Antibiotic Assaying by Use of a Direct Plate Technique

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The two standard methods of antibiotic assay, the cylinder plate technique (3) and the serial tube dilution-turbidometric technique (as described by Schlitter (4), Fisher (2), and Rammelkamp (method as modified by Eli Lilly Laboratories for Clinical Research), necessitate time-consuming, meticulous detail for their success. The method described in this paper utilizes a simpler technique, with complete assay results known in 18 to 24 hr.

A twofold serial dilution is performed on each sample of serum to be assayed (1 ml of serum to 1 ml of sterile saline) in 12 tubes, starting with a titer of 1:2 up to a titer of 1:4,096. A twofold serial dilution is also performed on the control antibiotics. The concentration of the first control tube is 100 μg/ml, made from the 1,000 μg/ml stock solution of the antibiotic being assayed. This tube is further diluted until the twelfth control tube has an antibiotic concentration of 0.0475 μg/ml. The initial antibiotic concentration can be varied, depending upon the sensitivity of the microorganism used in the assay.

The stock solution of the test microorganism is prepared as for the tube dilution method of assay (4), with an 18-hr-old culture which has an optical density of 1 or has 10% light transmittance at 600 absorbancy units (Spectronic-20 colorimeter, Bausch & Lomb, Rochester, N.Y.). This solution will contain approximately 600 million bacteria per ml. A 1:1,000 dilution is made from this original 18-hr-old stock culture solution, which will be used to seed the blood plates.

Blood plates (Optilux Blood Agar Plates, BBL; Trypticase Soy Agar with defibrinated sheep blood) are numbered and divided into four sections with a wax pencil, each section representing one particular tube in the serial dilutions. The entire plate is seeded with the bacteria by the same method used for routine sensitivity testing (1, 5).

After the twofold serial dilutions are made and the blood plates are seeded, 0.04 ml from each tube is dropped in the center of the plate area indicated for the particular tube. These plates are then incubated for 18 to 24 hr at 37 C and are read at the end of this incubation period.

The inhibiting quantity of control antibiotic to standard microorganism is the last drop area that shows no growth at the drop site. According to Fig. 1, there is obvious inhibition of bacterial growth at drop areas 1, 2, 3, and 4. Figure 2 shows decreasing inhibition, with number 8 the last area inhibiting bacterial growth. It is obvious from Fig. 3 that there is no inhibition of growth in any of the drop areas. Therefore, the actual inhibiting quantity of antibiotic has a concentration of 0.75 μg/ml (which is represented by tube 8).

The sample plates are read the same way. If area 3 of the sample is the last area that inhibits growth and has a 1:8 dilution, then the antibiotic concentration of this tube is 6.0 μg/ml (8 × 0.75 μg/ml). Therefore, the actual antibiotic concentration of the sample is greater than 6.0 μg/ml but less than 12.0 μg/ml.
This method was run many times simultaneously with the turbidimetric method as described by Rammelkamp and Schlicter and gave similar results.

The direct plate assay method described above avoids the laborious preparations required in the cylinder plate technique (3). In addition, it offers the following two advantages over the turbidimetric method: (i) many unknown inhibitory substances in blood, urine, etc. inhibit the test organism in a liquid medium, but do not diffuse into an agar medium, and (ii) the assay of turbid material may obscure the exact end point (3).

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LITERATURE CITED