ADVANCED INSTRUMENTS

WHITEPAPER

ANOXOMAT® III JAR SYSTEM: A GOLD STANDARD IN BACTERIAL CULTIVATION

SUPERIOR BACTERIAL CULTIVATION COMPARED TO ALTERNATIVE TECHNOLOGIES INCLUDING THE GASPAK GENERATING SACHET SYSTEM AND ANAEROBIC CHAMBERS

Dr. Jonah Riddell, Product Manager — Microbiology at Advanced Instruments

ABSTRACT

Combating false negative results and lags in productivity. False negative lab tests, misdiagnosis or delayed results can detrimentally impact patient outcomes leading to incorrect diagnosis or extended hospital stays. Patient outcomes can be assured by accurate isolation and identification of organisms in a timely manner within Microbiology laboratories. For this reason, among others, hospital and research laboratories value a system's ability to quickly create ideal conditions for the growth of anaerobic, microaerophilic, and capnophilic organisms. The Anoxomat III Jar System from Advanced Instruments creates suitable environments for bacterial cultivation and offers important advantages for laboratory managers over conventional technologies including the anaerobic chamber and the gas generating sachet system. Here we will summarize peer-reviewed publications comparing the Anoxomat System to other methods and display our own research in support of those findings. Over 250 publications in peer reviewed journals support and utilize the Anoxomat in their studies because of its unparalleled, reliable and efficient results assuring both the integrity of a laboratory's data and that no clinical results are missed as a result of false negatives.

CONTENTS

"This instrumentation is easy to use, low cost, and low maintenance. My staff love it!"

"It appears that our anaerobic recovery rate has increased. Our providers have noticed this and thus our anaerobic culture numbers have increased by about 25%."

Salina Regional Health Center, KS

BACKGROUND

A turning point in history

Frequently in peer-reviewed journals, the Anoxomat Jar System has been compared to other means of bacterial cultivation. The first and most pronounced of these studies was done by Brazier et al. in 1989, where the group compared the Anoxomat System with both an anaerobic cabinet and gaspak generating sachet system[2]. The results unequivocally favored the Anoxomat System and found that bacterial strains of anaerobic bacteria may grow significantly faster in the Anoxomat System than both the anaerobic cabinets tested and the gaspak generating sachet methods[2]. Comparisons of the Anoxomat System with the anaerobic chamber showed 51% of strains yielded larger colonies and 39% more growth after a 24-hour incubation. A strain of P. endodontalis failed to grow inside the anaerobic chamber or the gaspak jars after 48 hours of incubation but was successfully detected in the Anoxomat System[2]. This paper proved to be the cornerstone of a revolution in the field where the previously held position that anaerobic cabinets were superior to other methods began to be questioned.

"Highly Recommended"

"Ease of use and reproducibility of results are excellent. I would recommend this product for any microbiology department to ensure proper anaerobic conditions are met!"

Keesler Air Force Base, MS

A second later study by Summanen et al. in 1999 confirmed the previous results in a larger scale study. In this study, 52% of bacterial organism colonies were 0.1 — 10.0mm larger on Anoxomat plates at 24 hours compared to those incubated in anaerobic chambers[3]. Although less augmented at 48 hours, 33% of colonies were reported to be significantly larger on the Anoxomat System than the anaerobic chamber[3]. Shahin et al. in 2003 expanded with a larger comparison study of the Anoxomat System with respect to gaspak generating sachets[4]. The study again displayed evidence that in 67% of cases the Anoxomat showed superior results to the gaspak sachet generating system[4]. Results supported "superior anaerobiosis within the Anoxomat Jar System"[4].

From 1989 to 2020, the Anoxomat Jar System has been used in over 250 peer reviewed journals studying bacteria and cultured epithelial cells in a variety of environments; a majority of these studies were completed 2016-2020, showcasing the Anoxomat is doing nothing but gaining momentum as a mainstream technique. The Anoxomat has evolved to be a foundational technique supporting a broad range of studies including: traditional clinical bacterial testing, identification of novel bacterial strains, fundamental academic studies and bioburden research assessing bacterial loads on implants. Thus, in recent publications, the Anoxomat Jar system is treated as a "universally accepted standard of practice for microbiological cultures"[5,6]. This paper will demonstrate a summary of the peer-reviewed data enhanced by our own studies comparing the methodologies and highlighting the superior performance of the Anoxomat System.

RESULTS

Increased bacterial colony growth and colony numbers with the Anoxomat.

A summary of several publications comparing the utility of the Anoxomat vs. anaerobic cabinets and gaspak generating sachet systems are summarized in **Figure 1.0** [2,3,4]. Overall bacterial growth of 88 strains of anaerobic, microaerophilic and capnophilic bacteria are indicated by genus and name. Five strains showed measurable bacterial growth in the Anoxomat but had no growth in the anaerobic cabinets and two strains had no growth in the gaspak generating sachet system as indicated by the dark green color[2,3,4]. Overall 55% of strains showed an increased bacterial colony size in the Anoxomat to the Anaerobic cabinet at 24 hours and 46% in the Anoxomat over the gaspak generating sachet method (**Figure 2.0**). Additionally, the Anoxomat System performed in a superior manner to both the gas pack generating sachet system and the anaerobic cabinet system with an increased number of colonies generated in 20% and 35%, respectively. Internally we sought to expand the testing and confirm those results observed above within a case study.

In comparison to the gaspak system "The Anoxomat System provided superior growth, in terms of density and colony size, and achieved anaerobiosis more rapidly[4].

"Our laboratory has saved space and time for over ten years with the Anoxomat!"

"The Anoxomat III is an efficient instrument. It continues to provide anaerobic conditions for our anaerobic [jars] with consistent results."

Capital Region Medical Center, FL

Anoxomat case study yields greater bacterial growth and colony formation in distinct conditions.

Previous studies reported a growth advantage in colony size and number when culturing B. fragilis in the Anoxomat System. **Figure 3.0** supports that growing B. fragilis in the Anoxomat System in anaerobic conditions yielded average colony diameters that were 0.29mm larger compared to those obtained from the gaspak generating sachet system. Please refer to **Figure 7.1** for the individual data and **Figure 6.0** for the conditions in which the case study was performed. The average increase in 0.29mm is reflective of a colony size increase of approximately 10% in the Anoxomat System at 24 hours for B. fragilis. Although data was not available in previous studies, C. fetus was tested in the Anoxomat System under microaerophilic conditions and yielded average colony diameters that were 0.34mm larger than those obtained from the gaspak generating sachet system. Please refer to **Figure 7.2** for more information on the individual data and again to **Figure 6.0** for the experimental conditions.

To confirm that studies were appropriately controlled, N. gonorrhoeae was tested in capnophilic conditions as there was no difference observed in colony growth or size in previous studies. **Figure 3.0** indicates N. gonorrhoeae in the Anoxomat System under capnophilic conditions yielded colony sizes comparable to the gaspak generating sachet system (all observed differences were within 0.01mm). Please refer to **Figure 7.3** for more information on the individual data.

Overall, in both novel and repeated bacterial strains we observed superior bacterial colony growth within the Anoxomat Jar System.

Bacterial strain recovery was comparable to leading methods in clinical isolates.

Bacterial strain growth studies are a critical first step, but testing within clinical isolates is the critical confirmation step needed. A summary of studies done comparing bacterial growth experiments was done on clinical isolates and they were summarized in **Figure 4.0**[4]. As you can see in clinical isolates from four different sites, the Anoxomat grew the colonies indicated and the gaspak generating sachet system was not able to grow up the bacterial strains listed. Similarly, the Anoxomat was able to grow up the clinical strains listed below but the anaerobic chamber was not. The importance of missing results is critical when interpreting patient related data and missing bacterial identification in clinical isolates has detrimental effects on patient outcome.

Results from peer-reviewed journals support that in both bacterial clone growth experiments and clinical isolates, bacteria could be missed or the bacterial growth impaired in other systems compared to the superior results obtained with the Anoxomat System[2,3,4]. Focusing on some key strains of case studies confirmed an increased growth rate in the Anoxomat Jar System with B. fragilis in anaerobic conditions and C. fetus in microaerophilic conditions. Overall, results showcase the performance advantages of utilizing the Anoxomat System over other methodologies.

FIGURE 1.0

COMPARING ANOXOMAT METHODS TO CHAMBERS AND GASPAK METHODS[2,3,4]

Strains and Conditions					Anoxomat vs. Chamber		Anoxomat vs. Gaspak	
CONDITIONS	GRAM +/-	STRAIN / REF #	GENUS	BACTERIA NAME	COLONY SIZE INCREASE	# OF COLONIES	COLONY SIZE INCREASE	# OF COLONIES
ANAEROBIC	+	ATCC 35406	*Peptostreptococci*	*P. endodontalis*	+++	+++	+++	+++
ANAEROBIC	+	ATCC 23195, 8949	*Peptostreptococci*	*P. micros*	+++	+++	+++	+++
ANAEROBIC	+	R2727	*Clostridium*	*C. spiroforme*	+++	+++		
ANAEROBIC	-	7603	*Prevotelia*	*Prevotelia intermedia*	+++	+++	++	0
ANAEROBIC	+	R2794, 41B, ATCC 27606, 9002, 41, 9002B	*Clostridium*	*C. novyii A*	+++	0	-	0
ANAEROBIC	+	8899	*Eubacterium*	*E. biforme*	++	++	+	+
ANAEROBIC	-	8939, ATC25285 & R2699	*Bacteriodes*	*B. fragilis*	++	0	++	0
ANAEROBIC	-	8953	*Bacteroides*	*B. thetaiotaomicron*	++	++	0	0
ANAEROBIC	+	8878, ATCC 43593, R2377 & 8640	*Clostridium*	*C. difficile*	++	+	0	+
ANAEROBIC	-	ATCC 25556	*Fuscobacteria*	*F. necrogenes*	++	+	+	0
ANAEROBIC	-	ATCC29741 & R2314	*Bacteriodes*	*B. thetaiotaomicron*	++	0	+	0
ANAEROBIC	-	R2656	*Bacteriodes*	*B. vugatus*	++		+	
ANAEROBIC	+	88543	*Clostridium*	*C. beijerinckii*	++	0	-	0
ANAEROBIC	-	R2966	*Fuscobacteria*	*F. necrophorum*	++		-	
ANAEROBIC	-	8904 & R2841	*Bacteriodes*	*B. eggerthii*	+	++	0	0
ANAEROBIC	-	8969 & 9077	*Bilophila*	*B. wadsworthia*	+	+	+	0
ANAEROBIC	+	8938	*Peptostreptococci*	*P. intermedia*	+	+	0	+
ANAEROBIC	-	R2840	*Bacteriodes*	*B. oovatus*	+		+	
ANAEROBIC	+	8835	*Clostridium*	*C. clostridioforme*	+	+	+	-
ANAEROBIC	+	8692 & R3288	*Clostridium*	*C. ramosum*	+	+	0	0
ANAEROBIC	+	8980	*Eubacterium*	*E. ientum*	+	+	0	0
ANAEROBIC	-	R2966	*Fuscobacteria*	*F. nucleatum*	+		+	
ANAEROBIC	-	R2803 & 8320	*Fuscobacteria*	*F. russii*	+	+	0	0
ANAEROBIC	-	7928 & 8102	*Fusobacterium*	*F. gonidiaformans*	+	0	+	0
ANAEROBIC	-	R2514	*Bacteriodes*	*B. distasonis*	+			
ANAEROBIC	-	R3176	*Bacteriodes*	*B. melaninogenicus*	+			
ANAEROBIC	-	R2735	*Bacteriodes*	*B. merdae*	+			
ANAEROBIC	-	R3239	*Bacteriodes*	*B. praecutus*	+			
ANAEROBIC	-	R2567	*Bacteriodes*	*B. ureolyticus*	+			

Strains and Conditions					Anoxomat vs. Chamber		Anoxomat vs. Gaspak	
CONDITIONS	GRAM +/-	STRAIN / REF #	GENUS	BACTERIA NAME	COLONY SIZE INCREASE	# OF COLONIES	COLONY SIZE INCREASE	# OF COLONIES
ANAEROBIC	+	R3088 & 8467	*Clostridium*	*C. cadaveris*	+	0	0	0
ANAEROBIC	+	R3268	*Clostridium*	*C. chauvoei*	+			
ANAEROBIC	+	R2794	*Clostridium*	*C. fallax*	+			
ANAEROBIC	+	R2327	*Clostridium*	*C. putrificum*	+			
ANAEROBIC	-	R3122	*Fuscobacteria*	*F. monidiaformans*	+			
ANAEROBIC	-	ATCC 8501	*Fuscobacteria*	*F. varium*	+	0	0	0
ANAEROBIC	+	ATCC 29745	*Peptostreptococci*	*P. asaccarolyticus*	+	0	0	0
ANAEROBIC	+	8850	*Peptostreptococci*	*P. asaccarolyticus*	+	0	0	0
ANAEROBIC	+	R3238	*Peptostreptococci*	*P. prevotii*	+			
ANAEROBIC	-	ATCC 25260	*Porphyromonas*	*Porphyromonas asaccharolytica*	+	0	+	0
ANAEROBIC	-	R2560	*Veillonella*	*V. dispar*	+			
ANAEROBIC	-	8882	*Veillonella*	*Veillonella sp.*	+	0	0	0
MICROAEROPHILIC	-	8978 & ATCC 33236	*Campylobacter*	*C. gracilis*	+	0	+	0
MICROAEROPHILIC	-		*Campylobacter*	*C. pylori*	+			
MICROAEROPHILIC	-		*Campylobacter*	*Campylobacter sp*	+			
CAPNOPHILIC	+/-		*Gardnerella*	*Gardnerella Sp*	+			
CAPNOPHILIC	+	8829	*Lactobacillus*	*L. catenaforme*	+	0	0	0
MICROAEROPHILLIC	+	8828	*Lactobacillus*	*Lactobacillus species*	+	0	0	-
ANAEROBIC	+	R3277	*Clostridium*	*C. botulinum*			++	
ANAEROBIC	+	R3303	*Clostridium*	*C. sporogenes*	0		++	
ANAEROBIC	+	ATCC 15930 & 8166	*Peptostreptococci*	*P. loescheii*	0	+	+	+
ANAEROBIC	-		*Bacteriodes*	*B. capillosus*			+	
ANAEROBIC	+	R3187	*Clostridium*	*C butyricum*			+	
ANAEROBIC	+	8319 & R3267	*Clostridium*	*C. perfringens*	0	0	+	0
ANAEROBIC	+	ATCC 27337	*Peptostreptococci*	*P. anaerobius*			+	
ANAEROBIC	+	ATCC 33277 & 8925	*Peptostreptococci*	*P. gingivalis*	0	+	0	0
ANAEROBIC	+	R3234	*Peptostreptococci*	*P. magnus*	0		+	
ANAEROBIC	-	R2736	*Bacteriodes*	*B. caccae*	0			
ANAEROBIC	-	R2629	*Bacteriodes*	*B. disiens*	0			
ANAEROBIC	-	R2734	*Bacteriodes*	*B. stercoris*	0			
ANAEROBIC	-		*Bacteriodes*	*B. uniformis*			0	
ANAEROBIC	+	R3283	*Clostridium*	*C bifermentans*	0			

Strains and Conditions					Anoxomat vs. Chamber		Anoxomat vs. Gaspak	
CONDITIONS	GRAM +/-	STRAIN / REF #	GENUS	BACTERIA NAME	COLONY SIZE INCREASE	# OF COLONIES	COLONY SIZE INCREASE	# OF COLONIES
ANAEROBIC	-	8859	*Clostridium*	*C. clostridioforme*	0	+	-	0
ANAEROBIC	+	R3302	*Clostridium*	*C. paraputrificum*	0			
ANAEROBIC	+	ATCC 13124	*Clostridium*	*C. perfringens*	0	0	0	0
ANAEROBIC	+	R3055	*Clostridium*	*C. sordellii*	0			
ANAEROBIC	+	R2352	*Clostridium*	*C. tertium*	0			
ANAEROBIC	-	R3027	*Fuscobacteria*	*F. naviforme*	0			
ANAEROBIC	-	ATCC 35585	*Fusobacterium*	*F. sulci*	0	0	0	0
ANAEROBIC	+	R3330	*Peptostreptococci*	*P. assacharolyticus*	0			
ANAEROBIC	+	7784	*Peptostreptococci*	*P. intermedia*	0	0	0	0
ANAEROBIC	+	8926 & ATCC 29328	*Peptostreptococci*	*P. magnus*	0	-	+	0
ANAEROBIC	+	R3238	*Peptostreptococci*	*P. micros*	0			
ANAEROBIC	+	ATCC 33269	*Peptostreptococci*	*P. oralis*	0	-	0	0
ANAEROBIC	+	ATCC 27337	*Peptostreptococcus*	*Peptostreptococcus anaerobius*	0	0	0	0
ANAEROBIC	-	R2561	*Veillonella*	*V. atypica*	0			
ANAEROBIC	-	R2649	*Veillonella*	*V. caviae*	0			
ANAEROBIC	-	R2828	*Veillonella*	*V. criceti*	0			
ANAEROBIC	-	ATCC 10790	*Veillonella*	*V. paroula*	0	0	0	0
ANAEROBIC	-	R2648	*Veillonella*	*V. ratti*	0			
ANAEROBIC	-	R2647	*Veillonella*	*V. rodentium*	0			
MICROAEROPHILLIC	+		*Peptostreptococci*	*Streptococcus milleri*	0			
ANAEROBIC	-	ATCC 9817 & R2836	*Fuscobacteria*	*F. mortiferum*	0	0	-	0
ANAEROBIC	-	R2863	*Bacteriodes*	*B. bivius*	-			
ANAEROBIC	-	R3054	*Bacteriodes*	*B. hypermegas*	-			
ANAEROBIC	+	R3318	*Clostridium*	*C. septicum*	-			
ANAEROBIC	+	ATCC 25845 & 8931	*Peptostreptococci*	*P. melaninogenica*	-	0	-	+
CAPNOPHILIC	-		*Haemophilius*	*Haemophilius species*	-			
CAPNOPHILIC	-		*Neisseria*	*N gonorrhoeae*	-			
ANAEROBIC	-	ATCC 35896	*Fuscobacteria*	*F. alocis*	---	---	0	0

Figure 1.0: *Published results in peer-reviewed journals[2,3,4] are collectively summarized in the table. Conditions indicate the environments that the bacteria were cultured in during the published study and "Strain"/"Referance" refers to the ATCC order number or the reference in the publication that was used; some strains were not mentioned in publications and therefore were left blank. Colony size increase indicates the increase/decrease in size observed in the studies on average over the methodology mentioned (anaerobic chambers or gaspak generating sachets): Dark Blue — "Lost Data w/o Anoxomat" and no colonies observed in chamber/gaspak; Medium Blue — "Strong Increase" of Anoxomat colony size over 0.2mm increased; Light Blue — "Slight Increase" or the colony size observed was 0.0-0.2mm increase; 0 — "No Difference" observed in colony size; Light Cyan — "Slight Decrease" in colony size with the Anoxomat of 1.0-0.2mm; Dark Cyan — "Other Modality Better". Colony number indicates the increase/decrease in size observed in the studies on average over the methodology mentioned (anaerobic chambers or gaspak generating sachets): Dark Blue — no colony observed in chamber/gaspak; Medium Blue — over 5.0% increase in colony numbers; Light Blue is 0.5-5.0% increase; 0 — no difference observed in colony number; Light Cyan — decreased colony number by 0.5-5.0%; Dark Cyan — colonies not observed in the Anoxomat.*

FIGURE 2.0

ANOXOMAT GIVES INCREASED COLONY SIZE AND NUMBER

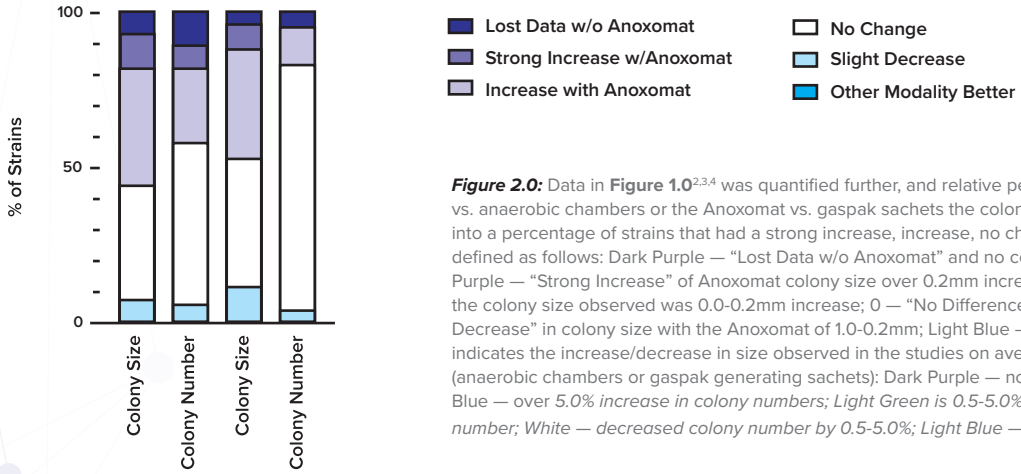

Figure 2.0: Data in **Figure 1.0**[2,3,4] was quantified further, and relative percentages calculated. For either the Anoxomat vs. anaerobic chambers or the Anoxomat vs. gaspak sachets the colony size and number of colonies were turned into a percentage of strains that had a strong increase, increase, no change, slight decrease or other modality better defined as follows: Dark Purple — "Lost Data w/o Anoxomat" and no colony observed in chamber/gaspak; Medium Purple — "Strong Increase" of Anoxomat colony size over 0.2mm increased; Light Purple — "Slight Increase" or the colony size observed was 0.0-0.2mm increase; 0 — "No Difference" observed in colony size; White — "Slight Decrease" in colony size with the Anoxomat of 1.0-0.2mm; Light Blue — "Other Modality Better". Colony number indicates the increase/decrease in size observed in the studies on average over the methodology mentioned (anaerobic chambers or gaspak generating sachets): Dark Purple — no colony observed in chamber/gaspak; Medium Blue — over *5.0% increase in colony numbers; Light Green is 0.5-5.0% increase; 0 — no difference observed in colony number; White — decreased colony number by 0.5-5.0%; Light Blue — colonies not observed in the Anoxomat.*

When growing B. fragilis and C. fetus, all Anoxomat jars yielded average colony diameters that were larger than those obtained from the gaspak generating system

FIGURE 3.0

ANOXOMAT HAS SUPERIOR COLONY SIZE AT 24-HOURS COMPARED TO GASPAK METHODOLOGY

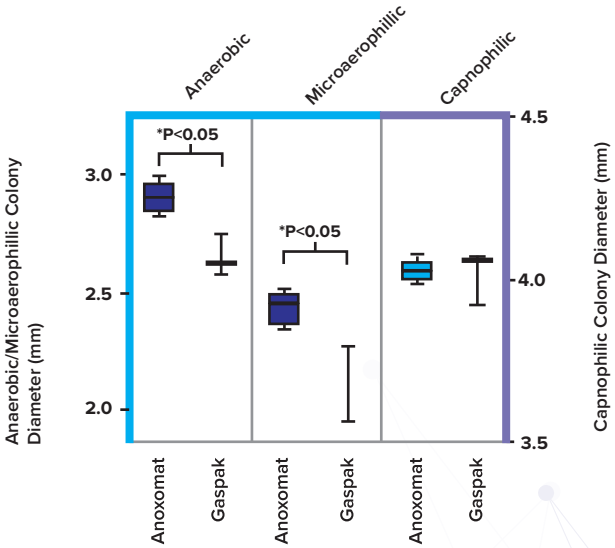

Figure 3.0: The data in **Figure 7.1-7.3** are summarized in this graphical representation of colony size information for each condition that was measured. Anaerobic and Microaerophillic data was plotted on the left axis and the Capnophillic on the right axis. P-values were calculated using a standard t-test and an unpaired Welch's corrections.* indicates a P-value less than 0.05.

"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" [2]

FIGURE 4.0

HOW THE MODALITIES PERFORM IN CLINICAL ISOLATES[4]

	Bacteria Missing in Isolates from Anaerobic Chambers	Bacteria Missing in Isolates from Gaspak Sachet Methods	Bacteria Missing in Isolates from Anoxomat
INTRA-ABDOMINAL ABSCESS	NA	NA	NA
ABDOMINAL TISSUE	*Sutterella Wadsworthensis*	*Bilophilia Species*	*Sutterella Wadsworthensis and Bilophilia Species*
ANTECUBITAL FOSSA ABSCESS	NA	NA	NA
APPENDIX TISSUE (GANGRENOUS APPENDIX)	NA	NA	NA
FOOT ABSCESS	*Bilophila Species and Bacteroides Species*	*Bacteroides and B. fragilis*	NA
HAND ABSCESS	NA	*C. clostridioforme*	NA
DECUBITUS ULCER, HIP	NA	NA	NA
NECROTIZING FASCIITIS (TISSUE)	NA	NA	NA
NECROTIZING FASCIITIS (ASPIRATE OF PUS)	NA	NA	*Prevotella Intermedia*
PERITONEAL FLUID	*Veilolonella Species and Prevotella Species*	*Velolonella Species, Clostridium Species and P. Intermedia*	*Campylobacter Gracilis*

Figure 4.0: Shahin et al. tested various clinical isolates for the presence or absence of bacterial strains. We have summarized their results in this table. Bacteria identification missing in the cultures was identified in the respective column or if all were identified then it was labeled NA.

In clinical isolates, The Anoxomat recovered 94% of clinical isolates tested and had better recovery than other modalities [4]

DISCUSSION

The test results demonstrate that the Anoxomat System is a superior alternative to the gaspak sachet system and anaerobic chambers. A change of a few mm's may seem trivial but has a significant impact on overall efficiency and turnaround of clinical results. Current users of the Anoxomat System indicate that they could analyze plates at 24 hours compared to 48 hours and therefore, the difference in size observed in mm's for published studies could have significant effects on testing timetables. The lack of growth of some bacterial strains indicates that false negatives could be reported and detrimentally affect patient outcomes. Confidence in results is paramount when you are reporting a negative result and any doubt in that test being truly negative is at the forefront of anyone reporting results to clinicians that mediate treatment options.

METHODS

Anoxomat Jar System

All studies were completed using the Anoxomat Jar system (Advanced Instruments; that functions using the McIntosh & Fildes Method for creating the desired environment **Figure 5.0**[1]. Four jar types were tested in this study, with 15 jars in total used to cultivate each of the three bacteria types in their corresponding conditions. Both the Anoxomat standard jar system and the ergonomic jar system were tested and gave similar results, but only the ergonomic jar system will be shown in the data, thus only the AJ9049 ergonomic jar system data will be presented. All Anoxomat jars were conditioned using a single Anoxomat III unit. The gaspak generating sachet system was tested according to the manufacturer's instructions and all jars in the test group were conditioned only using the gaspak sachet system from an undisclosed company. After conditioning, the oxygen concentration of each jar was measured using an OxySense® 325i optical oxygen analyzer.

Figure 5.0: The Anoxomat III Jar System is pictured in the figure, equipped with four jar attachments. The instrument can be used with ergonomic (blue handle jars) in 12/24 plate format or the standard jars (white lids) in 12/26 plate format. Additionally, a hard copy of the data for each run can be printed by the thermal printer, pictured (or dot matrix printer, not pictured); alternatively data can be scanned and recorded digitally with the track and trace package and/or the bar code scanner (bottom left).

APPENDIX

FIGURE 6.1

TEST ENVIRONMENTS

Representative bacteria were grown in anaerobic, microaerophilic, and capnophilic environments. **Figure 6.1** lists the atmospheric conditions, bacteria, and plate type for each test environment.

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*

Figure 6.1 and Figure 6.2: Bacteria were grown in their corresponding recommended conditions based on published literature. We have summarized above what is meant by Anaerobic, Microaerophilic and Capnophilic in terms of the environment, percentage oxygen present and the plate type that was used *during testing for the indicated bacteria.*

FIGURE 6.2

BACTERIAL TEST SCHEME

The average colony size (diameter in millimeters) of the bacteria grown in the Anoxomat System was compared to the gaspak generating sachet system multiple times and 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 6.2** summarizes the test scheme.

Environment	Bacteria	# of Plates	
		Anoxomat III Jar System	gaspak sachet system
ANAEROBIC	*B. fragilis*	18	9
	M. luteus (Control)	6	3
MICROAEROPHILIC	*C. fetus*	18	9
	B. fragilis (Control)	6	3
CAPNOPHILIC	*N. gonorrhoeae*	18	9
	B. fragilis (Control)	6	3

Table 6.2: To compare the Anoxomat Jar system with the gaspak generating sachet system we did 9 jars in total for each condition and they contained a differing number of plates depending on the capacity of the system. We have outlined how many plates were set up for each condition and listed where the indicated bacteria was tested.

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. (**Figure 6.0 and 7.0**)

ACCEPTANCE CRITERIA

Test results were evaluated against the following acceptance criteria:

- The Ergonomic Jar (AJ9049) shall maintain environments suitable for the equivalent growth for each of the three atmospheric conditions when compared to the jars containing the gaspak generating sachets. Growth equivalence will be determined by the average colony diameter.
- For all atmospheric conditions tested, the average colony diameter (mm) of plates grown in Anoxomat jars shall be greater than or equal to, or within the 95% confidence intervals of, the average colony diameters of bacteria grown in jars containing the gaspak generating sachets.
- 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

FIGURE 7.1

ANAEROBIC CONDITION (B. FRAGILIS)

Type	Jar #	O_2%	# of Plates	# of Colonies	Mean Dia. (mm)	SD	%CV
ANOXOMAT III	1	0	9	27	2.85	0.24	8
	2	0	9	27	2.94	0.267	9
	3	0.1	9	27	2.82	0.24	9
	4	0	9	27	2.9	0.252	9
	5	0	9	27	3	0.271	9
GASPAK GENERATING SACHET	1	N/A	9	27	2.58	0.296	11
	2	N/A	9	27	2.63	0.333	13
	3	N/A	9	27	2.63	0.275	10

FIGURE 7.2

MICROAEROPHILIC CONDITION (C.FETUS)

Type	Jar #	O_2%	# of Plates	# of Colonies	Mean Dia. (mm)	SD	%CV
ANOXOMAT III	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	2.5	0.38	15
GASPAK GENERATING SACHET	1	NA	9	27	2.27	0.27	12
	2	NA	9	27	2.07	0.235	11
	3	NA	9	27	1.95	0.402	21

FIGURE 7.3

CAPNOPHILIC CONDITION (N.GONORRHOEAE)

Type	Jar #	O_2%	# of Plates	# of Colonies	Mean Dia. (mm)	SD	%CV
ANOXOMAT III	1	10.1	9	24	4.09	0.332	8
	2	10	9	26	4.08	0.262	6
	3	10.1	9	27	4.03	0.249	6
	4	10	9	27	4.07	0.272	7
	5	10	9	27	4.13	0.208	5
GASPAK GENERATING SACHET	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 7.1-7.3: In the tables are listed the colony number and colony size information for each Jar/ test run that was done to compare the Anoxomat Jar System with the gaspak generating sachet system in anaerobic (7.1), microaerophilic (7.2) and capnophilic (7.3). The percentage of oxygen was measured as indicated with the sensor and reported here in O_2%. The number of plates were recorded here and 5 plus colonies from each plate were measured and reported as an average reading in mm's with an accompanying standard deviation (SD) and CV value to display the range of the data recorded originally.

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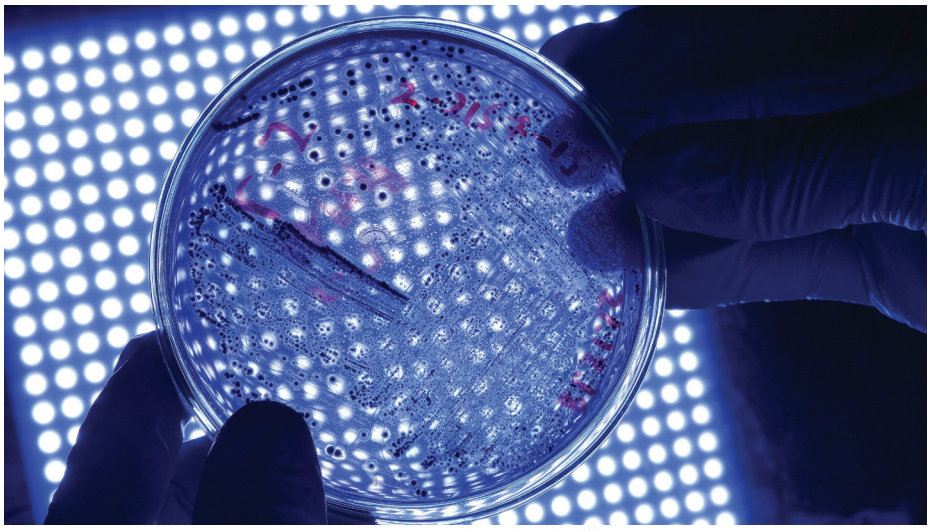
“Because of the way the Anoxomat jar works, I can open it up, take out a sample, and make it anaerobic again very fast.”

VA Wadsworth Medical Center, CA



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