Comparison of Quality Control Results with Use of Anaerobic Chambers Versus Anaerobic Jars. M. E. COX, R. J. KOHR, AND C. K. SAMIA. *From Anaerobe Systems, San Jose, California*

Anaerobic chambers and anaerobic jars are used to provide anaerobic conditions required to cultivate anaerobic bacteria. All operations and manipulations of cultures can be carried out inside the anaerobic chamber, thus avoiding any exposure to oxygen. However, many chamber users process samples in air and use the chamber only for incubation. When this process is used, plates can be passed immediately into the anaerobic chamber after manipulation in air. When using any of the anaerobic jar techniques, it is necessary to perform all manipulations in air. Once the plates are placed in an activated jar, anaerobic conditions are achieved in ~1 hour.

We evaluated the use of a Bactron IV anaerobic chamber (Sheldon Manufacturing, Cornelius, OR), the GasPak jar (BBL, Cockeysville, MD), and the AnaeroPack (Mitsubishi Gas Chemical, Tokyo) in the performance of routine quality control of prereduced anaerobically sterilized brucella blood agar medium supplemented with vitamin K_1 and hemin.

Six quality control organisms, *Bacteroides fragilis* (ATCC [American Type Culture Collection] 25285), *Clostridium perfringens* (ATCC 13124), *Fusobacterium necrophorum* (ATCC 25286), *Fusobacterium nucleatum* (ATCC 25586), *Peptostreptococcus anaerobius* (ATCC 27337), and *Porphyromonas levii* (ATCC 29147) were inoculated onto prereduced anaerobically sterilized brucella blood agar medium (Anaerobe Systems, San Jose, CA) according to the procedures outlined by the National Committee for Clinical Laboratory Standards [1]. Plates were streaked for isolation on four quadrants. For each run, four sets of plates were prepared as follows: (1) they were inoculated in air and incubated in the GasPak jar, (2) they were inoculated in air and incubated in the AnaeroPack, (3) they were inoculated in air and incubated in the Bactron IV anaerobic chamber, and (4) they were inoculated in the Bactron IV chamber and incubated in the Bactron IV chamber.

The plates were incubated for 48 hours at 36°C and examined for growth. Colonies were measured with use of an ocular scale mounted in a dissecting microscope. Table 1 shows the mean diameters of colonies of the six quality control isolates with the four different procedures and incubation conditions. Table 2 shows the growth scores for the six organisms. Growth was scored on a scale of 0 = no growth, 1+ = growth in the primary inoculation area only, 2+ = growth extending to the first zone of streaking, 3+ = growth extending to the second zone of streaking, 4+ = growth extending to the third zone of streaking.

The inoculum in this study contained ~1.5×10^4 cfu. In our experience, this inoculum will produce 4+ growth on a consistent basis. Growth of <3+ is considered a failure of growth requirements for our quality control purposes. Since many anaerobic infections are polymicrobic [2], successful isolation of each member of the mixed population frequently requires that the isolate grow at least 3+.

Table 1. Average colony size of the six quality control strains: comparison of four methods for cultivating anaerobic bacteria.

	Setup: incubation			
Organism	Air: GasPak	Air: AnaeroPak	Air: Chamber	Chamber: Chamber
Bacteroides fragilis	1.38	1.24	1.33	1.41
Clostridium perfringens	5.82	5.98	5.59	6.93
Fusobacterium necrophorum	2.78	2.45	2.82	2.79
Fusobacterium nucleatum	0.55	0.23	0.52	0.63
Peptostreptococcus anaerobius	1.99	1.81	2.13	2.26
Porphyromonas levii	0.53	0.3	0.31	0.63

NOTE. All values are mean diameter of the colony in mm.

The results of this study show that plates inoculated and incubated in the Bactron IV anaerobic chamber passed quality control requirements 100% of the time for all six isolates tested. Colonies tended to be larger with use of the Chamber-Chamber method than with the other three methods. The plates inoculated in air and incubated in the anaerobic chamber passed quality control 100% of the time for *Bacteroides fragilis, Fusobacterium necrophorum, Clostridium perfringens, Peptostreptococcus anaerobius,* and *Porphyromonas levii.* The Air inoculation-Chamber incubation resulted in a 19.2% failure of minimum growth requirements for *Fusobacterium nucleatum.*

The AnaeroPack passed quality control requirements 100% of the time for *Bacteroides fragilis, Clostridium perfringens,* and *Peptostreptococcus anaerobius.* The AnaeroPack failed minimum growth requirements 92.3%, 2.6%, and 16.7% of the time for *Fusobacterium nucleatum, Fusobacterium necrophorum,* and *Porphyromonas levii,* respectively. The GasPak jar passed quality control requirements 100% of the time for *Bacteroides fragilis, Fusobacterium necrophorum, Clostridium perfringens,* and *Peptostreptococcus anaerobius.* The GasPak failed minimum growth requirements 30.7% and 5.6% of the time for *Fusobacterium nucleatum* and *Porphyromonas levii,* respectively.

These data demonstrate that manipulating anaerobic bacteria in air can compromise optimal results. Since all of the procedures provided anaerobic conditions for incubation, the quality control failures observed must be a result of oxygen exposure during manipulation in air. A lower percentage of failures was observed with the Air-Chamber procedure than with both jar procedures. This difference could be due to the shorter air exposure in the Air-Chamber procedure. Optimal growth and larger colony size are

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Table 2. Growth of six quality control strains: comparison of four methods for cultivating anaerobic bacteria.

Organism, method	No growth	Growth score (%) 1+	2+	3+	4+	No. of times tested
Fusobacterium nucleatum						
Chamber-Chamber	0	0	0	33.3	66.7	36
Air-Chamber	0	0	19.2	38.5	42.3	26
GasPak	0	11.5	19.2	26.9	42.3	26
AnaeroPack	0	73.1	19.2	7.7	0	26
Bacteroides fragilis						
Chamber-Chamber	0	0	0	0	100	36
Air-Chamber	0	0	0	0	100	36
GasPak	0	0	0	0	100	36
AnaeroPack	0	0	0	0	100	36
Porphyromonas levii						
Chamber-Chamber	0	0	0	2.8	97.2	36
Air-Chamber	0	0	0	0	100	36
GasPak	0	0	5.6	0	94.4	36
AnaeroPack	8.3	5.6	2.8	5.6	83.3	36
Clostridium perfringens						
Chamber-Chamber	0	0	0	0	100	36
Air-Chamber	0	0	0	0	100	36
GasPak	0	0	0	0	100	36
AnaeroPack	0	0	0	2.8	97.2	36
Fusobacterium necrophorum						
Chamber-Chamber	0	0	0	5.6	94.4	36
Air-Chamber	0	0	0	2.8	97.2	36
GasPak	0	0	0	0	100	36
AnaeroPack	0	2.6	0	0	97.4	36
Peptostreptococcus anaerobius						
Chamber-Chamber	0	0	0	0	100	36
Air-Chamber	0	0	0	0	100	36
GasPak	0	0	0	0	100	36
AnaeroPack	0	0	0	0	100	36

NOTE. 1+ = growth in primary inoculation area only; 2+ = growth extending to the first zone of streaking; 3+ = growth extending to the second zone of streaking; and 4+ = growth extending to the third zone of streaking.

obtained by providing complete anaerobic conditions during processing, incubation, and examination of anaerobic cultures.

References

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2. Summanen P, Baron EJ, Citron DM, Strong CA, Wexler HM, Finegold SM. Wadsworth anaerobic bacteriology manual. 5th ed. Belmont, CA: Star Publishing, **1993**.