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 (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 with the other three methods. The plates inoculated in air and incubated in the Bactron IV anaerobic chamber passed quality control 100% of the time for all six isolates tested. Colonies were mounted in a dissecting microscope. Table 1 shows the mean diameters of colonies of the six quality control isolates with the Chamber-Chamber method. Organism GasPak AnaeroPack Chamber Chamber

<table>
<thead>
<tr>
<th>Organism</th>
<th>Setup: incubation</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides fragilis</td>
<td>1.38</td>
<td>1.24</td>
<td>1.33</td>
<td>1.41</td>
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<tr>
<td>Clostridium perfringens</td>
<td>5.82</td>
<td>5.98</td>
<td>5.59</td>
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<td>Fusobacterium necrophorum</td>
<td>2.78</td>
<td>2.45</td>
<td>2.82</td>
<td>2.79</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
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<td>0.23</td>
<td>0.52</td>
<td>0.63</td>
</tr>
<tr>
<td>Peptostreptococcus anaerobius</td>
<td>1.99</td>
<td>1.81</td>
<td>2.13</td>
<td>2.26</td>
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<tr>
<td>Porphyromonas levii</td>
<td>0.53</td>
<td>0.3</td>
<td>0.31</td>
<td>0.63</td>
</tr>
</tbody>
</table>

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

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 2 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 x 10^7 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 polymicrobial [2], successful isolation of each member of the mixed population frequently requires that the isolate grow at least 3+.

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 both jar procedures. This difference could be due to the shorter air exposure in the Air-Chamber procedure than with both jar procedures. The Air-Chamber failure rate for 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
Table 2. Growth of six quality control strains: comparison of four methods for cultivating anaerobic bacteria.

<table>
<thead>
<tr>
<th>Organism, method</th>
<th>No growth</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>No. of times tested</th>
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<td><strong>Fusobacterium nucleatum</strong></td>
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<td>0</td>
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<td>33.3</td>
<td>66.7</td>
<td>36</td>
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<td>38.5</td>
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<td>0</td>
<td>100</td>
<td>36</td>
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</tr>
<tr>
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<td>97.2</td>
<td>36</td>
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<td>5.6</td>
<td>0</td>
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</table>

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