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## Comparison of Recovery of Anaerobic Bacteria Using the Anoxomat<sup>®</sup>, Anaerobic Chamber, and GasPak<sup>®</sup> Jar Systems

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*The Anoxomat<sup>®</sup> system provides an automated evacuation-replacement technique to create an anaerobic or microaerophilic environment in a jar. We evaluated the Anoxomat<sup>®</sup> system for the growth of obligate anaerobes and for the recovery of anaerobic organisms from clinical specimens, and compared its performance to that of an anaerobic chamber and the GasPak<sup>®</sup> System. Of the 54 stock strains tested, the Anoxomat<sup>®</sup>, the chamber, and the GasPak<sup>®</sup> recovered 95%, 95% and 93% at 24 h, respectively. On 29 occasions (51%), the colonies on the Anoxomat<sup>®</sup> plates were slightly larger than those in the chamber and on 17 (30%) occasions larger than the colonies on the GasPak<sup>®</sup> jar plates. At 48 h, the Anoxomat<sup>®</sup>, the chamber, and the GasPak<sup>®</sup> recovered 93.5%, 94.4% and 88.9%, respectively; of 108 anaerobes isolated from 31 clinical specimens. Methylene blue indicators became decolorized (average of 10 tests) within 2 h inside the Anoxomat<sup>®</sup> jars, 2 h 10 min inside the anaerobic chamber, and 2 h 30 min inside the GasPak<sup>®</sup> jars.*

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### Introduction

Anaerobic methods include the use of anaerobic jars, plastic anaerobic bags, the PRAS (pre-reduced anaerobically sterilized) method of Hungate [1] (and modifications), and the anaerobic chamber. The last two techniques are rather time consuming, require complex or expensive equipment, and utilize PRAS media. They are essential for work with anaerobes of the normal flora as these organisms are often more oxygen-sensitive than clinical isolates. Comparative studies have shown that when clinical specimens are collected, transported, and processed properly, recovery of clinically significant anaerobes is as good with anaerobic jars as with the more complex methods [2,3]. To obtain reliable results with any anaerobic jar there must be adequate replacement of the oxygenated environment with an anaerobic atmosphere. This is usually achieved by introducing a gas mixture containing H<sub>2</sub> into the jar. In the presence of a catalyst, the oxygen combines with the H<sub>2</sub> to produce water, thus establishing anaerobiosis. The easiest system employs a commercially available gas generator envelope which releases H<sub>2</sub> and CO<sub>2</sub> when water is added. Alternatively, the 'evacuation-replacement' system may be used. Air is removed from

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the sealed jar by repeatedly drawing a vacuum and then filling the jar with an anaerobic gas mixture. Anaerobiosis is achieved more quickly by the 'evacuation-replacement' method [4]. The **Anoxomat**<sup>®</sup> (Mart BV<sup>®</sup> Microbiology Automation, Holland) uses an automated evacuation-replacement technique to create an anaerobic or microaerophilic environment in the jar. The anaerobic program repeats the evacuation-replacement cycle 3 times, during each cycle, 80% of the content of the jar is replaced by the anaerobic gas mixture. After the third cycle, any remaining oxygen and any oxygen liberated from the media in plates in the jar is removed by combining with H<sub>2</sub> with the aid of a palladium catalyst. This system also provides an internal quality assurance program that executes additional tests when selected: check for leaks, a test for adequate catalyst activity, and a test for adequate addition of replacement gas.

We evaluated the Anoxomat<sup>®</sup> system for: (1) the growth of obligate anaerobes; (2) the recovery of anaerobic organisms from clinical specimens; and (3) speed of reducing methylene blue. The performance of the Anoxomat<sup>®</sup> was compared to that of an anaerobic chamber and the GasPak<sup>®</sup> system. (This work was presented in part at the 92nd General Meeting of the American Society for Microbiology, 1992, Abstract no. 1834.)

## Materials and Methods

### *Anaerobic techniques*

Three anaerobic techniques were used in this study: (1) the Anoxomat<sup>®</sup> model WS8000, which has connections for three jars; (2) the anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, MI); (3) and the GasPak<sup>®</sup> system (BBL Microbiology Systems, Cockeysville, MI) using GasPak<sup>®</sup> gas generator envelopes which release H<sub>2</sub> and CO<sub>2</sub> when water is added. Palladium catalysts were used with all three systems. BBL GasPak<sup>®</sup> jars (volume approximately 2.4 L) were used with both the Anoxomat<sup>®</sup> and GasPak<sup>®</sup> systems; for the Anoxomat<sup>®</sup> system an adaptor was put on the jar lids. A gas mixture of 80% N<sub>2</sub>, 10% H<sub>2</sub> and 10% CO<sub>2</sub> was used with the Anoxomat<sup>®</sup> and the chamber techniques. The GasPak<sup>®</sup> system generates an atmosphere containing 25-30% H<sub>2</sub>, 4-7% CO<sub>2</sub>, and the balance N<sub>2</sub>. Anaerobiosis was monitored by methylene blue indicators (BBL); the achievement of anaerobiosis in all three systems was followed by checking every 0.5 h for reduction of the indicator.

### *Bacterial strains and growth conditions*

Altogether, 54 stock strains of obligate anaerobic bacteria representing different genera of clinical significance were tested (Table 1). Included were 20 ATCC strains and 34 strains from the Wadsworth Anaerobe Laboratory collection. Test organisms were either fresh isolates or had been kept frozen at -70°C in 20% skimmed milk. The frozen isolates were thawed and subcultured twice on supplemented Brucella blood agar (BBL, Microbiology Systems) to help ensure good growth and to check on the purity of the cultures. Suspensions equal to MacFarland standard 0.5 were made in reduced normal saline and aliquots of 10 µL were inoculated onto three sets of Brucella agar plates from these suspensions. One set of the plates was incubated in jars using the Anoxomat<sup>®</sup> system, the second set in jars using the GasPak<sup>®</sup> system, and the third set in an anaerobic chamber. Plates were incubated at 35-37°C and examined for amount of growth and colonial characteristics (size and morphology) after 24 and 48 h. A dissecting microscope supplied with an eyepiece calibrating device (0.1-mm divisions) was used to measure the colony sizes.

For recovery of anaerobic bacteria from clinical specimens, 31 specimens (Table 2) were evaluated using the three systems. Specimens were inoculated with a 10-µL loop on prereduced Brucella and BBE agar plates (Anaerobe Systems, San Jose, CA) and incubated as described above. Plates were examined after 48 h incubation and the recovery, amount (semiquantitative 1+ to 4+), and size of different colonial morphologies were recorded. The isolates were identified according to standard methods [5,6].

## Results

Table 1 lists the results of the comparison of amount of growth and average colony sizes recovered at 24 h incubation. Of the 54 stock strains tested, the Anoxomat<sup>®</sup>, the chamber, and GasPak<sup>®</sup> jars recovered 95%, 95% and 93%, respectively. The Anoxomat<sup>®</sup> failed to recover three organisms at 24 h incubation (*F. alocis* and 2 *C. novyii* strains). The chamber recovered these three organisms, and the GasPak<sup>®</sup> jar recovered one *C. novyii* that failed to grow in the Anoxomat<sup>®</sup> jar. Two strains (*P. micros*, and *P. endodontalis*) isolated by the Anoxomat<sup>®</sup> system failed to grow in the chamber and the GasPak<sup>®</sup> jar; further, one strain of *P. intermedia* failed to grow in the chamber. At 24 h, 22 (39%) of the strains yielded more growth on the Anoxomat<sup>®</sup> plates than on chamber plates and 7 (12%) more than in the GasPak<sup>®</sup> system. In the GasPak<sup>®</sup> system, 20 (35%) of the strains grew more heavily than in the chamber, and 5 (9%) grew more heavily than in the Anoxomat<sup>®</sup> system. The chamber yielded more growth than the two jar systems three times. On 29 occasions (51%), the colonies on the Anoxomat<sup>®</sup> plates were slightly larger than

those in the chamber and on 17 (30%) occasions larger than the colonies on the GasPak® jar plates. The chamber yielded larger colonies than the Anoxomat® system on 8 (14%) occasions, and on six occasions (10.5%) compared to the GasPak® jar; 25 (44%) and 11 (19%) strains grew larger in the GasPak® jars than in the chamber and Anoxomat®, respectively. After 48-h incubation, the colony sizes were more comparable among the three systems. Also, differences in the amount of growth at 48 h were not significant, except for *C. novyii* (9002), *P. endodontalis* (ATCC 35406), and *F. alocis* (ATCC 35896) all strains of which still failed to grow at 48 h in systems in which they failed to grow at 24 h.

Table 2 lists the 22 of the 31 specimens evaluated that yielded anaerobic growth, a total of 108 anaerobes was isolated at 48 h. The isolated genera include: *Bacteriodes*, *Bilophila*, *Campylobacter*, *Capnocytophaga*, *Clostridium*, *Desulfovibrio*, *Eubacterium*, *Fusobacterium*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Propionibacterium*, *Sutterella* and *Veillonella*. All three systems recovered all anaerobes from ten of the 22 specimens. The Anoxomat® system, the chamber, and the GasPak® system recovered 93.5%, 94.4%, and 88.9% of 108 anaerobes isolated, respectively. The amount of growth, when recovered was comparable with the three systems evaluated.

The methylene blue indicators became decolorized (average of 10 tests) within 2 h inside the Anoxomat® jars, 2 h 10 min inside the anaerobic chamber, and 2 h 30 min inside the GasPak® jars.

## Comparison of Anoxomat, Anaerobic Chamber, and GasPak Systems

Table 1. Comparison of mean colony sizes and semi-quantitative growth of anaerobic organisms after 24-h incubation in anaerobic chamber, GasPak<sup>®</sup> jar and Anoxomat<sup>®</sup> jar.

Strain No.	Organism	Mean Colony size (mm)			Semi-quantitative growth (CFU/mL)		
		Chamber	GasPak	Anoxomat	Chamber	GasPak	Anoxomat
8904	<i>Bacteroides eggerthii</i>	0.1	0.1	0.1	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>4</sup>
8939	<i>B. fragilis</i>	0.3	0.7	0.8	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>
ATCC 33236	<i>Campylobacter gracilis</i>	0.2	0.1	0.2	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>4</sup>
8978	<i>C. gracilis</i>	0.4	0.5	0.5	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8953	<i>B. thetaiotaomicron</i>	0.1	0.8	0.8	5 x 10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC29741	<i>B. thetaiotaomicron</i>	0.4	0.7	0.8	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8969	<i>Bilopila wadsworthia</i>	0.1	0.1	0.3	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>5</sup>
9077	<i>B. wadsworthia</i>	0.2	0.2	0.2	10 <sup>4</sup>	10 <sup>6</sup>	10 <sup>5</sup>
ATCC 35896	<i>Fusobacterium alocis</i>	0.2	NG <sup>a</sup>	NG <sup>a</sup>	10 <sup>4</sup>	NG <sup>a</sup>	NG <sup>a</sup>
8102	<i>F. gonidiaformans</i>	0.1	0.1	0.1	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>4</sup>
7928	<i>F. gonidiaformans</i>	0.4	0.6	0.8	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>
ATCC 9817	<i>F. mortiferum</i>	1.2	1.2	1.1	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 25556	<i>F. necrogenes</i>	0.4	0.5	0.7	5 x 10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 35585	<i>F. sulci</i>	0.3	0.3	0.3	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8320	<i>F. russii</i>	0.2	0.2	0.2	5 x 10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 8501	<i>F. varium</i>	1.2	1.5	1.5	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 25260	<i>Porphyromonas asaccharolytica</i>	0.1	0.1	0.2	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 35406	<i>P. endodontalis</i>	NG <sup>a</sup>	NG <sup>a</sup>	0.3	NG <sup>a</sup>	NG <sup>a</sup>	10 <sup>6</sup>
8925	<i>P. gingivalis</i>	0.1	0.1	0.1	5 x 10 <sup>6</sup>	10 <sup>4</sup>	10 <sup>4</sup>
ATCC 33277	<i>P. gingivalis</i>	0.1	0.1	0.1	5 x 10 <sup>2</sup>	10 <sup>6</sup>	10 <sup>6</sup>
7603	<i>Prevotella intermedia</i>	NG <sup>a</sup>	0.1	0.3	NG <sup>a</sup>	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>
7784	<i>P. intermedia</i>	0.1	0.1	0.1	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8938	<i>P. intermedia</i>	0.2	0.3	0.3	5 x 10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8166	<i>P. loescheii</i>		0.3	0.1		10 <sup>4</sup>	10 <sup>4</sup>
ATCC 15930	<i>P. loescheii</i>		0.2	0.2		10 <sup>5</sup>	10 <sup>5</sup>
8931	<i>P. melaninogenica</i>	0.1	0.2	0.2	5 x 10 <sup>6</sup>	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>
ATCC 25845	<i>P. melaninogenica</i>	0.4	0.4	0.3	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 33269	<i>P. oralis</i>	0.1	0.1	0.1	5 x 10 <sup>6</sup>	10 <sup>4</sup>	10 <sup>4</sup>
8854	<i>Clostridium beijerinckii</i>	3.4	3.8	3.0	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8467	<i>C. cadaveris</i>	1.8	1.8	1.8	3 x 10 <sup>4</sup>	3 x 10 <sup>4</sup>	3 x 10 <sup>4</sup>
8835	<i>C. clostridioforme</i>	0.4	0.4	0.5	2 x 10 <sup>2</sup>	8 x 10 <sup>2</sup>	4 x 10 <sup>2</sup>
8859	<i>C. clostridioforme</i>	0.1	0.2	0.1	5 x 10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8878	<i>C. difficile</i>		0.5	1.8		2 x 10 <sup>2</sup>	6 x 10 <sup>6</sup>
8640	<i>C. difficile</i>		1.3	1.5		6 x 10 <sup>6</sup>	10 <sup>4</sup>
8922	<i>C. innocuum</i>	0.9	1.1	1.0	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>
41	<i>C. novyii A</i>	0.7	1.0	0.7	8 x 10 <sup>4</sup>	10 <sup>5</sup>	5 x 10 <sup>4</sup>
41 <sup>b</sup>	<i>C. novyii A</i>	0.6	0.8	0.7	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
9002	<i>C. novyii A</i>	1.0	NG <sup>a</sup>	NG <sup>a</sup>	10 <sup>4</sup>	NG <sup>a</sup>	NG <sup>a</sup>
9002 <sup>b</sup>	<i>C. novyii A</i>	0.6	0.6	0.5	8 x 10 <sup>4</sup>	10 <sup>5</sup>	7 x 10 <sup>4</sup>
ATCC 27606	<i>C. novyii A</i>	0.6	0.8	NG <sup>a</sup>	5 x 10 <sup>2</sup>	5 x 10 <sup>6</sup>	NG <sup>a</sup>
8319	<i>C. perfringens</i>	2.8	2.5	2.8	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 13124	<i>C. perfringens</i>	5.0	5.0	5.0	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8692	<i>C. ramosum</i>	0.8	1.2	1.2	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>
8899	<i>Eubacterium bifforme</i>	0.5	0.9	1.0	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	10 <sup>6</sup>
8980	<i>E. ientum</i>	0.1	0.4	0.3	8 x 10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8828	<i>Lactobacillus species</i>	0.1	0.2	0.2	5 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	10 <sup>4</sup>
8829	<i>L. cateniforme</i>	0.1	0.2	0.2	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 27337	<i>Peptostreptococcus anaerobius</i>	0.2	0.2	0.2	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8850	<i>P. asaccarolyticus</i>	0.2	0.4	0.4	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 29745	<i>P. asaccarolyticus</i>	0.1	0.3	0.3	5 x 10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8926	<i>P. magnus</i>	0.1	0.1	0.2	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 29328	<i>P. magnus</i>	0.5	0.3	0.3	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>
8949	<i>P. micros</i>	0.1	0.1	0.1	5 x 10 <sup>4</sup>	3 x 10 <sup>4</sup>	3 x 10 <sup>4</sup>
ATCC 23195	<i>P. micros</i>	NG <sup>a</sup>	NG <sup>a</sup>	0.1	NG <sup>a</sup>	NG <sup>a</sup>	10 <sup>4</sup>
8882	<i>Veillonella sp.</i>	0.3	0.5	0.5	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
ATCC 10790	<i>Veillonella paroula</i>	0.3	0.3	0.3	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>

<sup>a</sup>NG = no growth

<sup>b</sup>Strains run on two occasions

Table 2. Recovery of anaerobes from clinical specimens at 48-h incubation

Specimen Source	Total				Anoxomat	Organism not recovered
	anaerobes	Chamber	GasPak Jar			
Intra-abdominal abscess	3	2	3	2		<i>Sutterella wadsworthensis</i> [2+] (Anoxomat, Chamber)
Intra-abdominal abscess	6	6	5	5		<i>Bilophila</i> sp. [1+] (Anoxomat, GasPak)
Abdominal tissue	1	1	1	1		
Antecubital fossa abscess	5	5	5	5		
Appendix tissue (gangrenous appendix)	6	4	5	6		<i>Bilophila</i> sp. [1+] (Chamber) <i>Bacteroides</i> sp. [1+] (Chamber, GasPak) <sup>b</sup> <i>B. fragilis</i> [1 col] (GasPak)
Appendix tissue (gangrenous appendix)	2	2	1	2		
Appendix tissue (gangrenous appendix)	5	5	5	5		
Appendix tissue (gangrenous appendix)	3	3	2	2		<i>Clostridium</i> sp. [3+] (Anoxomat, GasPak)
Appendix tissue (gangrenous appendix)	5	5	4	4		<sup>b</sup> <i>Bilophila</i> sp. [5 col] (Anoxomat, Gaspak)
Appendix tissue (gangrenous appendix)	4	4	4	4		
Appendix tissue (gangrenous appendix)	13	13	13	13		
Appendix tissue (gangrenous appendix)	7	7	7	7		
Foot abscess	7	7	6	7		<i>C. clostridioforme</i> [3+] (GasPak)
Hand abscess	3	3	3	3		
Decubitus ulcer, hip	1	1	1	1		
Necrotizing fasciitis (tissue)	1	1	1	0		<i>Prevotella intermedia</i> [2+] (Anoxomat)
Necrotizing fasciitis (aspirate of pus)	4	4	4	4		
Periodontal abscess	7	6	5	7		<i>Veillonella</i> sp. [+3] (Chamber, GasPak) <i>Clostridium</i> sp. [2+] (GasPak)
Periodontal abscess	8	7	7	6		<i>P. intermedia</i> [4+] (GasPak) <i>Prevotella</i> sp. [4+] (Anoxomat, Chamber), <i>Campylobacter gracilis</i> [4+] (Anoxomat)
Peritoneal fluid	5	4	4	5		<i>Desulfovibrio</i> [2+] (Chamber, GasPak)
Peritoneal fluid	7	7	5	7		<i>Fusobacterium nucleatum</i> [3+] (GasPak), <i>Fusobacterium</i> sp. [2+] (GasPak)
Peritoneal fluid	5	5	5	5		
Total:	108	102	96	101		
		(94.4%)	(88.9%)	(93.5%)		

<sup>a</sup> [1+]: 10<sup>3</sup>-10<sup>4</sup> CFU/mL; [2+]: 10<sup>4</sup>-10<sup>5</sup> CFU/mL; [3+]: 10<sup>5</sup>-10<sup>6</sup> CFU/mL; [4+] > 10<sup>6</sup> CFU/mL.

<sup>b</sup>Note: only rare colonies present.

<sup>c</sup>P value is 0.14 compared to chamber method.

## Discussion

The Anoxomat<sup>®</sup> system employs the evacuation-replacement technique to create an anaerobic environment in a jar. With this method, according to the manufacturer, anaerobiosis is achieved in 1 to 2 h, although oxidation-reduction indicators may take longer to decolorize. Imhof and Heinzer compared seven commercial anaerobic systems, including the GasPak<sup>®</sup>, and the evacuation-replacement method for their ability to decrease oxygen concentration in an anaerobic jar [4]. They found the evacuation-replacement method the most rapid and reliable system for generating anaerobic environment. In our study, the average time for achievement of anaerobiosis by the Anoxomat<sup>®</sup> system was 0.5 h faster than the GasPak<sup>®</sup> jars and 10 min faster than the anaerobic chamber, as judged from decolorization of methylene blue indicators.

Brazier and Smith [7] evaluated the Anoxomat<sup>®</sup> system in comparison with two types of anaerobic cabinets, and reported superior growth of anaerobic bacteria with the Anoxomat<sup>®</sup> system. The results of our comparative study also suggest that growth of anaerobic bacteria may be faster inside a jar in the Anoxomat<sup>®</sup> system than in a chamber or the GasPak<sup>®</sup> jar. We found that of the strains tested, 51% yielded slightly larger colonies and 39% slightly more

growth after 24-h incubation with the Anoxomat<sup>®</sup> as compared with the plates from the anaerobic chamber. One strain each of *C. novyii* and *F. alocis* were not recovered from plates in the Anoxomat<sup>®</sup> or GasPak<sup>®</sup> jars after 48-h incubation, and one *P. endodontalis* strain failed to grow inside the anaerobic chamber. The colonies on the chamber plates were generally slightly smaller than those from either the Anoxomat<sup>®</sup> or GasPak<sup>®</sup> jar plates. After 48-h incubation the colony sizes were more comparable between the three systems evaluated (data not shown).

Recovery of isolates from clinical specimens at 48 h was comparable between the anaerobic chamber and the Anoxomat<sup>®</sup> systems (94.4% and 93.5%, respectively), and somewhat poorer with the GasPak<sup>®</sup> jar system (88.9%) (difference not statistically significant). Two of the specimens only yielded a few colonies of certain anaerobes; the fact that these organisms were isolated on some plates and not others could be due to the uneven distribution of organisms in the specimen rather than the incubation method. Also, it is possible that in some cases when heavy growth with multiple organisms was recovered, minute colonies might have been overgrown. In general, more than 48-h incubation is recommended for the optimal recovery of anaerobes from clinical specimens.

In our experiences, the Anoxomat<sup>®</sup> system is easy to use; the system has never malfunctioned in three years. As this system provides an internal quality assurance (leak checks, catalyst activity and adequate addition of replacement gas), the jars filled with this system rarely 'fall'. We use transparent plastic jars with the system (Anoxomat<sup>®</sup> or converted BBL GasPak<sup>®</sup> jars) and always include a redox indicator. The Anoxomat<sup>®</sup> company also offers a solid, impervious jar, but it is not possible to follow the establishment of anaerobiosis in this system.

The results of this study, and a previous study [7], suggest that the Anoxomat<sup>®</sup> system appears to be a good alternative to a chamber for clinical laboratories with limited space or unavailability of chambers. It also offers an option to laboratories using conventional jar systems with various gas generating kits or the manual evacuation-replacement method. The initial cost of the Anoxomat<sup>®</sup> is high, but the continuing cost of its use is small.

#### Acknowledgements

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