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Micromethod (Spot-Plate) Determination of In Vitro Antibiotic Susceptibility

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ABSTRACT

BEARGIE, R. A. (University of Oklahoma Medical Center, Oklahoma City), E. C. BRACKEN, AND H. D. RILEY, JR. Micromethod (spot-plate) determination of in vitro antibiotic susceptibility. Appl. Microbiol. **13**:279–280. 1965.—The spot-plate method for determining in vitro antibiotic susceptibility is a modified version of the standard tube dilution technique, with the same principle of twofold dilution. Results of the spot-plate method are compared with those of the standard tube dilution technique. It is proposed that the spot-plate method is an equally accurate, but more rapid and economical, technique for determining in vitro antibiotic susceptibility.

It is generally agreed that the most accurate technique for determination of in vitro antibiotic susceptibility is the serial tube dilution method. Because of the ever-increasing emergence of antibiotic-resistant organisms, there is a pressing need for an equally accurate, but more rapid and economical, method of determining in vitro antibiotic susceptibility. The purpose of this communication is to report experience with a modified version (spot-plate method) of the tube dilution technique with the same principle of twofold dilution.

MATERIALS AND METHODS

A clear glass spot-plate (Pyrex brand) containing nine wells was used. Each well had a 22-mm diameter and a depth of 7 mm for an approximate volume capacity of 0.5 ml. The basic dilution volume was 0.1 ml, and the method proceeded in the same manner as that described in the standard tube dilution technique (Randall and Grove, 1955). The spot-plate method for in vitro antibiotic susceptibility testing was conducted as follows. Twofold serial dilutions of the antibiotic were prepared by placing 0.1 ml of Brain Heart Infusion broth in each of the nine wells. To the first well, 0.1 ml of the antibiotic standard solution was added and mixed; 0.1 ml was then transferred from the first well to the second well. This same procedure was continued until the ninth well was reached, at which time the last 0.1 ml was discarded. Inoculum broth (0.1 ml) was added to each of the wells in the series. The antibiotic standards used in these studies contained 200 μg/ml so that the first well contained 50 μg/ml, the second well 25 μg/ml, etc., with the ninth well containing 0.195 μg/ml. When the spot-plates were prepared and ready for incubation, each well contained 0.2 ml.

A sheet of plastic wrap (Saran, Dow Chemical Co., Midland, Mich.) was laid over the glass plate, and each well was effectively sealed by gently pressing the plastic around the rim. The plate was incubated at 37 C for 18 hr or overnight. As shown in Fig. 1, the clear background provided good contrast for visual determination of minimal inhibitory concentration. Contamination was determined by staining smears of the minimal inhibitory dilution as well as those immediately above and below this dilution. When contamination was doubtful, the tube or well under suspicion was subcultured for identification of organisms.

Initial experience with the spot-plate method involved antibiotic susceptibility studies which were requested on 34 organisms isolated from clinical material and processed through the Infectious Disease Laboratory, the Children's Memorial Hospital of the University of Oklahoma Medical Center. Depending upon the anticipated antibiotic susceptibility of the organism under study, each culture was tested with 8 to 10 of a total of 16 antibiotic standards routinely used in this laboratory for in vitro antibiotic susceptibility testing. In the initial study, 294 antibiotic-organism combinations were tested by both the standard tube technique and the spot-plate method. Comparison of the two methods was not possible when the minimal inhibitory concentration fell outside the limits of the dilutions used, i.e., greater than 50 μg/ml or less than 0.1 μg/ml. Of course, results of sets found contaminated in either the tube or the spot-plate could not be used for comparison.

After this preliminary study, the method was extended to a larger group of organisms. To determine the reproducibility of results, three test

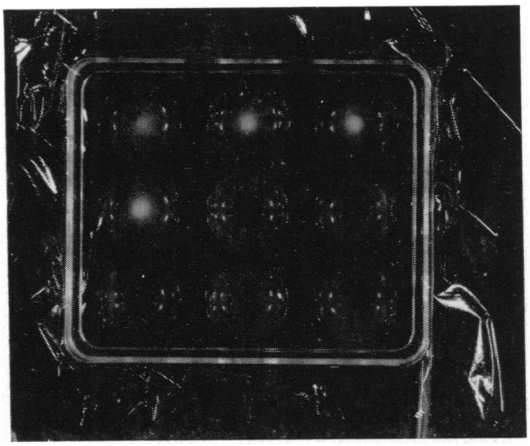

FIG. 1. *Spot-plate with plastic wrap cover used in determining in vitro antibiotic susceptibility, in this instance, with Salmonella species.*

TABLE 1. *Comparison of standard tube dilution technique with the spot-plate method of in vitro antibiotic susceptibility testing with 34 organisms isolated from clinical material (group A) and three selected organisms (group B)*

Serial dilutions	Group A	Group B
Number of sets studied*	294	364
Number of sets outside limits of dilutions used	163	21
Number of standard tube dilutions contaminated	19 (6.5%)	8 (2.4%)
Number of spot-plate dilutions contaminated	10 (3.4%)	5 (1.5%)
Number of sets available for comparison	105	332
Sets agree at same dilution	32 (30.5%)	108 (32.5%)
within 1 dilution	77 (73.3%)	250 (75.3%)
within 2 dilutions	97 (92.4%)	290 (87.3%)
within 3 dilutions	102 (97.1%)	320 (96.4%)
within 4 dilutions	105 (100%)	326 (98.2%)
within 5 dilutions		332 (100%)

* A set includes an antibiotic-organism combination tested by both the standard tube and the spot-plate techniques.

microorganisms *Escherichia coli* B4, *Sarcina lutea*, and *Staphylococcus aureus*) were selected for further study. Each organism was subjected to 30 consecutive runs with three antibiotics by both the tube and the spot-plate methods. Each antibiotic-organism combination had a minimal inhibitory concentration well within the limits of the dilutions used.

RESULTS

In the initial experience with the 34 clinical isolates, 105 sets of serial dilutions were available for comparison (Table 1, group A). Minimal inhibitory concentrations agreed within two dilutions in 92.4% of the sets available for comparison. Contamination occurred in 6.5% of the tube and in 3.4% of the spot-plate dilutions.

Further studies to determine the reproducibility of results with the three selected test organisms lent an additional 332 sets for comparison (Table 1, group B). The minimal inhibitory concentrations agreed within two dilutions in 87.3% of the sets available for comparison. Contamination occurred in 2.4% of the tube and in 1.5% of the spot-plate dilutions.

DISCUSSION

A technique similar to the spot-plate method was utilized in antibody titration studies as early as 1931 and has been used in serum antibiotic assays since 1947 (Goslings, *personal communication*). Because it has not received published consideration for in vitro antibiotic susceptibility testing, it seems appropriate to bring the method to the attention of those who might benefit from the economy of time and materials that it provides.

The spot-plate method has been employed in the Infectious Disease Laboratory for 1 year. The conservation of glassware and its maintenance offers distinct advantages. Laboratory personnel are particularily impressed with the ease and speed of setting up spot-plate determinations. It is our opinion that the spot-plate, as compared with the standard tube dilution technique, offers an equally accurate and more rapid and economical method of in vitro antibiotic susceptibility testing.

ACKNOWLEDGMENT

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