PANCREATIC DIGEST CHOCOLATE BLOOD AGAR FOR THE ISOLATION OF THE GONOCOCCUS

MARIE L. KOCH

Departments of Bacteriology and Gynecology, Johns Hopkins Medical School, Baltimore, Maryland

Received for publication April 3, 1948

Since Neisseria gonorrhoeae was first cultivated by Bumm (1885) on placental blood serum, beef serum, or sheep serum, many media have been devised and tested for the isolation of the gonococcus from suspected cases of gonorrhea. Prior to 1933 the medium most used was the Douglas (1914) agar. This was a tryptic digest of beef enriched with either ascitic fluid, hydrocele fluid, beef serum, or defibrinated blood, and frequently with the addition of 1 per cent glucose. In 1910 Cohen and Fitzgerald first introduced the use of chocolate blood agar for the isolation of Hemophilus influenzae, but it was not until 1927 that McLeod and his associates used chocolate agar for the isolation of the gonococcus. The chocolate agar was prepared either by using a base of "filtered, sterilized, 75 C meat extract" or by using Wright's bouillon, which is made by extracting meat and peptone together.

Wherry and Oliver (1916) reported that the gonococcus grew best when grown in an atmosphere of approximately 10 per cent carbon dioxide. This was again recommended by McLeod (1934), although Erickson and Albert (1922), Cook and Stafford (1921), and Torrey and Buckell (1922) had considered reinforcing the air with carbon dioxide to be unnecessary for the isolation of the gonococcus.

Leahy and Carpenter (1936), using chocolate agar prepared from a Douglas agar base and incubating cultures for 48 hours at 37 C in an atmosphere containing 10 per cent carbon dioxide, isolated gonococci in 34 per cent of 302 specimens. By using the oxidase test, 14 per cent of 146 cultures that would otherwise have been reported negative were found to be positive. Also by incubating cultures in 10 per cent carbon dioxide they were able to isolate 15 per cent more positive cultures than were recovered from duplicate cultures incubated aerobically, under conditions that were otherwise identical.

Finger, Ghon, and Schlaganhauser (1894) stated that the gonococcus grew best in a slightly acid medium. They used litmus as an indicator and the degree of acidity was not determined. Swartz, Shohl, and Davis (1920) showed the acid end point of the gonococcus, in vitro, to be pH 6.6.

The report to be presented in this paper comprises a study of the value of pancreatic digest of beef heart as described by Brown (1948) for supporting the growth of the gonococcus in pure culture and as a medium for the isolation of the organism from cervical secretions.
STUDIES ON THE GROWTH OF PURE CULTURES OF NEISSERIA GONORRHOEAE IN PANCREATIC DIGEST BROTH AND ON PANCREATIC DIGEST CHOCOLATE BLOOD AGAR

Six freshly isolated cervical strains of the gonococcus were tested for their ability to grow in pancreatic digest broth having various pH values and on pancreatic digest chocolate blood (human, 10 per cent) agar, pH 7.2. A series of tubes containing 5.0 ml of the broth to be tested and 0.1 ml of a 24-hour broth culture (pH 7.2) were incubated at 37 C for 24 hours. The tubes were then thoroughly shaken and 0.1 ml from each tube was transferred to a corresponding pancreatic digest chocolate blood agar plate. The plates were spread, incubated for 24 hours, and the colonies counted.

The six strains reacted in the same manner. Prolific growth was obtained in broth at pH 7.2 as evidenced by innumerable colonies per plate. At pH 6.8 the growth was reduced approximately 50 per cent as compared with growth in broth at pH 7.2. At pH 6.6, 6.4, 6.0, and 5.8 all plates were sterile. A duplicate set of plates was made and incubated in an atmosphere of approximately 10 per cent carbon dioxide. The results were the same as described above.

After 48 hours' incubation the colonies are quite characteristic. They are of medium size, round, smooth, convex, and have glistening "wet"-appearing surfaces. The colonies have a tendency to stick to the needle, forming threads, when being transferred.

STUDIES ON PANCREATIC DIGEST CHOCOLATE BLOOD AGAR FOR THE ISOLATION OF NEISSERIA GONORRHOEAE FROM CERVICAL SECRETIONS

This study comprises a total of 580 cervical cultures from 411 patients and a total of 452 cervical smears from 283 patients. All plates were incubated for 48 hours at 37 C in an atmosphere of approximately 10 per cent carbon dioxide. Oxidase tests were routinely made on all plates.

Group I. The results of cervical cultures from dispensary patients suspected of having gonorrhea. The cases are divided according to diagnosis.

<table>
<thead>
<tr>
<th>Total number of cultures</th>
<th>405</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cultures</td>
<td>153 (37.7 per cent)</td>
</tr>
<tr>
<td>Negative cultures</td>
<td>252 (62.3 per cent)</td>
</tr>
</tbody>
</table>

**Acute gonorrhea**

<table>
<thead>
<tr>
<th>Total number</th>
<th>86</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cultures</td>
<td>37 (43.0 per cent)</td>
</tr>
<tr>
<td>Negative cultures</td>
<td>49 (57.0 per cent)</td>
</tr>
</tbody>
</table>

**Urethritis**

<table>
<thead>
<tr>
<th>Total number</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cultures</td>
<td>8 (61.5 per cent)</td>
</tr>
<tr>
<td>Negative cultures</td>
<td>5 (38.5 per cent)</td>
</tr>
</tbody>
</table>

**Cervicitis**

<table>
<thead>
<tr>
<th>Total number</th>
<th>155</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cultures</td>
<td>64 (41.2 per cent)</td>
</tr>
<tr>
<td>Negative cultures</td>
<td>91 (58.8 per cent)</td>
</tr>
</tbody>
</table>
Salpingitis
Total number .............................................. 125
Positive cultures ........................................... 37 (19.5 per cent)
Negative cultures .......................................... 88 (80.5 per cent)

Pelvic inflammatory disease
Total number .............................................. 26
Positive cultures ........................................... 7 (26.9 per cent)
Negative cultures .......................................... 19 (73.1 per cent)

Group II. This series comprises cervical cultures from ward and dispensary patients, all having clinical and physical signs of acute gonorrhea. At the time of culturing, the pH of the cervical mucus was tested by using a series of buffer solutions and indicators as described by Brown (1924). The following results were obtained:

Total number of cultures .................................... 175
Positive cultures ........................................... 113 (64.2 per cent)
Negative cultures .......................................... 62 (35.8 per cent)

Cultures taken when the cervical mucus pH range was 7.5 to 6.7
Total number .............................................. 129
Positive cultures ........................................... 109 (86.0 per cent)
Negative cultures .......................................... 20 (14.0 per cent)

Cultures taken when the cervical mucus pH range was 6.6 to 5.2
Total number .............................................. 46
Positive cultures ........................................... 4 (8.7 per cent)
Negative cultures .......................................... 42 (91.3 per cent)

A comparison of the method of cervical smear examinations and cervical cultures. From groups I and II a total of 452 cervical smears were examined. All smears were stained by Burke's modification (1922) of the gram stain. In this series 96 or 21.2 per cent of the smears were positive, whereas the gonococcus was isolated from 230 or 50.8 per cent of the same group of patients.

DISCUSSION

When pancreatic digest chocolate blood agar was used for the isolation of the gonococcus in a series of 580 cervical cultures, 266 or 45.9 per cent were positive. When the patients were divided into two groups, the group having only cultures (405) yielded 153 or 37.7 per cent positive cultures. The group having the pH of the cervical mucus tested at the time of culturing (175) yielded 113 or 64.2 per cent positive cultures. This simply means that if cultures are taken when the pH range of the cervical mucus is 7.5 to 6.7 (the optimal growth range of the organism) the chances of isolating the gonococcus are greatly increased. Conversely, if cultures are taken when the pH of the cervical mucus is acid (6.6 to 5.2), the chances of isolating the organism are greatly decreased. It is shown by the figures that, in a total of 62 negative cultures in this group, 42 or 67.7 per cent were obtained when the pH was 6.6 and below, pH 6.6 corresponding to the
acid death point of the gonococcus when grown \textit{in vitro}. Many of the patients who were again cultured during the estrogenic phases of their subsequent menstrual cycles yielded positive cultures (Koch, 1947).

In the group of patients having cervical smear examinations and cultures, the cultural method resulted in the discovery of 29.6 per cent more gonococcal infections than by the use of smear examinations alone. This should re-emphasize the invalidity of negative smears.

\textbf{ACKNOWLEDGMENT}

The author wishes to thank Dr. J. Howard Brown for his valuable assistance during the course of this investigation.

\textbf{SUMMARY}

Pancreatic digest chocolate blood agar supported a prolific growth of the gonococcus in pure culture after 24 hours' incubation at 37 C, both aerobically and in an atmosphere containing approximately 10 per cent carbon dioxide.

The medium proved to be of great value in the isolation of the organism from cervical secretions, especially in cases of acute gonorrhea from which 57.4 per cent positive cultures were obtained.

Negative cervical cultures do not indicate the absence of foci of infection or the lack of a sufficiently favorable medium upon which to isolate the organism. Other factors influencing negative cultures may be involved, an important one being the pH of the cervical mucus at the time of cultivating.

The cultural method for the diagnosis of gonococcal infections is far superior to smear examinations.

\textbf{REFERENCES}


Erickson, M. J., and Albert, H. 1922 Cultivation of the gonococcus. J. Infectious Diseases, 30, 268–278.


Wherry, W. B., and Oliver, W. W. 1916 Adaptations to certain tensions as shown by gonococcus and other parasitic and saprophytic bacteria. J. Infectious Diseases, 19, 288-298.