Evaluation of Four Methods for Isolation of Neisseria gonorrhoeae

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Bio-Bag (Marion Laboratories, Kansas City, Mo.) type C is made up of a zip-lock plastic bag which contains a Thayer-Martin plate and a crushable CO2-generating ampoule. This system was compared with the candle extinction jar, Gono-Pak (Nasco, Fort Atkinson, Wis.), and JEMBEC (GIBCO Diagnostics, Lawrence, Mass.) systems to determine their efficiency and reliability for the isolation of Neisseria gonorrhoeae. A total of 191 anal and 130 urethral specimens were tested. There were 104 isolates of N. gonorrhoeae (24 anal and 80 urethral). The candle jar and Bio-Bag systems each detected 98 (94%) of the isolates. The Gono-Pak and JEMBEC systems detected 102 (98%) and 100 (98%) of the 104 isolates, respectively. These differences are not statistically significant. The Bio-Bag has the advantage of immediate CO2 release as compared with the Gono-Pak and JEMBEC systems, where CO2 production is dependent on the release of moisture from the medium. The Bio-Bag is a useful system, especially in situations where it is not convenient to use a candle jar.

Thayer-Martin medium incubated in candle extinction jars has been the standard reference system for the isolation and identification of Neisseria gonorrhoeae since its introduction in 1964 (5). Use of candle jars in a clinic or a physician's office is not always possible due to insufficient incubator space and difficulty in transportation to a laboratory. This led to the development of the Gono-Pak (Nasco, Fort Atkinson, Wis.) (1) system, wherein the Thayer-Martin plate is placed in a plastic bag along with a CO2-generating (citric acid-sodium bicarbonate) tablet, and the bag is sealed. The disadvantage of this system is that generation of CO2 is accomplished by release of water from the medium. Thus, the efficacy of the system is dependent on the freshness of the medium. The JEMBEC (GIBCO Diagnostics, Lawrence, Mass.) (3) system was developed utilizing the same principles as the Gono-Pak system. Modified Thayer-Martin medium is dispensed in a rectangular plastic container which has a well into which a CO2-generating tablet is placed. (The tablet is the same as for the Gono-Pak system.) Activation is also dependent on the freshness of the medium.

The Bio-Bag (Marion Laboratories, Kansas City, Mo.) type C system eliminates dependence on the freshness of the medium and allows for immediate CO2 release into the surrounding enclosed environment. Thayer-Martin medium is placed in a zip-lock type of plastic bag of 0.76-mm thickness. A CO2 generator containing one 130-mg sodium bicarbonate tablet and one 0.5-ml ampoule of 1.56 N hydrochloric acid is packaged in a plastic shell. The ampoule is crushed for activation. This provides rapid and consistent amounts of CO2 for 48 h.

This study was initiated to determine the efficiency and reliability of the Bio-Bag type C system for the isolation of N. gonorrhoeae in comparison with the candle extinction jar, Gono-Pak, and JEMBEC systems. Colony count and size were also evaluated.

MATERIALS AND METHODS

The study group consisted of men attending a clinic for gay men at the Fenway Community Health Center in Boston, Mass. The clinic was selected because it is an excellent facility with clients who have a high rate (15 to 20%) of infection with N. gonorrhoeae. Specimens for cultures were obtained from the urethra and anal canal as indicated by epidemiological history. Cultures were not taken from a specific site solely for the purpose of the study. The urethral specimens for culture were obtained by inserting a urethrogenital Calgiswab (GIBCO Diagnostics) approximately 2.5 cm into the anterior urethra. Anal specimens were obtained by inserting a sterile, cottontipped swab approximately 2.5 cm into the anal canal (7).

Each specimen was placed immediately into a tube containing 1.0 ml of tryptic soy broth (GIBCO). After agitation, the swab was pressed against the wall of the tube to express all excess fluid. The swab was then discarded. Three Thayer-Martin plates and one JEM-
BEC plate were inoculated. Four swabs were introduced successively into the broth medium and inoculated onto the respective plates in random order. The swab was rotated 360° in a "Z" pattern over one-third to one-half of the medium, expressing as much of the fluid as possible. This inoculum was then cross-streaked with a sterile bacteriological loop. The plates were then introduced into their respective CO₂ environments. One Thayer-Martin plate identified by patient name was placed into the candle jar. One Thayer-Martin plate numbered consecutively (1, 2, 3, etc.) was placed into a Whirl-Pak bag (Nasco, Fort Atkinson, Wis.) of 0.76-mm thickness. A citric acid-sodium bicarbonate (100 mg) tablet was placed in the bag, and the bag was sealed. The third Thayer-Martin plate, labeled with the patient's clinic number, was placed in a Bio-Bag of 0.76-mm thickness. A CO₂-generating ampoule was placed in the bag, and the bag was sealed. The generator was crushed by squeezing firmly. An immediate bubbling reaction was observed. A 100-mg citric acid-sodium bicarbonate tablet was placed into the well of the JEMBEC plate (modified Thayer-Martin medium), labeled with the patient's date of birth. The plate was placed in a plastic zip-lock bag of 0.76-mm thickness, and the bag was sealed. All of the systems except JEMBEC utilized Thayer-Martin medium from the same lot and shipment.

The plates were incubated overnight at 35 to 36°C and delivered to the State Laboratory Institute the next day. Cultures were examined after 24 and 48 h of incubation (4, 6), noting number of colonies and colony size. Oxidase-positive colonies with morphology typical of Neisseria which contained gram-negative cocci were examined by using a fluorescent-antibody test. Organisms which gave a positive reaction were considered to be N. gonorrhoeae.

The conjugate used for the fluorescent-antibody technique was manufactured by Difco Laboratories, Detroit, Mich. Before use, quality control was performed by means of titration (4, 6). The following organisms were utilized: N. gonorrhoeae (four strains), N. meningitidis (two strains), one strain each of N. lactamica, N. perflava, N. flava, N. subflava, N. sicca, N. flavescens, Branhamella catarrhalis, Staphylococcus epidermidis, Escherichia coli, and Enterobacter cloacae. In accordance with the Center for Disease Control protocol (6), the working dilution selected (1:32) along with the surrounding dilutions (1:16 and 1:64) must show clear-cut results with no cross-reactivity. Thus, the four strains of N. gonorrhoeae showed 4+ fluorescence at 1:16, 1:32, and 1:64 dilutions. The N. meningitidis, saprophytic Neisseria, and other bacteria showed no cross-reactivity. Two strains of N. gonorrhoeae were used routinely as positive controls, one strain of N. meningitidis was used as a negative control, and E. cloacae was used as the nonspecific staining control.

RESULTS

Cultures were obtained from one or more sites from 277 men. Cultures were obtained from both the urethra and the anal canal from 44 men, from the urethra only from 86 men, and from the anal canal only from 147 men. Table 1 compares the rates of isolation of N. gonorrhoeae in the four systems. Of 104 isolates detected by any of the systems, 102 (98.1%) were isolated with Gono-Pak, 100 (96.2%) with JEMBEC, and 98 (94.2%) each with the candle jar and Bio-Bag systems. There were no significant differences.

Table 2 compares colonial size of gonococcal isolates in the candle jar system with that in each of the other three systems after 48 h of incubation. Most of the specimens resulted in colonies of equal size in each of the systems. Larger colonies, however, were seen more often in each of the other systems than in the candle jar system. Colony count showed even less variation.

Fifty-two additional urethral cultures were evaluated to test the efficacy of individually packaged CO₂ gas generators. (The original evaluation used generators stored in glass jars with a desiccant.) The three test systems were Thayer-Martin/candle jar, Thayer-Martin medium with an individually packaged gas generator in a Whirl-Pak bag (Nasco), and Thayer-Martin medium in a Bio-Bag. Forty-eight isolates of N. gonorrhoeae were detected in all three systems. Four cultures were negative in all systems.

### Table 1. Comparison of candle jar, Gono-Pak, Bio-Bag type C, and JEMBEC systems for the recovery of N. gonorrhoeae

<table>
<thead>
<tr>
<th>System</th>
<th>Anal canal (%)</th>
<th>Urethra (%)</th>
<th>Total no. (%) (n = 321)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thayer-Martin/candle jar</td>
<td>22 (91.7)</td>
<td>76 (95.0)</td>
<td>98 (94.2)</td>
</tr>
<tr>
<td>Gono-Pak</td>
<td>23 (95.8)</td>
<td>79 (98.8)</td>
<td>102 (98.1)</td>
</tr>
<tr>
<td>Bio-Bag</td>
<td>22 (91.7)</td>
<td>76 (95.0)</td>
<td>98 (94.2)</td>
</tr>
<tr>
<td>JEMBEC</td>
<td>21 (87.5)</td>
<td>79 (98.8)</td>
<td>100 (96.2)</td>
</tr>
<tr>
<td>Totals detected by any system</td>
<td>24</td>
<td>80</td>
<td>104</td>
</tr>
</tbody>
</table>

* P = 0.3 in all cases.

### Table 2. Comparison of colonial size of N. gonorrhoeae isolates (n = 98) in candle jar system compared with those in the Gono-Pak, Bio-Bag, and JEMBEC systems

<table>
<thead>
<tr>
<th>Other system (no. of isolates)</th>
<th>Culture positive only in other system</th>
<th>Culture positive only in other system</th>
<th>Larger colonies in other systems</th>
<th>Colones same size</th>
<th>Larger colonies in candle jar</th>
<th>Culture positive only in candle jar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gono-Pak (102)</td>
<td>6</td>
<td>31</td>
<td>61</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bio-Bag (98)</td>
<td>5</td>
<td>15</td>
<td>69</td>
<td>9</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>JEMBEC (100)</td>
<td>4</td>
<td>28</td>
<td>60</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The candle extinction jar was the standard reference system for this study. The Gono-Pak and JEMBEC systems were also used as references based on their proven efficacy (1–3). The Thayer-Martin plates used in the candle jar, Gono-Pak, and Bio-Bag systems were all from the same lot and had been subjected to routine quality control procedures (4, 6) before use. The modified Thayer-Martin medium/JEMBEC system also was subjected to quality control (4, 6) before use. The plates in the four systems were inoculated at the same time by one person, incubated in the same incubator for the same length of time, and picked up and delivered to the laboratory by one messenger. Readings after 24 and 48 h of incubation were performed at the same time by one observer. Thus, the only variables were the source of CO2 and the container (jar or bag).

These data show no significant differences between the Bio-Bag type C system and the other three systems. For the isolation of N. gonorrhoeae, the Bio-Bag has the advantage of immediate, consistent release of CO2 and is not dependent upon the freshness (moisture content) of the medium as are the Gono-Pak and JEMBEC systems.

LITERATURE CITED