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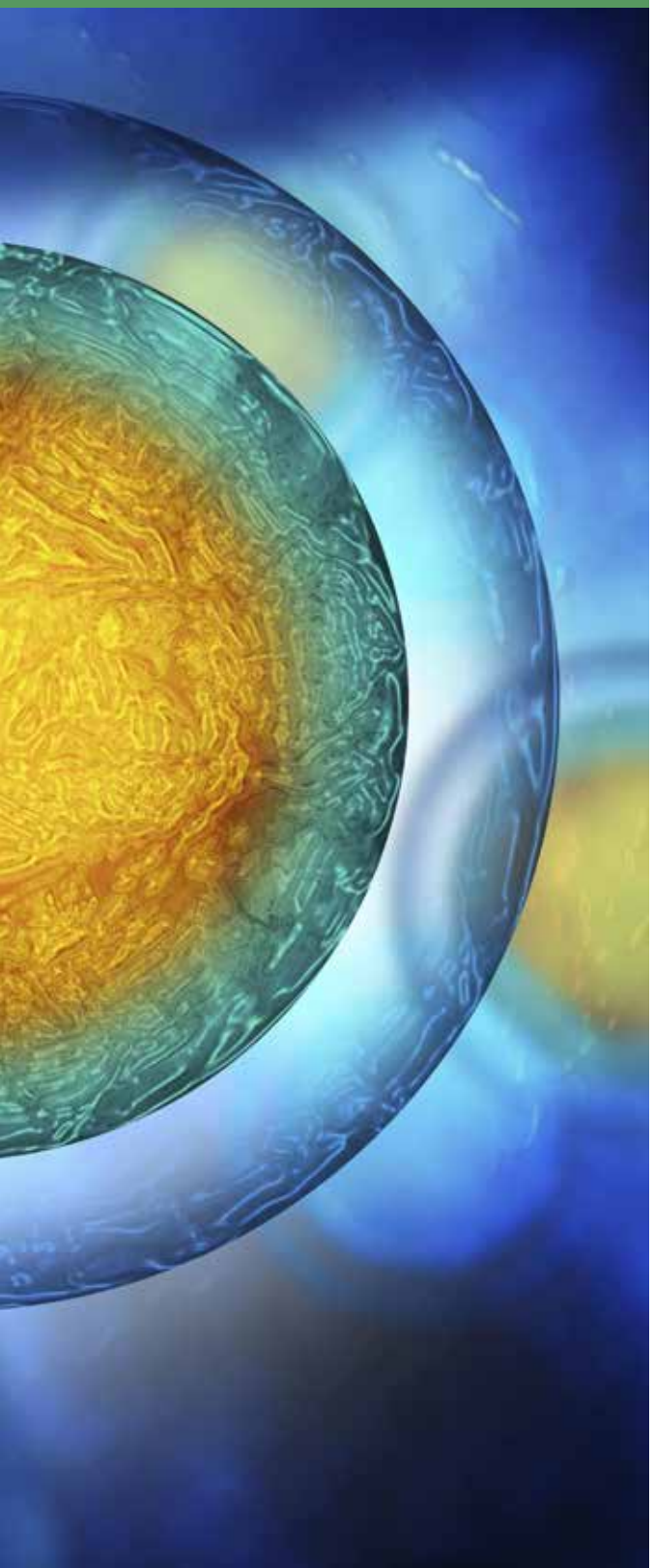

Advanced™ Anoxomat® III and Ergonomic Jars: an alternative to the Anaerobic Chamber and GasPak™ System

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COMBATING A LOSS IN PRODUCTIVITY

Microbiology laboratories are always striving to streamline workflow and find superior practices for culturing bacteria. Because laboratories must correctly diagnose illness and safeguard patient health, it is vital that the method used yields accurate isolation and identification of organisms in a timely manner.

Hospital and research laboratories value a system's ability to quickly create ideal conditions for the growth of most anaerobic, microaerophilic, and capnophilic organisms. Traditional approaches have centered on the use of anaerobic chambers or gas generating sachet systems, such as the BD GasPak™ System. However, the Advanced Anoxomat III system from Advanced Instruments is an extremely attractive alternative. Its automated McIntosh and Filde's[1] evacuation and replacement method for creating suitable environments for bacterial cultivation offers important advantages for laboratory managers over these conventional technologies.

> "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

Why bacterial cultivation is important

Culturing bacteria effectively is essential to the biotechnology, pharmaceutical, medical, food safety, and alternative energy industries.

In the clinical setting, laboratories are under increasing pressure to determine the presence or absence of a wide array of pathogens quickly and reliably. Patient specimens must be cultured in a variety of atmospheric environments if patients are to receive the correct medical care in a timely manner. This need for speed and reliability places a premium on flexible, fast, and accurate results.

Proper specimen collection, transport, and isolation of bacteria are critical to the analysis and identification of pathogens, especially given today's economic challenges and rising patient care costs.

Detecting the correct microbial agents helps clinicians to prescribe the appropriate antibiotics at the right dosage, minimizing the risk of antibiotic resistance.

In the biopharmaceutical setting, bacterial culturing provides a means by which to assess microbial resistance to antibiotics. Researchers armed with a better understanding of the physiological growth of bacteria add essential value to the biotechnology industry, which in turn leads to improvements in human health and overall wellbeing.

An absolutely critical component of bacterial processing is generating the environment for bacterial growth. Three methods typically used to create suitable atmospheric conditions for growing non-aerobic bacteria are anaerobic chambers, gas generating sachet systems, and automated jar systems — such as the Advanced Anoxomat III.

Anaerobic chambers

Anaerobic chambers, the oldest technique still in use today, are airtight enclosures designed to attain an environment suitable for the cultivation of bacteriologic microorganisms.

Most chambers are windowed glove boxes with external controls (Figure 1). These chambers can be up to four times the size of the Anoxomat III — a considerably large footprint on the already crowded modern lab bench.

Figure 1. Anaerobic Chamber

Anaerobic chambers can generally provide a reliable means for growth of microorganisms, but their cumbersome size and slower anaerobiosis restrict them to laboratories where large volumes of samples are tested in a single specific environmental condition. Anaerobic chambers also require fairly complex and costly equipment repairs should the system malfunction.

In laboratories where space and time constraints are critical, the Anoxomat III provides flexibility for creating different environments — unlike anaerobic chambers which are unable to cultivate microaerophilic and anaerobic organisms using the same system.

Gaspaks

The BD GasPak System, the most commonly used gas generating sachet system, uses a self-contained sachet (Figure 2) to replace the ambient oxygenated environment in a standard box and/or jar with a specific gas constitution.

The system requires the use of a different sachet to create each of the 3 available environments (anaerobic, microaerophilic, and capnophilic).

Figure 2. Gaspak System

GasPaks may take between 2-24 hours to achieve the desired environment and once conditioned, the jar cannot be opened to add or remove plates for the remainder of the culturing process.

Unlike with the Anoxomat system, if the jar or box is unsealed during the culturing process, the atmosphere is compromised and needs to be reconditioned with a new sachet, resulting in a waste of materials and time.

Of the 54 stock strains tested, 51% of the colonies grown with the Anoxomat system were larger than the chamber, and 30% were larger than the GasPaks. In clinical isolates, the Anoxomat recovered 94%, the chamber recovered 94%, Gaspaks recovered 89%.[2]

The results of our comparative study also suggest that growth of anaerobic bacteria may be faster inside a jar in the Anoxomat system than in a chamber or a GasPak jar.[2]

Anoxomat III

The Anoxomat III (Figure 3) is an automated system that can create an anaerobic, microaerophilic, or custom atmospheric recipes (such as capnophilic) in jars using an automated evacuation and replacement method.

The Anoxomat III is able to generate an anaerobic environment within minutes, compared to up to 24 hours when using the BD GasPak System. The user programming function allows labs to develop custom environment recipes using any combination of gases connected to the Anoxomat III. The Anoxomat III's compact design (roughly the size of a desktop printer) saves on valuable lab space and time with the ability to connect up to 4 jars at once. The Anoxomat jars are available in a variety of sizes and styles, with each able to hold different numbers of stacked culture plates.

With the Anoxomat III, gas mixtures are automatically delivered into the jars accurately, with gas constituents remaining stable within 0.5% of the delivered concentration(s) up to 48 hours incubation based on internal testing at AI. The low gas consumption of the Anoxomat III means a substantially lower cost of ownership when compared to laboratory expenditures for anaerobic chambers (gas supplies) and GasPaks, resulting in a rapid return on investment with the Anoxomat III.

Transitioning from manual to automated systems is a key strategy in creating a more responsive and effective microbiology laboratory. The Anoxomat III, an automated system, provides guaranteed improvements in timeliness and efficiency.

The automatic quality assurance program of the Anoxomat III provides each recipe with worry-free assurance by detecting possible leakage in the jar prior to running and ensures appropriate anaerobic catalyst activity, thus eliminating the guesswork and frustration attributed to such pain points.

The Anoxomat III provides an unmatched flexibility to technicians of any skill level. It allows the ability to generate environments in the jars in minutes — rather than hours when compared to other methods — and plates can be added or removed, and environments reconditioned, with minimal impact to the cultures being grown.

The Anoxomat III can easily trace and track samples for quality control by featuring an optional barcode scanner and printer to simplify the process of record-keeping.

The Anoxomat system combats major productivity losses by eliminating inherent risks in bacteria recovery in the following ways.

- Ensures anaerobic, microaerophilic, and custom growth environments are uncompromised through built-in safeguards
- Minimizes disruption when transferring plates into incubators through the improved portability of the Anoxomat jars
- Provides reliable process documentation

Figure 3. Advanced™ Anoxomat® III

INTRODUCTION

Recently, Advanced Instruments updated the Ergonomic Jar design to improve its overall usability in the laboratory setting. The new Ergonomic Jar, available with the Anoxomat III, features a transparent cover for ease of use while inspecting plates as well as a latching mechanism to provide improved seal integrity. The following study provides technical data demonstrating the new Ergonomic Jars' equivalence to previous proven Standard Jars and shows continued high quality of results when using the new Ergonomic Jars.

This study compared the performances of the Ergonomic Jars (AJ9049 and AJ9050), the Standard Jars (AJ9025), and the BD BBL GasPak 100 jars.

Test environments

Representative bacteria were grown in anaerobic, microaerophilic, and capnophilic environments.

Table 1 on page 6 lists the atmospheric conditions, bacteria, and plate type for each test environment.

Jar types

Four jar types were tested in this study, with 15 jars in total used to cultivate each of the three bacteria types.

Table 2 on page 6 summarizes the jar types.

Jar conditioning

All Anoxomat jars were conditioned using a single Anoxomat III unit. The GasPak 100 jars were conditioned using GasPak sachets.

After conditioning, the oxygen concentration of each jar was measured using an OxySense® 325i optical oxygen analyzer.

Test scheme

The average colony size (diameter in millimeters) of the bacteria grown in the Ergonomic Jars was compared to the average colony size of both the Standard Jars and the GasPak 100 jars.

Incubation times were identical for each of the jar types tested across all three bacteria species.

Negative control plates were incubated alongside test plates to assure the appropriate gas constituents were present in each of the jars.

Table 3 on page 6 summarizes the test scheme.

In comparison to the GasPak jar system "The Anoxomat System provided superior growth, in terms of density and colony size, and achieved anaerobiosis more rapidly."[3]

Table 1. Test environments

Environment	Atmosphere	Bacteria	Plate type
Anaerobic	≤ 0.2% or detectable level of oxygen present	*Bacteroides fragilis*	Columbia Agar
Microaerophilic	~ 6% oxygen present	*Campylobacter fetus*	Chocolate II Agar
Capnophilic	> 5% carbon dioxide present	*Neisseria gonorrhoeae*	Chocolate II Agar

Table 2. Jar types

Jar Type	AI Part Number	Volume	# Jars	Conditioning
Anoxomat Ergonomic	AJ9049	12 plates	5	Anoxomat III
Anoxomat Ergonomic	AJ9050	24 plates	5	Anoxomat III
Anoxomat Standard	AJ9025	12 plates	2	Anoxomat III
BD GasPak 100	not applicable	12 plates	3	BD GasPak sachets

Table 3. Bacterial test scheme

Environment	Bacteria	# Plates			
		Anoxomat Ergonomic 24 (AJ9049)	Anoxomat Ergonomic 12 (AJ9050)	Anoxomat Standard 12 (AJ9025)	BD GasPak
Anaerobic	*B. fragilis*	9	18	9	9
	M. luteus (control)	3	6	3	3
	C. fetus	9	18	9	9
	B. fragilis (control)	3	6	3	3
Capnophilic	*N. gonorrhoeae*	9	18	9	9
	B. fragilis (control)	3	6	3	3

ACCEPTANCE CRITERIA

Test results were evaluated against the following acceptance criteria:

- The Ergonomic jars (AJ9049 and AJ9050) shall maintain environments suitable for the equivalent growth for each of the three atmospheric conditions when compared to the Standard jars (AJ9025) and when compared to the GasPak 100 jars. Growth equivalence will be determined by the average colony diameter size.
- For all three atmospheric conditions tested, the average colony diameter size (in mm) of plates grown in Ergonomic jars shall be greater than or equal to, or within the 95% confidence intervals of, the average colony diameters of bacteria grown in both the Standard jars and the GasPak 100 jars.
- The Ergonomic jars will pass all quality control/quality assurance (QC/QA) tests performed by the Anoxomat III during conditioning.
- The Anoxomat III will condition the Anoxomat jars to within ± 0.3% of the displayed oxygen concentration using the OxySense System per recipe type:
    - Anaerobic condition: ≤ 0.2% (or undetectable) oxygen
    - Microaerophilic condition: 6.0% ± 0.3% oxygen
    - Capnophilic condition: 10.0% ± 0.3% oxygen

DATA ANALYSIS

The diameters of three to five bacterial colonies per plate were measured using a digital caliper. Diameters were recorded in millimeters. Only isolated colonies were measured.

Oxygen concentration measurements were recorded prior to each Anoxomat jar incubation period.

Descriptive statistics and representative graphs were generated using Minitab® software with 95% confidence intervals for each of the jar types as part of the comparison.

RESULTS

Anaerobic condition

When growing *B. fragilis,* all Anoxomat jars (AJ9049, AJ9050, and AJ9025) yielded average colony diameters that were 0.29 mm, 0.11 mm, and 0.22 mm larger, respectively, than those obtained from the GasPak 100 jars.

Ergonomic 12-plate Jar produced an average colony diameter 0.18 mm larger than the average colony diameter of the Standard 12-plate Jar. However, the Ergonomic 24-plate Jar yielded an average colony diameter that was lower than the Standard 12-plate Jar (0.11 mm smaller). The discrepancy between the Ergonomic type jars may be due to the difference in jar volume.

See Table 4 and Figure 4 (page 8).

Microaerophilic condition

When growing *C. fetus*, all Anoxomat jars (AJ9049, AJ9050, and AJ9025) yielded average colony diameters that were 0.34 mm, 0.15 mm, and 0.26 mm larger, respectively, than those obtained from the GasPak 100 jars.

The Ergonomic 12-plate Jar produced an average colony diameter that was 0.08 mm larger than the Standard 12-plate Jar. However, the Ergonomic 24-plate Jar yielded an average diameter that was lower than the Standard 12-plate Jar (-0.11 mm smaller). The discrepancy between the Ergonomic type jars may be due to the difference in jar volume as observed in the anaerobic condition or due to the lower number of the countable colonies observed in the 12-plate jars.

See Table 5 and Figure 5 (page 9).

Capnophilic condition

When growing *N. gonorrhoeae*, all jar types yielded colony sizes colony sizes comparable to one another (all observed differences were within 0.01 mm). No jar type clearly produced the largest average colony for the capnophilic condition.

See Table 6 and Figure 6 (page 10)

> "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

When growing *B. fragilis*, all Anoxomat jars yielded average colony diameters that were larger than those obtained from the BD GasPak 100 jars.

Table 4. Anaerobic Condition Test Results Summary

JAR TYPE	JAR #	ANAEROBIC CONDITION (*B. fragilis*)					
		O_2%	# Plates	# Colonies	Mean Dia. (mm)	SD	%CV
Anoxomat Ergonomic 24	#1	0.0%	18	54	2.77	0.378	14
	#2	0.0%	18	54	2.82	0.258	9
	#3	0.0%	18	54	2.68	0.280	10
	#4	0.0%	18	54	2.74	0.239	9
	#5	0.1%	18	54	2.60	0.298	11
Anoxomat Ergonomic 12	#1	0.0%	9	27	2.85	0.240	8
	#2	0.0%	9	27	2.94	0.267	9
	#3	0.1%	9	27	2.82	0.240	9
	#4	0.0%	9	27	2.90	0.252	9
	#5	0.0%	9	27	3.00	0.271	9
Anoxomat Standard 12	#1	0.1%	9	27	3.00	0.265	9
	#2	0.2%	9	27	2.67	0.277	10
BD GasPak 100	#1	NA	9	27	2.58	0.296	11
	#2	NA	9	27	2.63	0.333	13
	#3	NA	9	27	2.63	0.275	10

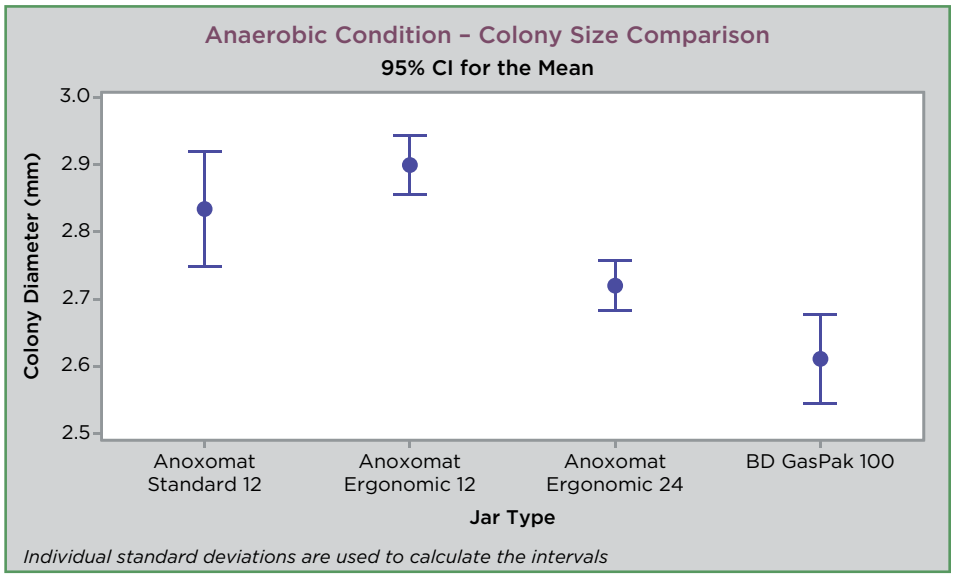

Figure 4. Colony Size Comparison for Anaerobic Condition

Table 5. Microaerophilic Condition Test Results Summary

JAR TYPE	JAR #	MICROAEROPHILIC CONDITION (*C. fetus*)					
		O_2%	# Plates	# Colonies	Mean Dia. (mm)	SD	%CV
Anoxomat Ergonomic 24	#1	6.0%	7	21	2.02	0.203	10
	#2	6.0%	10	30	2.12	0.250	12
	#3	5.8%	9	27	2.39	0.250	10
	#4	5.8%	14	28	2.32	0.276	12
	#5	6.0%	13	33	2.40	0.287	12
Anoxomat Ergonomic 12	#1	6.1%	6	16	2.52	0.297	12
	#2	6.1%	8	17	2.46	0.203	8
	#3	6.1%	3	5	2.36	0.306	13
	#4	5.9%	3	9	2.36	0.256	11
	#5	6.2%	3	9	2.50	0.380	15
Anoxomat Standard 12	#1	5.9%	5	11	2.63	0.259	10
	#2	6.1%	7	16	2.10	0.446	21
BD GasPak 100	#1	NA	9	27	2.27	0.270	12
	#2	NA	9	27	2.07	0.235	11
	#3	NA	9	27	1.95	0.402	21

When growing *C. fetus*, all Anoxomat jars yielded average colony diameters that were larger than those obtained from the BD GasPak 100 jars.

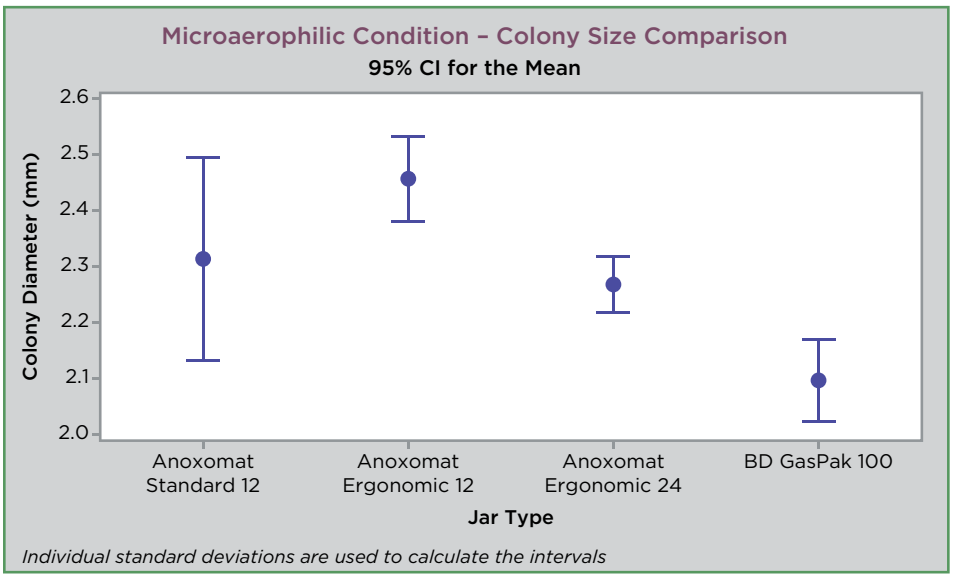

Figure 5. Colony Size Comparison for Microaerophilic Condition

> 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

Table 6. Capnophilic Condition Test Results Summary

JAR TYPE	JAR #	CAPNOPHILIC CONDITION (*N. gonorrhoeae*)					
		O_2%	# Plates	# Colonies	Mean Dia. (mm)	SD	%CV
Anoxomat Ergonomic 24	#1	9.8%	18	48	4.01	0.324	8
	#2	9.9%	18	47	4.07	0.234	6
	#3	9.9%	18	39	4.07	0.234	6
	#4	9.9%	18	43	4.05	0.243	6
	#5	9.8%	18	43	4.12	0.267	6
Anoxomat Ergonomic 12	#1	10.1%	9	24	4.09	0.332	8
	#2	10.0%	9	26	4.08	0.262	6
	#3	10.1%	9	27	4.03	0.249	6
	#4	10.0%	9	27	4.07	0.272	7
	#5	10.0%	9	27	4.13	0.208	5
Anoxomat Standard 12	#1	9.9%	9	21	4.12	0.267	6
	#2	9.9%	9	23	4.03	0.313	8
BD GasPak 100	#1	NA	9	26	4.13	0.311	8
	#2	NA	9	25	3.96	0.279	7
	#3	NA	9	25	4.12	0.361	9

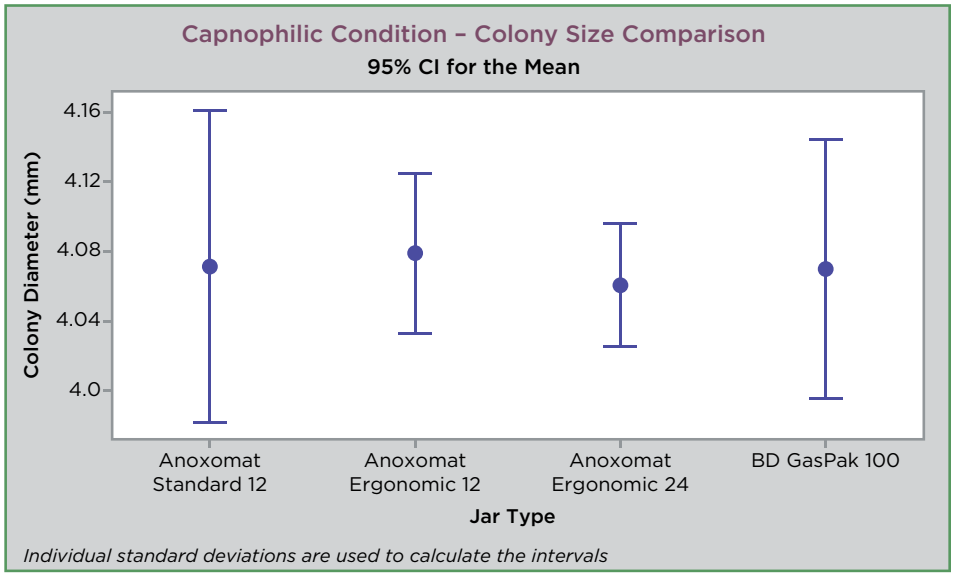

Figure 6. Colony Size Comparison for Capnophilic Condition

CONCLUSION

The test results demonstrate that Ergonomic Jars are equivalent to Standard Jars and are a superior alternative to the GasPak 100 jars conditioned with GasPak sachets. The two types of Ergonomic Jars (AJ9049 and AJ9050) successfully maintained atmospheric conditions for cultivating anaerobic, microaerophilic, and capnophilic bacteria. The Ergonomic Jars did not yield any QC/QA errors throughout testing the three atmospheric conditions.

The Ergonomic Jars yielded average colony diameters that were equivalent to or better than both the Standard Jars and the GasPak 100 jars across all 3 atmospheric conditions/bacteria tested. Equivalence was established by observing the overlapping 95% confidence intervals of the average colony diameters of each bacteria and jar type.

The 24-plate Ergonomic Jars yielded a slightly lower average colony diameter for the anaerobic and microaerophilic conditions (*B. fragilis* and *C. fetus*, respectively) than the 12-plate Standard Jars. This difference in average colony diameter may be due to the difference in the jar volumes, as the phenomenon was observed for both the anaerobic and microaerophilic bacteria species tested.

Both the 12-plate and 24-plate Ergonomic jars perform comparably to the 12-plate Standard jars when growing bacteria under the anaerobic, microaerophilic, and capnophilic atmospheric conditions.

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