automatically flushed twice with nitrogen and a third time with the anaerobic gas mixture. Anaerobic conditions in the chamber are indicated by a colorless methylene blue solution with chamber air slowly bubbled through it (2). The solution is blue in the presence of air.

BBL GasPak jars (2.5 liters) were used for both jar techniques. The lid is a polycarbonate plastic that supports a double-screened catalyst chamber containing palladium pellets, an O-ring gasket, and a sealing clamp. Before use, the pellets were reactivated in a hot-air oven at 160°C for 2 h.

BBL. A BBL GasPak Anaerobe envelope was placed in a jar, and 10 ml of water was added to the envelope. A fresh palladium catalyst was added to the jar with a disposable anaerobic indicator strip. Within 2 h of incubation at 35°C, the oxygen concentration was <1% and the carbon dioxide concentration was >4% but <10%.

Oxoid. Oxoid AnaeroGen requires neither catalyst nor water. In addition, no hydrogen is produced. AnaeroGen is activated on contact with air, generating a carbon dioxide level between 9 and 13% and reducing the oxygen level in the jar to below 1% within 30 min.

GasPak disposable anaerobic indicator strip. Both jar methods utilized the GasPak disposable anaerobic indicator strip, which consists of a foil envelope containing a pad saturated with methylene blue solution. The pad is blue in the presence of oxygen and colorless in the absence of oxygen. Anaerobic conditions are measured by reaching a redox potential of −100 mV within 60 min and −300 mV within 2 h in media of pH 7 at room temperature, in a properly operating jar system.

Inoculation. Cultures were taken from lyophilized stocks and passed twice on anaerobic blood agar plates before use. The Centers for Disease Control and Prevention laboratory is a reference laboratory and does little primary isolation from clinical material. Colonies from pure isolates were suspended in Lombard-Dowell broth to a McFarland standard of 1, and 0.01 ml was streaked for isolation on three anaerobe blood agar plates (Carr-Scarborough Microbiologicals, Stone Mountain, Ga.). One plate was placed in the glove box, one was used with the AnaeroGen, and one was used with the GasPak. Both jars were sealed and incubated at 35°C. The hour was noted when the indicator strip was decolorized in the jar systems.

The three sets of plates were read at 48 and 72 h, and some containing slow growers were read again at 7 days. A numerical coding system that combines the degree of growth and colony size is used at the Centers for Disease Control and Prevention. Criteria for recording the degree of growth on anaerobe blood agar

TABLE 1. Growth differences of selected strains by three anaerobic methods

Test organism	Growth score[a]					
	48 h			72 h		
	Chamber	GasPak	Anaero*Gen*	Chamber	GasPak	Anaero*Gen*
Actinomyces gerencseriae	0	4	4	4	7	7
Actinomyces israelii	1	4	4	4	7	7
Actinomyces odontolyticus	1	4	4+	4	7	7
Bacteroides ureolyticus	7	7+	7	7	7+	7
Bacteroides ureolyticus	7	7+	7	7	7+	7
Bacteroides ureolyticus	4	7	7	7	8	7
Bacteroides gracilis	4	7	4	7	7+	4
Bacteroides gracilis	0	7	0	7	7+	4
Bifidobacterium adolescentis	4	7	7	4	7	7
Bilophila wadsworthia	0	0	4	4	0	7
Bilophila wadsworthia	4	4	4	7	7	4
Campylobacter curvus/rectus	7	7	4	7	7+	7
Campylobacter mucosalis	4	7	7	4	7+	7
Campylobacter concisus	4	7	4	4	7+	7
Campylobacter curvus/rectus	4	4	4	7	7+	7
Eubacterium alactolyticum	4	4	7	4	7	7
Lactobacillus minutus	7	0	7	7	4	7
Mobiluncus curtissii subsp. *curtissii*	0	4	4	4	7+	7
Mobiluncus mulieris	0	4	4	4	7	7+
Mobiluncus mulieris	0	4	4	4	7	7
Porphyromonas asaccharolytica	0	7	4	0	8	7
Selenomonas noxia	0	0	4	4	7	7+

[a] 0, no growth; 1, sparse (<30 per plate) and tiny (<1-mm diameter) colonies; 4, moderate (30 to 300 per plate) and tiny (<1-mm diameter) colonies; 4+, most abundant growth with a score of 4; 7, abundant (>300 per plate) and tiny (<1-mm diameter) colonies; 7+, most abundant growth with a score of 7; 8, abundant (>300 per plate) and small (1- to 3-mm diameter) colonies.

were as follows: abundant, >300 colonies; moderate, 30 to 300 colonies; and sparse, <30 colonies. Colony size was also noted as follows: large, ≥5 mm; small, 1 to 3 mm; and tiny, <1 mm.

RESULTS

We compared the speed of growth and quality of growth of 135 anaerobic bacteria in two anaerobic atmosphere-generating systems, Oxoid Anaero*Gen* and BBL GasPak, with the same characteristics of growth in the Coy anaerobe chamber.

For 132 of the 135 strains tested, better initial growth at 48 h was noted with the jar methods than with the anaerobe chamber. At 72 h, 113 of the 135 strains showed equal growth (Table 1). At 7 days, only marginal differences in growth patterns were noted.

All 19 strains of *Clostridium* species grew equally well in the three atmospheres. The anaerobe chamber supported the best demonstration of double-zone hemolysis for *Clostridium perfringens*. At 7 days there was little difference in growth patterns among the three methods for the clostridia.

Actinomyces odontolyticus and *Actinomyces naeslundii* demonstrated better pigment production in the Oxoid system than in the anaerobe chamber or the BBL system. *Actinomyces israelii*, *Actinomyces gerencseriae*, and *A. odontolyticus* grew more slowly in the chamber at 48 h and 72 h, but all strains of *Actinomyces* grew equally well by the three methods at 7 days.

For 13 *Bacteroides* strains and the 8 *Eubacterium* strains, the quality and speed of growth at 48 and 72 h were marginally better for some species in either the GasPak or the Anaero*Gen* jar than in the anaerobe chamber. *Bacteroides gracilis* and *Bacteroides ureolyticus*, reclassified as *Campylobacter gracilis* and species incertae sedis, respectively (1, 3, 4), grew better in the BBL system at 48 and 72 h. These species have been in a uncertain taxonomic position, possibly more closely related to *Campylobacter* than to *Bacteroides*, and the H_2 gas in the BBL system may be an important growth factor for these organisms.

Of the five strains of *Eubacterium lentum*, three grew better at 48 h in the jar systems than in the anaerobe chamber or the Oxoid system. At 72 h and 7 days there was no difference in growth patterns among the three methods for *E. lentum*.

Motile gram-negative rods were studied as a group. The quality of growth and speed of growth varied somewhat among the three methods at the first and second readings but were satisfactory at 7 days for this fastidious group. *Campylobacter concisus*, *Campylobacter mucosalis*, and two of the five strains of *Campylobacter curvus/rectus* (Table 1) grew somewhat better in the BBL system than in the Oxoid system, possibly because of H_2 present in the GasPak hydrogen-plus-carbon dioxide envelope. The five strains of *Desulfomonas pigra* and *Desulfovibrio desulfuricans* had more consistent growth at 48 h in the anaerobe chamber than by the jar methods, and on two occasions they were nonviable by the jar methods. The *Mobiluncus* group as a whole grew better at 48 h by jar methods than in the anaerobe chamber, but at 7 days the qualities of growth by the three methods were equal.

For some strains of the *Selenomonas* group, growth and recovery were slow or nonviable with the GasPak. One of two strains of *Selenomonas noxia* showed better quality of growth and speed of growth in the Oxoid system at 48 h, 72 h, and 7 days. *Selenomonas fluggei* grew equally well in the three systems. *Bilophila wadsworthia* had to be held for 7 days to obtain good quality of growth because of the slow-growing nature of this genus.

Eight strains of *Peptostreptococcus* sp., one strain of *Veillonella parvula*, two strains of *Acidaminococcus fermentans*, and seven of the eight strains of *Bifidobacterium* species showed good speed of growth and quality of growth at each of the three

TABLE 2. Occasions on which growth in the Anaero*Gen* was better than growth in the chamber at 48 h

Test organism	Growth score[a]	
	Anaero*Gen*	Chamber
Actinomyces gerencseriae	4	0
Actinomyces israeli[b]	4	1
Actinomyces odontolyticus[b]	4+	1
Bacteroides ureolyticus[c]	7	4
Bifidobacterium adolescentis	7	4
Bilophila wadsworthia[b]	4	0
Campylobacter mucosalis	7	4
Eubacterium alactolyticum	7	4
Mobiluncus mulieris	4	0
Mobiluncus mulieris	4	0
Mobiluncus curtissii subsp. *curtissii*[c]	4	0
Porphyromonas asaccharolyticus	4	0
Selenomonas noxia	4	0

[a] 0, no growth; 1, sparse (<30 per plate) and tiny (<1-mm diameter) colonies; 4, moderate (30 to 300 per plate) and tiny (<1-mm diameter) colonies; 4+, most abundant growth with a score of 4; 7, abundant (>300 per plate) and tiny (<1-mm diameter) colonies.
[b] One of two strains tested.
[c] One of three strains tested.

time intervals, indicating that the overall performance for these taxa was highly satisfactory by all three methods.

For the majority of test organisms there was no significant difference in the speed or quality of growth achieved by any system.

The Anaero*Gen* system was better than the anaerobe chamber at the 48-h reading for 13 of 135 isolates (Table 2). The GasPak system was also better than the anaerobe chamber at the 48-h reading for 13 of 135 isolates (Table 3). The Anaero*Gen* was better than the GasPak on 4 occasions (4 of 135), and the GasPak was better than the Anaero*Gen* on 5 occasions (5 of 135), with 126 of 135 isolates showing equal growth at 48 h (Table 4).

The Anaero*Gen* produced better growth than the GasPak at 72 h on two occasions, and the GasPak produced better growth than the Anaero*Gen* at 72 h on three occasions (Table 1),

TABLE 3. Occasions on which growth in the GasPak system was better than growth in the chamber at 48 h

Test organism	Growth score[a]	
	GasPak	Chamber
Actinomyces israeli[b]	4	1
Actinomyces gerencseriae	4	0
Actinomyes odontolyticus[b]	4	1
Bifidobacterium adolescentis	7	4
Bacteroides gracilis	7	0
Bacteroides gracilis	7	4
Bacteroides ureolyticus[c]	7	4
Campylobacter concisus	7	4
Campylobacter mucosalis	7	4
Mobiluncus mulieris	4	0
Mobiluncus mulieris	4	0
Mobiluncus curtissii subsp. *curtissii*[c]	4	0
Porphyromonas asaccharolytica	7	0

[a] 0, no growth; 1, sparse (<30 per plate) and tiny (<1-mm diameter) colonies; 4, moderate (<300 per plate) and tiny (<1-mm diameter) colonies; 7, abundant (30 to 300 per plate) and tiny (<1-mm diameter) colonies.
[b] One of two strains tested.
[c] One of three strains tested.

TABLE 4. Significant differences in the growth of anaerobes in the Anaero*Gen* and GasPak systems observed at 48 h

Test organism	Growth score[a]	
	Anaero*Gen*	GasPak
Bacteroides gracilis	4	7
Bacteroides gracilis	0	7
Bilophila wadsworthia[b]	4	0
Campylobacter concisus	4	7
Campylobacter curvus/rectus[c]	4	7
Eubacterium alactolyticum	7	4
Lactobacillus minutus[b]	7	0
Peptostreptococcus asaccharolyticus	4	7
Selenomonas noxia	4	0

[a] 0, no growth; 1, sparse (<30 per plate) and tiny (<1-mm diameter) colonies; 4, moderate (30 to 300 per plate) and tiny (<1-mm diameter) colonies; 7, abundant (>300 per plate) and tiny (<1-mm diameter) colonies.
[b] One of two strains tested.
[c] One of five strains tested.

indicating there was no difference in the performance of the two envelope systems.

DISCUSSION

The Oxoid Anaero*Gen* and BBL GasPak jar systems are designed to support the growth of anaerobes and facultative anaerobes. The anaerobe chamber gave the most consistent growth, with all isolates viable at 7 days of incubation. The chamber also allows interim examination of plates without exposure to air. The BBL GasPak, because of its provision of H_2, may be a better method for the strains of *Campylobacter* tested (*C. concisus*, *C. mucosalis*, *C. rectus/curvus*, and *C. gracilis*) and for *B. ureolyticus* organisms which require H_2 as an electron donor (1).

This study indicates that the Anaero*Gen* is an effective product for creating an anaerobic atmosphere in a jar. The Anaero*Gen* never failed to reduce the methylene blue indicator in the jar, whereas 10% failures were observed with the GasPak. The catalyst pellets were properly reactivated at each time of use, and the failed jar and jar lids could be switched for successful use for the next set of concurrently tested organisms. The indicator strip was reduced faster with the Anaero*Gen* than with the GasPak.

The Anaero*Gen* system was easy to use, having the advantage of not requiring the addition of water or the maintenance of the palladium catalyst. The list price for the Anaero*Gen* is $24 per box of 10, and that for the GasPak is $17.60 per box of 10.

The anaerobic jar, with either envelope, was more efficient than the anaerobe chamber at initiating growth by the first reading at 48 h. While the GasPak and the Anaero*Gen* have 4 to 10% and 9 to 13% CO_2, respectively, the anaerobic gas used in the chamber had 5% CO_2. Since CO_2 is stimulatory to many anaerobes, this shortage could be one explanation for the slower growth observed in the chamber. All systems were tested concurrently for a given set of organisms. Proper humidity was maintained in the chamber, the GasPak created excess humidity in the jar bottom, and the Anaero*Gen* exhibited a dryer atmosphere.

By 7 days, all three methods were essentially equal in their ability to support anaerobic growth. Most of the time there was no noticeable difference in growth or in culture outcome. The Anaero*Gen* atmosphere-generating system is highly effective in

supporting the growth of anaerobes and is easier to use than the GasPak.

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