Effect of Holding Temperature on Isolation of Neisseria gonorrhoeae

KAYE D. EVANS, ELLENA M. PETERSON, JANIS I. CURRY, J. R. GREENWOOD, AND LUIS M. DE LA MAZA
Division of Medical Microbiology, Department of Pathology, University of California Irvine Medical Center, Orange, California 92668, and Health Care Agency, County of Orange, Santa Ana, California 92706

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The effect of holding temperature on the recovery of Neisseria gonorrhoeae was studied. From 300 specimens tested, Thayer-Martin medium plates inoculated and incubated in the presence of CO₂ at 35, 22, and 4°C for 24 h before incubation at 35°C yielded 100, 96, and 95% of all isolates ultimately recovered from 82 positive specimens. Although there was a decrease in the quantity of organisms recovered, initial incubation of specimens under refrigeration or at room temperature yielded ≥95% of the positive specimens.

The recommended procedure for the isolation of Neisseria gonorrhoeae involves inoculation of a specimen in the patient care area directly onto a nutritive growth medium that is at room temperature, such as Thayer-Martin (TM), Martin-Lewis, or New York medium, and immediate incubation at 35 to 37°C in an atmosphere of 3 to 10% CO₂ (3, 5). In situations in which specimens must be transported over long distances, to maximize recovery and survival of N. gonorrhoeae the inoculated medium should be incubated at 35 to 37°C for 18 to 24 h before being mailed or transported to the microbiology laboratory (3, 5).

Unfortunately, many clinics, including those at remote locations, using the services of regional microbiology laboratories are unable to provide the initial incubation at 35 to 37°C before submitting inoculated media to the regional laboratories. The conditions under which these specimens are transported are often beyond the control of the laboratories involved. Transporting a specimen at 35°C requires expensive special handling and equipment. Ambient temperature or wet ice is often the condition under which specimens are transported. For these reasons, a study was undertaken to evaluate the effect on the recovery of N. gonorrhoeae of different holding temperatures for inoculated plates held in an atmosphere of increased CO₂.

A total of 300 specimens, of which 138 (46%) were from males and 162 (54%) were from females, were included in this study. All the specimens were taken from patients seen at the Special Diseases Clinic (Sexually Transmitted Diseases Clinic) of the Orange County Health Department. Body sites cultured were: genital, 268 of 300 specimens (89%); rectal, 12 of 300 specimens (4%); and throat, 20 of 300 specimens (7%). Within 1 h of collection, the specimens were inoculated onto one chocolate agar plate and three TM agar plates. All the plates were at room temperature when inoculated, and the order of inoculation of the TM agar plates was changed every 100 samples. Upon inoculation, the chocolate agar plate and one TM agar plate were immediately placed in a candle jar and held in a 35°C incubator. The other two TM agar plates were placed in candle jars and held at 22 or 4°C. Candle jars were held at their respective temperatures (35, 22 or 4°C) for 18 to 24 h. All the jars were then placed in an incubator at 35°C. All the plates were examined for the presence of N. gonorrhoeae at 48 h after inoculation, and all the plates were reincubated in candle jars at 35°C. At 96 h after inoculation, all the plates were examined again for the presence of N. gonorrhoeae. From all plates, colonies which resembled N. gonorrhoeae were reinoculated to chocolate agar. Identification of N. gonorrhoeae was confirmed by Gram stain, oxidase reaction, and carbohydrate utilization by using the Minitest system (BBL Microbiology Systems, Cockeysville, Md.).

N. gonorrhoeae was isolated from 82 of the 300 (27%) samples examined. TM plates initially incubated at 35°C grew N. gonorrhoeae from all 82 (100%) positive specimens. Chocolate agar plates which were initially incubated at 35°C grew N. gonorrhoeae from 80 (97.6%) positive specimens. TM plates held at room temperature (22°C) for 18 to 24 h after inoculation grew N. gonorrhoeae from 79 (96.3%) positive specimens, and TM plates initially refrigerated overnight yielded N. gonorrhoeae from 78 (95.1%) positive specimens.

The quantity of N. gonorrhoeae growing on each TM plate was compared with that on chocolate agar (Table 1). The same amount of growth was present in 69% (55 of 80) of the TM plates held at 35°C, 33% (26 of 80) of the TM plates held at 22°C, and 26% (21 of 80) of the TM plates held at 4°C. However a 50% reduction in growth was seen in 5% (4 of 80) of the 35°C TM plates, 35% (28 of 80) of the 22°C TM plates, and 44% (35 of 80) of the 4°C TM plates.

Although the ultimate quantity of N. gonorrhoeae organisms recovered was compromised, the number of specimens from which organisms were recovered from plates held at room temperature or under refrigeration did not differ dramatically from the number from which organisms were recovered from plates held at recommended temperatures. In fact, 95% of the isolates were recovered from plates initially held at 4°C for 24 h. These results complement those reported by Ratner et al. (6) and Chapel et al. (2), who studied the effect of inoculating specimens for N. gonorrhoeae isolation onto refrigerated versus room temperature plates followed by immediate incubation at 37°C. In both studies, they found that, whereas there was a decrease in the size and number of colonies isolated from specimens inoculated onto a cold agar plate, there was a final recovery of 99 and 98%, respectively, of all isolates from the cold plate as compared with the room temperature plate. We had 5% negative specimens with cold incubation, but in our study the plates were held for 24 h at 4°C, compared with the
from two specimens. With both specimens, *N. gonorrhoeae* grew on all three TM plates. Both specimens had other flora that grew on chocolate agar in a quantity greater than that of *N. gonorrhoeae* on the 37°C TM plate. In some studies, vancomycin (4 μg/ml)-susceptible strains comprised 2 to 10% of all *N. gonorrhoeae* isolates (1, 4); however, we did not recover any in our patient population.

In summary, the practice of transporting cultures under suboptimal temperatures does not have a significant effect on the overall recovery of *N. gonorrhoeae* from a population with a high incidence of the organism. Whether this also holds true for a low-incidence population remains to be established.

**LITERATURE CITED**


