Ammonium Bicarbonate as a Replacement for Carbon Dioxide in Transgrow Bottles for Primary Isolation of *Neisseria gonorrhoeae*

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Transgrow bottles with medium containing ammonium bicarbonate and Transgrow bottles gassed with 10% carbon dioxide performed equally well in detecting *Neisseria gonorrhoeae* in 434 clinical specimens. It appears that incorporation of ammonium bicarbonate into the medium increased the efficiency of the manufacturing process while maintaining the effectiveness of the medium.

The importance of a partial CO2 environment for enhancing primary growth of the gonococcal strains was first reported by Wherry and Oliver (10). Chapin (3) introduced the candle jar extinction method as a means of providing this atmosphere, and in 1947 Spink and Keefer (8) confirmed that this method supplied adequate CO2 for primary isolation of gonococci. However, the candle jar is breakable, cumbersome, and difficult to transport and requires a large incubator space. In 1971, Martin and Lester (6) described a transport medium for *Neisseria gonorrhoeae* called Transgrow, which is prepared in screw-capped bottles and charged with CO2 after preparation. In 1973, Catlin (1) incorporated sodium bicarbonate into a chemically defined medium for isolation of *N. gonorrhoeae* but still incubated the medium in a CO2-enriched atmosphere. Morse and Bartenstein (7) incorporated sodium bicarbonate into a liquid medium for isolation of *N. gonorrhoeae*, with incubation in an ambient atmosphere. In 1975, Talley and Baugh (9) suggested that the addition of bicarbonate to a culture medium could replace the ambient CO2 requirement. In 1977, Jones and Talley (4) reported that incorporation of sodium bicarbonate into a nutritionally correct medium for *N. gonorrhoeae* satisfied the CO2 requirements as well as ambient CO2 did for some but not all gonococcal strains tested. Since some problems with the use of sodium bicarbonate were encountered by these workers, perhaps owing to the additional sodium, we decided to evaluate the use of ammonium bicarbonate.

Transgrow is produced and distributed by a number of state and local health departments for transport of *N. gonorrhoeae* cultures. Approximately 3 × 10⁶ Transgrow bottles are so produced or are commercially prepared and purchased annually for use in a national gonorrhea screening program. Charging bottles with CO2 is a time-consuming, costly, and labor-intensive operation that is subject to error. The presence of either inadequate or excessively high concentrations of CO2 in Transgrow bottles was responsible for problems with gonococcal isolation in comparative studies (5). Cylinders with incorrect gas mixtures that failed to provide the appropriate atmosphere for growth of the gonococci have been received from suppliers. Ensuring that each Transgrow bottle is charged with adequate CO2 and that such an atmosphere is maintained within the bottle remains a constant problem. Tipping or inverting the bottles during inoculation will cause loss of gaseous CO2. Therefore, an alternative to providing an ambient CO2 atmosphere in Transgrow bottles, one that would increase manufacturing efficiency and provide a consistent product effective for recovery of the gonococci was sought.

A 10% solution of ammonium bicarbonate (10.0 g/100 ml distilled water) was prepared and filter sterilized on the day of use. Then, as a substitute for ambient CO2, 10 ml of this solution was added to each 1,000 ml of Transgrow medium to give a 0.1% final concentration of ammonium bicarbonate.

In this study, equal quantities of Transgrow medium (modified Martin-Lewis formulation) were prepared with and without ammonium bicarbonate. Ammonium bicarbonate was added at the time of preparation to half of the medium before bottling. The other half of the medium was bottled and gassed with a 10% CO2-90% air mixture. The lots were subjected to quality control procedures before use (2). The shelf life of both lots was found to be at least 3 months when the lots were stored at 2 to 8°C.
TABLE 1. Isolation of *N. gonorrhoeae* from 434 clinical specimens

<table>
<thead>
<tr>
<th>Results with CO₂-gassed Transgrow medium</th>
<th>Results with Transgrow medium + NH₄HCO₃</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>129</td>
<td>10</td>
<td>291</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Three county health departments participated in this study by providing clinical specimens. Duplicate swabs were taken from each patient so that one swab could be used to inoculate each type of medium at these collection sites.

The inoculated bottles were returned to the laboratory either by courier on the day of collection or through the mail after 16 to 18 h of incubation at 36 ± 0.5°C at the collection site. Upon arrival at the laboratory, all bottles were incubated at 36 ± 0.5°C in a non-CO₂ environment and observed for growth after 24 and 48 h of incubation. Organisms were identified by oxidase reaction, Gram stain, and carbohydrate degradation.

Of the 434 patient specimens examined, 143 yielded *N. gonorrhoeae* isolates, 139 in the ammonium bicarbonate system and 133 in the CO₂-gassed Transgrow medium. Growth varied from confluence to ca. 25 colonies (Table 1). Bottles of Transgrow medium containing ammonium bicarbonate detected the same 129 positive specimens as did bottles gassed with 10% CO₂. In 10 cases, only the ammonium bicarbonate medium detected *N. gonorrhoeae*, and in 4 cases, only the gassed medium detected *N. gonorrhoeae*. The McNemar test was used to determine whether there was a statistically significant difference in the rate of recovery on one medium versus the other. The number of isolations in Transgrow with ammonium bicarbonate was not significantly different than that obtained with gassed Transgrow. These findings indicate that the ammonium bicarbonate-containing Transgrow medium was comparable to the CO₂-charged Transgrow medium for primary isolation of *N. gonorrhoeae*. This was the first evaluation of a bicarbonate medium in which primary cultures were used. These results are in essential agreement with those of Jones and Talley (4) for a bicarbonate medium in a sealed jar. It appears that incorporation of ammonium bicarbonate into Transgrow medium for primary isolation of gonococci provides a means for increasing the efficiency of the manufacturing process while maintaining the effectiveness of the medium.

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LITERATURE CITED