



Effectively Culturing Anaerobic Bacteria



INTRODUCTION

To obtain correct diagnoses and safeguard patient health, modern clinical laboratories must ensure accurate, timely isolation and identification of anaerobic bacteria and related organisms.

Traditional approaches to this problem have centered on the use of anaerobic chambers or gas bags. However, the Anoxomat anaerobic system from Spiral Biotech, an Advanced Instruments company, is an attractive alternative. Its automatic evacuation/replacement method offers many important advantages for laboratory managers over conventional gas bag or anaerobic chamber technologies.

Use of this system is steadily growing in the clinical marketplace. Hospital and research laboratories alike are finding that the system can quickly create ideal conditions for the growth of most anaerobic, microaerophilic, and capnophilic organisms.

Comparative studies have demonstrated that the Anoxomat provides significant benefits. Its innovative approach can save consumables, costs, time, and effort for laboratory managers and their staffs. Users report that the system's fast, easy operation makes their work process more flexible. Finally, Anoxomat's built-in quality assurance features help guarantee reliable results.

THE CLINICAL NEED

Hospital infection rates have increased sharply in recent years, and show little signs of abating. Clinicians must ascertain the presence or absence of a wide array of pathogens. Ideally, these determinations should be made before patients leave the hospital, avoiding costly callbacks plus delayed diagnosis and treatment.

This puts growing pressure on laboratories to quickly and reliably culture a variety of clinically important organisms from patient specimens, in varying test environments. Capnophilic organisms demand elevated concentrations of carbon dioxide. Microaerophiles require reduced oxygen concentrations. Obligate anaerobes flourish only when there's no oxygen at all.

With conventional culturing techniques, this range means a laboratory must maintain multiple devices or solutions, such as separate anaerobic chambers or a variety of gas bags, in inventory. Also of concern is the tendency of conventional technologies to complicate handling and the general work process for laboratory personnel — with negative impacts on time, costs, and error rates. Overall, this clinical need places a premium on flexible, fast, accurate results.



ANAEROBIC CHAMBERS

An anaerobic chamber is an airtight enclosure that is designed to attain an oxygen-free environment for the purposes of bacteriologic cell culture. Most chambers are desktop-sized, windowed glove boxes that feature external controls. Accessorized with vacuum pumps and recirculation blowers, the chamber is connected to gas cylinders. These typically contain nitrogen and hydrogen, required to create the appropriate internal anaerobic atmosphere.

Variations in gases and cycling details can render such chambers also appropriate for cultivation of microaerophiles and capnophiles. Laboratories may maintain a range of incubators in which given atmospheric conditions are fixed or variable.

Generally, chambers provide reliable means for growth of the required organisms. A chamber's advantages remain most obvious in very-high-volume laboratories, where thousands of similar samples must be processed in a given period.

However, chamber technology is the oldest such technique still in common use. It's relatively cumbersome and time-consuming to use. It provides relatively slow anaerobiosis. It requires relatively complex and costly equipment. And it demands a large footprint in today's space-hungry laboratory.

The chamber method necessitates the use of comparatively expensive pre-reduced anaerobically sterilized (PRAS) media. It entails a substantial consumption of other consumables, chiefly gases, and calls for costly, inconvenient servicing.

GAS BAGS

The gas bag method achieves replacement of the ambient oxygenated environment in a standard jar with an anaerobic atmosphere by using a self-contained, flexible plastic gas generator envelope. The bag releases H_2 and CO_2 when water is added, in the presence of a standard palladium catalyst sachet, establishing anaerobiosis.

Gas bags take up the smallest footprint of any of the current anaerobic isolation technologies. However, the bags themselves are disposable, one-use items, so this method

incurs the most significant consumables cost. Expense is further magnified when microaerophiles and capnophiles must be tested in addition to anaerobes, requiring separate stocks of different gas bags. In addition, some users report significant rates of jar failure using gas bags.

In normal functioning, gas bags take from 1 to 4 hours to achieve anaerobic conditions. For the remainder of the culturing process, the jar cannot be disturbed — for instance to add or remove samples — or the entire process must be redone.



Finally, testing (see Figure 2) seems to indicate that a number of organisms grow more slowly in gas bags than with other technologies, displaying smaller colony sizes especially at early intervals. And with gas bags more than with other methods, some organisms may fail to be recovered at all.

ANOXOMAT SYSTEM

This alternative to the two technologies above uses an automated evacuation/replacement method to create an anaerobic, microaerophilic, or capnophilic environment in an attached standard jar. One to three jars may be used, in varying sizes holding various quantities of stacked culture plates.

“Our glove box was breaking down a lot, and it was also ergonomically very difficult for our techs to work in it.”

— Angelika Lichtenfeld,
Calgary Lab Services

“Our hood was no longer any good. So we were using set-up bags, and they’re expensive ... Now the techs don’t have to open up all those bags.”

— Jeanine Palmer,
Sharp Memorial
Hospital, San Diego



The Anoxomat's automated system repeats its evacuation/replacement cycle three times. During each cycle, 80 percent of the jar's content is removed by the unit's vacuum and replaced by an anaerobic gas mixture from a connected gas cylinder. After the third cycle, any small amount of remaining oxygen — as well as any residual oxygen liberated from the media in the plates inside the jar — is removed via a standard palladium catalyst sachet.

Users report that one cycle achieves a microaerophilic environment within 2 minutes. Three cycles produce an anaerobic atmosphere within 3 minutes. The system indicates the end of each cycle by an audible signal.

The system also executes additional quality assurance checks: for leaks, for adequate catalyst activity, and for adequate addition of replacement gas.

“The Anoxomat was an easier operation ... On gases alone, in the first year of operation, it probably saved us around \$8,000.”

*— Frank Hollis,
Hackensack Medical
Center*



COMPARATIVE TESTING

Several researchers have examined the efficacy of differing isolation techniques. This testing usually evaluates the growth of obligate anaerobes, as well as the recovery of anaerobic organisms from clinical specimens. Studies emphasize that clinical specimens must routinely be collected, transported, and processed properly.

In typical testing, plates are incubated at temperatures around the range of 35° to 37° C. After a suitable period, for example at 24 and 48 hours, the plates are examined for mean size of colony growth and for characteristics such as colony size and morphology.

One study [1] tested 54 stock strains of obligate anaerobic bacteria, representing different genera of clinical significance. One set of plates each was incubated in jars using the Anoxomat system, another in jars using a gas bag technique, and the third set using an anaerobic chamber.

Results of this study suggest faster growth of anaerobic bacteria inside a jar in the Anoxomat system compared to the other two technologies. At 24 hours, of the strains

tested, 51% yielded larger colonies in the Anoxomat than in the chamber, and 30% yielded more growth using the Anoxomat than the gas bags. (See Figure 1.)

After 48 hours, recovery of isolates from clinical specimens became more comparable between the chamber (94.4%) and the Anoxomat system (93.5%). These contrasted with a poorer showing from the gas bag technique (88.9%).

Another study [2] evaluated growth in 50 species of anaerobes, 29 strains of capnophiles, and 11 strains of microaerophiles. In this case, Anoxomat's automatic evacuation/replacement performance was compared against anaerobic chambers, carbon dioxide incubators, and manual evacuation/replacement.

COMPARISON OF ANOXOMAT®, ANAEROBIC CHAMBER, AND GASPAK® SYSTEMS

Figure 1. Comparison of mean colony sizes and semi-quantitative growth of anaerobic organisms after 24-hour incubation in anaerobic chamber, GasPak jar, and Anoxomat® jar. Note: this is a partial list of study results.

Strain No.	Organism	Mean colony size (mm)			Semi-quantitative growth (CFU/mL)		
		Chamber	GasPak	Anoxomat	Chamber	Gas Bag	Anoxomat
8904	<i>Bacteroides eggerthii</i>	0.1	0.1	0.1	10 ²	10 ⁴	10 ⁴
8939	<i>B. fragilis</i>	0.3	0.7	0.8	5 x 10 ⁴	5 x 10 ⁴	5 x 10 ⁴
8978	<i>C. gracilis</i>	0.4	0.5	0.5	10 ⁵	10 ⁵	10 ⁵
ATCC29741	<i>B. thetaiotaomicon</i>	0.4	0.7	0.8	10 ⁵	10 ⁵	10 ⁵
8102	<i>F. gonidiaformans</i>	0.1	0.1	0.1	10 ⁴	10 ⁴	10 ⁴
7928	<i>F. gonidiaformans</i>	0.4	0.6	0.8	5 x 10 ⁴	5 x 10 ⁴	5 x 10 ⁴
ATCC 9817	<i>F. mortiferum</i>	1.2	1.2	1.1	10 ⁵	10 ⁵	10 ⁵
ATCC 25556	<i>F. necrogenes</i>	0.4	0.5	0.7	5 x 10 ⁴	10 ⁵	10 ⁵
ATCC 35585	<i>F. sulci</i>	0.3	0.3	0.3	10 ⁵	10 ⁵	10 ⁵
ATCC 25260	<i>Porphyromonas asaccharolytica</i>	0.1	0.1	0.2	10 ⁵	10 ⁵	10 ⁵
ATCC 35406	<i>P. endodontalis</i>	NG ^a	NG ^a	0.3	NG ^a	NG ^a	10 ⁶
ATCC 33277	<i>P. gingivalis</i>	0.1	0.1	0.1	5 x 10 ²	10 ⁶	10 ⁶
7603	<i>Prevotella intermedia</i>	NG ^a	0.1	0.3	NG ^a	5 x 10 ⁴	5 x 10 ⁴
7784	<i>P. intermedia</i>	0.1	0.1	0.1	10 ⁵	10 ⁵	10 ⁵
8859	<i>C. clostridioforme</i>	0.1	0.2	0.1	5 x 10 ⁴	10 ⁵	10 ⁵
8878	<i>C. difficile</i>	0.5	1.8	1.3	2 x 10 ²	6 x 10 ⁶	8 x 10 ⁶
8640	<i>C. difficile</i>	1.3	1.5	1.8	6 x 10 ⁶	104	2 x 104
8922	<i>C. innocuum</i>	0.9	1.1	1.0	5 x 10 ⁴	5 x 10 ⁴	5 x 10 ⁴
8319	<i>C. perfringens</i>	2.8	2.5	2.8	10 ⁵	10 ⁵	10 ⁵
ATCC 13124	<i>C. perfringens</i>	5.0	5.0	5.0	10 ⁵	10 ⁵	10 ⁵
ATCC 27337	<i>Peptostreptococcus anaerobius</i>	0.2	0.2	0.2	10 ⁵	10 ⁵	10 ⁵
8850	<i>P. asaccharolyticus</i>	0.2	0.4	0.4	10 ⁵	10 ⁵	10 ⁵
ATCC 29745	<i>P. asaccharolyticus</i>	0.1	0.3	0.3	5 x 10 ⁴	10 ⁵	10 ⁵
ATCC 23195	<i>P. micros</i>	NG ^a	NG ^a	0.1	NG ^a	NG ^a	10 ⁴

a NG = no growth



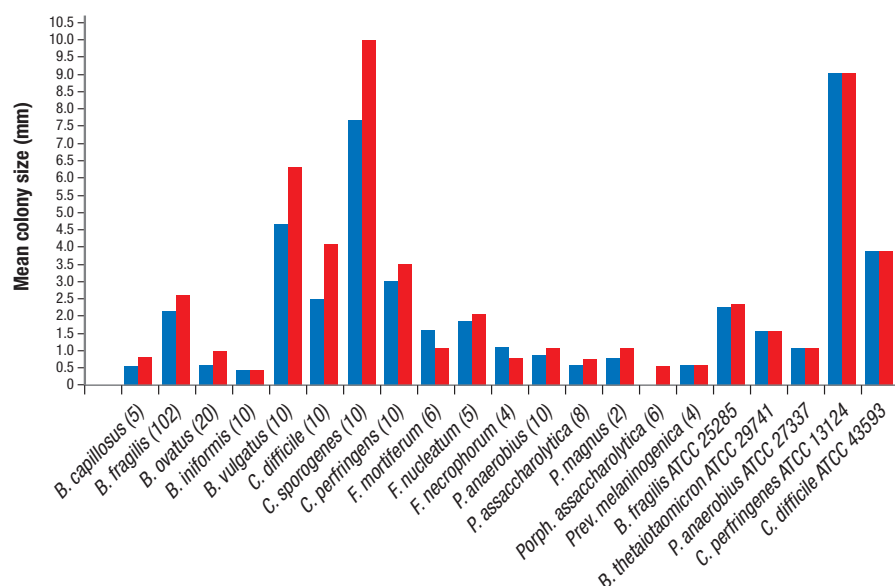
Again, the Anoxomat posted superior numbers. At 24 hours, for anaerobes, the Anoxomat equaled the chamber's growth in 42% of cases, and surpassed it in another 52%. Even at 48 hours, the Anoxomat attained larger colony growth in 33% of anaerobic colonies. The Anoxomat matched the chamber equally in growth of capnophiles and microaerophiles. This study also made note of the versatility of the Anoxomat system in growing a full range of nonaerobic clinically important organisms.

A most recent study [3] featured Anoxomat in a direct comparison against gas bag technology only. A total of 227 obligate anaerobic bacteria — representing different genera — were investigated for growth on plates. Strains were scored for density, colony sizes, susceptibility zones of antibiotic inhibition, and speed of anaerobiosis (as measured by reduction of a test strip indicator).

The results here conclusively demonstrate that the Anoxomat system generally provides faster growth than the gas bag technique. Of 227 strains, at 48 hours 67% were larger in the Anoxomat jar, with 12% of comparable size and only 21% larger in the gas bag jar. (See Figure 2.)

Figure 2. Comparison of mean colony sizes in gas bag anaerobic jar (n) and Anoxomat jar (n). The differences in colony sizes were most apparent with *C. sporogenes* (3 mm), *C. difficile* (1.7 mm), and *B. vulgatus* (2 mm).

The study finds no significant difference in zones of inhibition, but notes that the Anoxomat achieved both better colony size and density, plus significantly faster anaerobiosis.



ANOXOMAT ADVANTAGES

As several comparative studies and extensive user experience demonstrate, the Anoxomat system is proven *highly effective at cultivating pathogens*. It can attain growth performance that easily equals or exceeds that of chambers or gas bags. Some users report recovering organisms they've never been able to grow before, such as the most fastidious bacteria.

The system provides the *most rapid achievement of anaerobic conditions*. Anaerobiosis can be reached in a few minutes, as opposed to 1 to 4 hours for gas bags or chambers.

Anoxomat offers *exact, repeatable environmental conditions*. Its gas mixtures stay within 0.5% of desired values. This high precision, combined with ensured reproducibility, allows microbiologists to draw accurate conclusions from repeated findings.

The system also demonstrates *substantial cost-of-ownership savings*. Using 10 or 12 gas cylinders per year and requiring an expensive service contract, an anaerobic chamber's ongoing costs for a single year may pay the entire purchase price of an Anoxomat system.

A comparison with the gas bag method is equally dramatic. Consider a typically busy laboratory in a large hospital utilizing gas bags for anaerobe isolation. This lab might use 15 bags per day, with associated indicator strips, deployed in standard glass jars. At \$1.89 per bag and indicator, disposables cost more than \$28 per day, or over \$10,300 per year.

But when this laboratory switches to the Anoxomat system, a volume of 15 jars a day — with a low fill cost of only 14 cents per jar — translates to using only 6 or 7 gas cylinders per year. Approximate annual gas cost: less than \$700. Total savings difference versus gas bag technology: more than \$9,500 per year.

(Note: the above examples assume 90% anaerobic to 10% microaerophilic work. Pricing based on 200 ft³ anaerobic gas mixture.)

Anoxomat can even help users *avoid purchasing expensive pre-reduced media*. If desired, the system can remove oxygen from media right in the user's lab. Many lab managers are happy to escape paying this pre-reduced premium.

Finally, the Anoxomat Mark II model introduces a number of *significant improvements* to an already successful technology.

Its touchscreen interface and intuitive software increase *ease of use* for a technology that already provides substantial walkaway convenience.

The improved model's innovative *automatic quality assurance features* increase confidence and prevent delays. They replace older technology's reliance on color indicator strips, which take 2-4 hours to turn and are subject to frequent failure. Anoxomat Mark II is programmed to perform quality assurance checks that ensure the jar has reached an airtight status, and to look for pressure drops warning of leaks. Another QA test makes sure the jar's catalyst — a standard palladium metal sachet — is working to bind any residual oxygen after evacuation cycling.

The system also offers *unmatched flexibility*. Users can take plates in and out of jars, then return the jars to anaerobic environments in minutes, not hours. Also, the system can quickly and accurately culture anaerobes, capnophiles, and microaerophiles. For any small-volume or mid-volume laboratory isolation work, Anoxomat is clearly the instrument of choice.

Anoxomat systems are now in use in hundreds of laboratories worldwide. Successful clinical applications include hospitals, universities, and public health facilities. The system is also providing superior performance in industrial applications including food and beverage, water supply, and pharmaceutical uses.

“Because of the way the Anoxomat jar works, I can open it up, take out a sample, and make it anaerobic again very fast.”

— Dr. Hannah Wexler,
VA Wadsworth Medical
Center, Los Angeles

“We saved a considerable amount in gases ... I think we're using a quarter of what we've used ... We're looking at purchasing a second Anoxomat.”

— Angelika Lichtenfeld,
Calgary Lab Services

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(800) 554-1620 or +1 (781) 320-9000

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