Viability of Neisseria gonorrhoeae in Stuart’s Transport Medium

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Abstract

Stuart’s transport medium (STM) was evaluated for its efficacy as a transport medium for N. gonorrhoeae in 55 Thai female patients. The endocervical specimens were inoculated into STM and incubated for various durations before further inoculating onto Thayer-Martin (TM) medium. One of the specimens was directly streaked on TM medium and immediately incubated as control. Viability of N. gonorrhoeae in STM was reduced 3.64%, 7.27%, 9.09%, 16.98%, 22.22%, 32.08% and 45.45% when specimens were incubated in STM for 6, 9, 12, 15, 18, 21 and 24 hours respectively, as compared with control specimens. There was no difference in viability between the control and the tested specimens inoculated after 6 hours in STM. In our opinion, the decrease in growth rate of N. gonorrhoeae after 9 hours incubation in STM by 7.3% was not statistically significant. Thus the specimen could be kept in STM for up to 9 hours before subculture onto TM medium (key words: Viability, Stuart’s transport media, N. gonorrhoeae)

INTRODUCTION

The cultural method is essential for the diagnosis of gonococcal infection in the female. Where facilities for cultures are available the clinical specimens are directly inoculated onto the selective medium. The well-known selective medium is TM medium which contains polymyxin B or vancomycin, colistin and nystatin. Riddell and Buck have also added trimethoprim to increase the selectivity of the medium. There is the limitation that the specimens must be inoculated onto the medium immediately. Whenever possible, where facilities for culture are not readily available, the agent has to be kept alive in a transport medium before inoculation onto the TM medium.
Stuart’s transport medium (STM) is usually employed as it can keep Neisseria gonorrhoeae viable up to 24 hours. The viability of gonococci deteriorates progressively after 24 hours in STM. There are very few field studies about the appropriate time that the specimens should be held before culturing onto TM medium. The purpose of the present study is to compare the results obtained when the specimens are inoculated directly onto TM medium with those obtained when the specimens are held in STM at various intervals of time under the local environmental conditions before subculture on TM medium.

MATERIALS AND METHODS

Fifty-five female patients attending at the Bangrak Hospital from January 17, 1983 to February 18, 1983 were enrolled in the study. They had Gram negative intracellular diplococci in the Gram’s stained smear of the specimens collected from the endocervix. None of them was pregnant and no one was less than 15 years old. Nine specimens were collected from each patient, using the loop for the first specimen and charcoal swabs for the rest. The loop was immediately inoculated onto TM medium which was incubated in a candle jar at 35°C for 24-48 hours. The remaining 8 charcoal swabs were placed in STM as follows:

1. The first collection of specimens was put in the control tube.
2. The second collection of specimens was kept in STM tube for 24 hours.
3. The third collection of specimens was kept in STM tube for 21 hours.
4. The fourth collection of specimens was kept in STM tube for 18 hours.
5. The fifth collection of specimens was kept in STM tube for 15 hours.
6. The sixth collection of specimens was kept in STM tube for 12 hours.
7. The seventh collection of specimens was kept in STM tube for 9 hours.
8. The eighth collection of specimens was kept in STM tube for 6 hours.

The specimens from the control tubes were inoculated onto TM medium within 15 minutes and were used as control. The other specimens were removed from STM and were inoculated onto TM medium at 6, 9, 12, 15, 18, 21 and 24 hours respectively.

After inoculation, the plates were incubated in the candle jars with moistened filter papers at the bottom and kept in the incubator at 35°C for 24-48 hours. After that period the cultures were examined and the identification of Neisseria gonorrhoeae was based on the typical colonial morphology, Gram’s stained smears showing Gram negative diplococci and a positive oxidase test. Isolates were confirmed as gonococci by sugar fermentation reactions.

RESULTS

Table 1 shows the results obtained when the specimens were kept in STM for various intervals of time. It was found that the percentage growth was reduced by 3.64%, 7.27%, 9.09%, 16.98%, 22.22%, 32.08% and 45.45% at 6, 9, 12, 15, 18, 21 and 24 hours respectively when compared with the control. The percentage loss of growth was increased according to the period of time that the specimens were held before culturing (Figure 1). There was no difference between the control and the tested specimens inoculated at 6 hours. (Sign test P Value > 0.1586). However, we cannot state whether the control and the tested specimens inoculated at 9 hours intervals are different or not, because of the statistical result (P = 0.05). The difference was statistically significant for the results of the positive culture when the specimens were kept in STM at the other intervals of time (12, 15, 18, 21 and 24 hours respectively).

Table 1 Comparison of the results obtained when the specimens were kept in the STM for various intervals of time.

<table>
<thead>
<tr>
<th>Time intervals between the Specimens collection and the inoculation onto TM medium</th>
<th>Control</th>
<th>6hr</th>
<th>9hr</th>
<th>12hr</th>
<th>15hr</th>
<th>18hr</th>
<th>21hr</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of positive cultures</td>
<td>55</td>
<td>53</td>
<td>51</td>
<td>50</td>
<td>44</td>
<td>42</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>No. of negative cultures</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>12</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>No. of contaminate</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>% rate loss</td>
<td>0%</td>
<td>3.64%</td>
<td>7.27%</td>
<td>9.09%</td>
<td>16.98%</td>
<td>22.22%</td>
<td>32.08%</td>
<td>45.45%</td>
</tr>
<tr>
<td>P value</td>
<td>-</td>
<td>0.16</td>
<td>0.05</td>
<td>0.01</td>
<td>0.0013</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>
Infect Dis Antimicrob Agents

DISCUSSION

In the present study, it was observed that when the specimens were held in STM for various intervals of time within 24 hours, the longer the specimens were held, the greater the loss of growth (Figure 1). The highest yield of positive results was observed when the specimens were inoculated within 6 hours after being held in STM. After 6 hours, the isolation rate began to fall. This result is in agreement with Kandhari et al. (1972). There are very few field studies about the appropriate time that the specimens should be held in the STM. Physicians in Sweden, as in the other countries in Scandinavia, usually have to send the specimens to the laboratory by post, and the transport time is usually 16-24 hours or longer. In 1973 Danielsson et al compared the yield with both selective and non-selective gonococcal culture media inoculated in the clinic and after transport of specimens. They found that after the specimens were held in the STM for 18-20 hours, 9% of the female would have escaped the diagnosis of gonorrhoea if only selective culture media had been used, and as many as 12% if only nonselective culture media had been used. However, WHO stated that the swabs can be placed in STM, and kept in the refrigerator if the delay before plating has not exceeded 12-18 hours. Hosty et al (1974) noted a loss of 44% of positive women after the specimens were held in STM for 24 hours while in this investigation we observed a 45%-reduction, which was not much different. This is in contrast to Kandhari et al (1972), who found that the rate of positive cultures had only 21.7% loss at 24 hours.

In this study we were unable to state whether the results obtained in the control group and the tested group are different or not when the specimens were inoculated at 9 hour intervals, because of the statistical result (Sign test P value = 0.05). In the determination of the percentage loss of growth, we found a loss of only 7.27% of positive women. Thus our findings indicate that the specimens may be held in STM for up to nine hours without significant loss of viability of N. gonorrhoeae. This may apply to regular practice and should encourage private practitioners to employ the cultural method in the diagnosis of gonorrhoea in the female. This would be of great benefit in the control of gonorrhoea.

ACKNOWLEDGEMENT

The authors feel grateful to Dr. Mukda Trishnanda, chairman of Epidemiology Projects, Mahidol University for his valuable suggestions and his permission to establish the study in this journal. We wish to thank the personnel of the Bangrak VD clinic, VD Control Division, for their valuable help with the preparation of the patients and preparation of the media during the study.

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