ENUMERATION OF PLEUROPNEUMONIA-LIKE CULTURES

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Received for publication December 3, 1959

Kelley and Morton (J. Bacteriol., 67, 129, 1954) and Smith (Appl. Microbiol., 4, 254, 1956) observed that use of colony counts or turbidimetric methods of counting pleuropneumonia-like organisms are difficult at best. In some studies on the limitations of “coding” of DNA (Morowitz and Cleverdon, Biochim. et Biophys. Acta, 34, 578, 1959), we repeatedly used the Bausch and Lomb Spectronic 20 at 625 μ, and replicate subcultures were made of serial decimal dilutions. The diluent was 9.0 ml of the growth medium warmed to 37°C after incubation for 48 hr to assure sterility. Pyrex screw-capped tubes (15 by 150 mm) were used so the dilution could be made by 6 careful inversions. (Inclusion of glass beads to increase the mixing with so few inversions appeared not to influence the counts.) The replicate culture medium was the growth medium, 2.0 ml (in 13 by 100 mm pyrex tubes, closed with aluminum caps) and warmed to 37°C. From each dilution, 5 replicates of 1 ml were made, using a separate 1.0-ml pipette for each. It was suitable to use a 5-ml pipette for the 5 replicates.

After 6 days at 37°C in a humid air incubator, the tubes were visually scored (this culture produces abundant acid from glucose), and the numbers converted to counts from the tables of Halvorson and Ziegler (J. Bacteriol., 25, 101, 1933) or of Fisher and Yates (Statistical tables for biological, agricultural and medical research, 2nd ed., 1943), or by recourse to the Poisson equation. Table 1 shows typical data of growth of the culture with calculated values.

### TABLE 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>No. of Positive Tubes after 6 Days</th>
<th>Most Probable Number per Mi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8}</td>
<td>Halvorson-Ziegler Fisher-Yates Poisson</td>
</tr>
<tr>
<td>0</td>
<td>0.080 5 5 2 0 0</td>
<td>4.9 X 10^6 2.77 X 10^6</td>
</tr>
<tr>
<td>120</td>
<td>0.060 5 5 4 1 0</td>
<td>1.7 X 10^6 3.31 X 10^6</td>
</tr>
<tr>
<td>260</td>
<td>0.085 5 5 5 5 0</td>
<td>2.4 X 10^6 3.04 X 10^7</td>
</tr>
<tr>
<td>380</td>
<td>0.170 5 5 5 2 0</td>
<td>5.4 X 10^6 2.36 X 10^7</td>
</tr>
<tr>
<td>490</td>
<td>0.199 5 5 5 3 0</td>
<td>9.2 X 10^6 2.27 X 10^8</td>
</tr>
<tr>
<td>610</td>
<td>0.258 5 5 5 1 0</td>
<td>3.3 X 10^7 1.92 X 10^8</td>
</tr>
<tr>
<td>780</td>
<td>0.486 5 4 2 0</td>
<td>2.2 X 10^8 2.08 X 10^9</td>
</tr>
<tr>
<td>1420</td>
<td>0.625 4 0 0 0</td>
<td>2.3 X 10^8 3.09 X 10^9</td>
</tr>
</tbody>
</table>

“most probable number” procedure, although it was considered unsatisfactory by Smith. The culture used was *Mycoplasma gallisepticum* strain A 5969, which had undergone at least 600 passages in broth. It was grown in tryptose broth (Difco) at pH 8.0, with 1 per cent PPLO serum fraction, phenol red 0.002 per cent, generally also with 5 g per L tris(hydroxymethyl)amino-methane, and occasionally with penicillin, 100 units per ml.

For studies of growth, inoculated tubes were incubated statically in a water bath, stirred so there was some unintentional agitation. At intervals the optical density was observed with a

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Additional use was made of this procedure in measuring the killing of the culture by γ-rays from Co⁶⁰, details of which will be reported elsewhere. The culture in plain or ground-glass stoppered pyrex tubes was exposed in a treatment cup containing ice for cooling. At intervals, a tube was removed, chilled, and subjected within 1½ hr to the counting procedure. Table 2 shows typical results, calculated only from the tables of Halvorson and Ziegler. The occurrence of growth of apparently low concentrations of viable cells of this culture lends support to the endpoint titration as a procedure for estimation of population, and although considerable preparation is required, it appears no more tedious than agar plate counts.

ANTIBODY RESPONSE OF MAN TO CANINE DISTEMPER VIRUS

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Received for publication December 7, 1959

Canine distemper virus antibody has been found in the sera of children and adults with a past history of measles. (Carlström, Acta Paediatrica, 45, 180, 1956). Recently, Adams et al. (Virology, 7, 352, 1959) reported that the administration of egg-adapted canine distemper virus (Lederle) to children conferred a degree of protection against natural infection with measles, but no information is available on antibody levels in man after canine distemper vaccination. The following study was undertaken to establish the clinical and immunological response of children to live, egg-adapted, canine distemper virus.

Twenty-six children between 2 and 12 years of age received a 1-ml dose subcutaneously of a 10 per cent chorioallantoic membrane suspension containing 10⁴.₄ EID₉₀ per ml of canine distemper virus. Another group of 5 children were given a similar preparation but with the virus inactivated by 1:4000 formaldehyde. No clinical reactions were observed. Sera were collected at intervals after inoculation and titrated for (a) distemper virus neutralizing antibody by neutralization of 200 to 300 EID₉₀ of the Onderstepoort strain of virus and (b) for measles complement-fixing antibody. The results expressed as reciprocals of the titers are shown in table 1.

The prevaccination sera of the children had canine distemper virus antibody titers ranging from 1:10 and 1:61 with a geometric mean of 20. Three to four weeks after vaccination there was an increase in titer in the majority of cases rang-