EFFECT OF CULTURE MEDIUM ON PREPARATION OF SEROLOGICAL REAGENTS FOR THE n FACTOR OF *STAPHYLOCOCCUS AUREUS*

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The n factor of *Staphylococcus aureus* (Haukenes and Oeding, Acta Pathol. Microbiol. Scand. 49:237, 1960) was originally demonstrated as a component of the old "e" factor of Oeding. Methods were described for preparing specific antiserum for n factor by use of serum for Cowan III (serotype aben) and adsorption with strains F21 (bcim) and 2095 (abc). Haukenes and Oeding (personal communication) used a nutrient agar prepared from natural materials for growth of cells for immunization and adsorption.

In our laboratory, we have used both Trypticase Soy Agar (TSA; BBL) and nutrient agar (Difco) for growing cells used for immunization and adsorption in separate series. For slide agglutination tests, nutrient agar was used because of a tendency for more strains to agglutinate spontaneously from the TSA.

In our first series, rabbits were immunized with TSA-grown cells, and the serum was adsorbed with cells from TSA. Agglutination antisera were produced successfully for factors a, b, c, e, h, i, k, and m, and fluorescent-antibody reagents were produced for factors a, b, c, h1, i, k, and m (Cohen and Oeding, J. Bacteriol. 84:735, 1962); but several attempts to prepare reagents for n factor ended in failure.

Since the initial work, we have received a small sample of n serum from Oeding. Strains 2095 and F21 from both TSA and nutrient agar were tested for agglutination in n factor serum. Both strains failed to agglutinate when grown on nutrient agar, but strain 2095 agglutinated weakly (+) when grown on TSA. When nutrient agar-grown cells were used in adsorption, antisera for the n factor were produced successfully from two lots of Cowan III serum from rabbits immunized with nutrient agar-grown cells, and one lot of Cowan III serum produced in the original series with TSA-grown cells. The latter serum had been used in our earlier unsuccessful attempts.

Fluorescein-labeled Cowan III globulin was adsorbed with strains 2095 and F21 from nutrient agar. The adsorbed labeled globulin agglutinated strains Cowan III and 1503 strongly but was negative with 2095, F21, and Cowan I. Fluorescent staining was observed for strains 17A, 1503, Cowan III, 3647, 2253, and 28. Strains Wood 46, Cowan I, Cowan II, S365, F21, 2095, and 3189 were negative. Except for the staining of strain 17A, the pattern of reactions agrees with that of agglutination reactions reported by Haukenes and Oeding. Strain 17A also agglutinated in our n antiserum. Strain DA629-3 (serotype en) stained brilliantly with this n factor fluorescent-antibody reagent. Preliminary results with other diagnostic strains indicate a fair degree of correlation between agglutination and fluorescent staining with the n factor reagents, although the intensity of staining is much better with some diagnostic strains than with others.

In conclusion, strain 2095 appears to produce a small amount of n antigen when grown on TSA. When 2095 was grown on nutrient agar, little if any n antigen was exhibited, and the cells were suitable for use in the adsorption procedure for the production of both n factor serum and n fluorescent-antibody reagent. Recently, Hofstad and Oeding (personal communication) have prepared the n factor serum from 1503 serum (serotype aemm) by adsorption with strains 2095, Cowan I, and 137. Preliminary work with this new procedure for n factor serum production resulted in successful preparation of n serum when nutrient agar-grown cells were used. However, the new procedure did not yield a higher titered serum than that produced with Cowan III antiserum.

These experiments indicate that the choice of culture medium for adsorption can be critical in preparation of serological reagents for *S. aureus*. Preliminary results with the k factor serum suggest that the medium for growth of immunizing cells influences the reaction spectrum of the k factor serum. The k serum prepared from rabbits immunized with TSA-grown cells ex-
hhibited a broader spectrum of reactions than serum from nutrient agar-grown cells. Specific recommendations concerning culture medium for use in preparing serological reagents for *S. aureus* would facilitate the development of a universal system for serological typing of staphylococci.

**INFLUENCE OF MILK ON THE GROWTH OF COAGULASE-NEGATIVE STAPHYLOCOCCI ON TELLURITE GLYCINE AGAR MEDIUM**

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During studies involving quantitative determination of coagulase-positive staphylococci in mastitic milk, discrepancies occurred between counts obtained on the tellurite glycine (TG) agar medium (Difco) of Zebovitz, Evans, and Niven (J. Bacteriol. 70:686, 1955) and on the Staphylococcus Medium No. 110 (Difco) of Chapman (J. Bacteriol. 51:409, 1946). This suggested that the milk itself might have an effect on the growth of staphylococci in certain selective media. Accordingly, an experiment was conducted to study the influence of milk on the growth of staphylococci on TG agar medium.

Ten coagulase-negative isolates were used. The organisms were grown in Brain Heart Infusion (BHI) broth (Difco) for 18 hr at 37 C, and 0.1-ml samples of a 10^6 saline dilution of each organism were inoculated into separate tubes containing 5 ml of sterile whole milk and also into doubling dilutions of milk in saline (1:64). Tubes containing 5 ml of sterile saline were also inoculated with a 0.1-ml sample of a 10^6 saline dilution of each organism; these served as controls. Five plates were made with 0.1-ml samples from these tubes on TG agar medium and BHI agar by spreading the inoculum with the aid of a bent glass rod. After incubation at 37 C for 24 hr, the colonies were counted.

The results indicated that 61 to 95% (average, 82%) of the organisms which grew on BHI agar also grew on TG agar medium in the presence of whole milk. In contrast, no growth took place on TG agar medium in the absence of milk. The representative results of five cultures with varying concentrations of milk (Table 1) indicated that the presence of milk diluted 1:32 or more did not permit the growth of coagulase-negative staphylococci on TG agar medium. The conclusion is that TG agar medium is of little use for the selective quantitative estimation of coagulase-positive staphylococci in mastitic milk, since, unless the milk samples are cultured in broth prior to plating on TG medium or diluted at least 1:32 before direct plating, coagulase-negative staphylococci will also grow on TG agar medium.

Table 1. Influence of dilution of milk on growth of coagulase-negative staphylococci

<table>
<thead>
<tr>
<th>Culture no.</th>
<th>Count on tellurite glycine agar medium*</th>
<th>Milk</th>
<th>Dilution of milk in saline</th>
<th>Saline</th>
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<tbody>
<tr>
<td>1</td>
<td>73 60 42 19 2 0 0 0</td>
<td>1:2</td>
<td>1:4 1:8 1:16 1:32 1:64</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>79 71 53 45 26 9 0 0</td>
<td>1:2</td>
<td>1:4 1:8 1:16 1:32 1:64</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>81 75 54 45 31 7 0 0</td>
<td>1:2</td>
<td>1:4 1:8 1:16 1:32 1:64</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>89 36 20 19 14 0 0 0</td>
<td>1:2</td>
<td>1:4 1:8 1:16 1:32 1:64</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>79 76 40 19 1 0 0 0</td>
<td>1:2</td>
<td>1:4 1:8 1:16 1:32 1:64</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>80 63 41 29 15 3 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as per cent count on Brain Heart Infusion Agar.

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