Rapid Method for Determining Serum Bactericidal Activity

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To screen large numbers of sera, a method was devised which utilizes the Steers-Foltz replicator which is usually used to determine minimal inhibitory concentration for antibiotics. Each of the wells (9 by 15 mm) of the replicator is filled with 0.06 ml of serum, 0.02 ml of a 10^4 suspension of organisms, and 0.02 ml of diluent (tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 8.4). The mixtures are incubated for 3 h, and samples are taken at 0, 1, 2, and 3 h by stamping duplicate nutrient agar plates (approximately 0.004 ml from each well). Plates are incubated overnight, and bactericidal activity is estimated by visual inspection of bacterial growth at each site for each sampling time. Results obtained with 28 serum-organism pairs paralleled standard pipetting-pour plate methods. The replicator method for determining bactericidal activity allows for the testing of a large number of samples and requires negligible amounts of serum.

Currently available methods for determining serum bactericidal activity involve either multiple pipettings and pour-plate dilutions or relatively sophisticated equipment. To screen large numbers of sera for bactericidal activity, a method was devised utilizing the Steers-Foltz Replicator (1), a device usually used to determine minimal inhibitory concentration for antibiotics. The purpose of this report is to describe this method as well as to present data from a comparative study of 28 serum-organism pairs using this replicator method and a standard pour-plate technique.

MATERIALS AND METHODS

The Steers-Foltz replicator consists of a sliding tray, a well plate, and an inoculating plate. The well plate has 36 wells (9 by 15 mm) arranged in 6 rows of 6 wells each. This plate fits on the left side of the sliding tray. The right side of the tray is arranged to hold a square plastic petri dish which has a grid of 36 squares stamped on the bottom corresponding to the wells in the well plate. The inoculating plate has 36 rods arranged in the same manner and is attached to a piston on the base. In operation, the suspensions to be plated are placed in the appropriate wells, the inoculating rods which pick up approximately 4 μl of each are dipped into the wells, the slide is moved to the left, and the petri dish is stamped.

To prepare the organisms for study, 1 ml of an overnight culture in brain heart infusion broth (Difco) was added to 9 ml of fresh broth and incubated with gentle shaking for 90 min in a 37 C water bath. This log-phase suspension was washed three times in 0.06 M NaCl and resuspended in a small volume of 0.05 M tris(hydroxymethyl)aminomethane - (Tris)hydrochloride buffer at pH 8.4. It was then standardized photometrically by using the Tris buffer as a diluent to give a final concentration of approximately 3 × 10^4 colony-forming units per ml. All organisms studied were Enterobacteriaceae. Complement was obtained from a 1:5 dilution in Tris buffer of a freshly thawed sample of commercially prepared fresh frozen guinea pig complement (Gibco) which contained no preservatives.

Initially, a template (Fig. 1) was prepared showing the position of the serum-organism pairs, each pair with its controls requiring four wells. The number and type of controls used in any particular investigation were dictated by the variables appropriate for that investigation. The study used in describing this method involved sera which had been stored at −20 C for 1 to 4 years. For this reason, a complement control (2nd row) was used to assure that any lack of bactericidal activity exhibited by a given serum was not the result of a decrease in complement titer due to the long storage. To differentiate between those organisms which were sensitive to the bactericidal activity of normal serum and those that were resistant to that activity, a normal serum control (3rd row) was used. These normal sera were obtained from healthy laboratory personnel 3 h prior to use.

A third control (4th row) was introduced to assure that the diluent had no antibacterial activity and also to evaluate the inoculum. The sequence of addition and amount used for each of the reagents are listed in Fig. 1.

The contents of the wells can be mixed by a vertical motion of the inoculating rods or, alternatively, a sterile 5-mm magnetic stirring bar can be placed in
represented essentially no bactericidal activity with the 3rd-h growth obviously greater than the original inoculum.

RESULTS

To evaluate the method, 28 serum-organism pairs were tested by using the replicator method and the pour plate technique for Taylor et al. (2). Bacterial suspension, serum, and diluent were identical for both methods. The tests were run on the same day, and the results with each method were obtained independently. To have a common ground for comparing the two methods, the ranking system published by Taylor et al. (2) was used. The comparison of these two methods was made with patient serum without added complement.

When the 28 serum-organism pairs were studied with the two methods, the results obtained differed by one or two ranks in only 3 serum-organism pairs, whereas in 25 of the 28 pairs there was no difference in rank. By using Spearman's correlation coefficient for ranked pairs, a positive correlation of 0.997 was obtained between the two methods ($P < 0.001$).

DISCUSSION

The data presented suggest that the Steers-Foltz replicator can be used to determine serum bactericidal activity. The method is rapid and simple and allows for the screening of large numbers of sera with multiple controls. The results obtained with the replicator method compare favorably with a standard technique which requires considerably more patient serum.

Further studies are necessary to determine if this method can be used in a more quantitative manner to measure bactericidal antibody titers, but at the present time it appears useful for rapid detection of the presence or absence of serum bactericidal activity.

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LITERATURE CITED
