Effect of Plate Size and Location of Disc on Zone Diameter in the Disc Antimicrobial Susceptibility Test

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An established standard antimicrobial disc susceptibility test recommends the use of a 150-mm petri dish. Many workers substitute 100-mm plates and use various types of mechanical dispensers. A comparison of zone diameters was made by testing Staphylococcus aureus and Escherichia coli against penicillin and chloramphenicol with the use of these different sized plates and dispensers. Zone diameters were consistently smaller at the edges of plates than in the center (0.4 to 1.2 mm). Significant small differences of up to 1.2 mm or no differences were observed when the mean diameters from these various plates were compared. All of the zone diameter measurements fell within accepted values for analytical variability in this procedure. There is no evidence that any individual combination of plates, dispensers, or disc locations provides greater precision in zone diameter measurement. More difficulty was encountered in measuring zones at the periphery of small plates. This suggests that busy clinical laboratory workers might not produce work of comparable quality with the smaller plates.

The antimicrobial susceptibility test described by Bauer et al. (1) makes use of a plastic petri dish 150 mm in diameter. Antibiotic-impregnated discs are dispensed onto the surface of Mueller-Hinton agar 4 to 6 mm in depth manually or by use of commercially available dispensers. It is possible to position up to a dozen discs on this plate with 35 mm between disc centers and 16 mm from disc edges to the plate edge. It has not been established whether the results would be significantly different if tests were performed on a plate 100 mm in diameter. Additive and interfering effects have been described when zones are allowed to overlap. Workers have questioned whether a decrease in distance between the disc and the plate edge causes a change in zone diameter and, in addition, whether it might reduce the precision of measurements. Unpublished reports have suggested that zone diameters produced around discs on the 100-mm plate were smaller than those obtained by testing the same cultures simultaneously on 150-mm plates. We established an investigation to determine whether there were differences in the precision and accuracy of zone diameter measurements with different sized plates and dispensers.

MATERIALS AND METHODS

Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) cultures were maintained on tryptic digest-casein-soy-agar. Several colonies from freshly grown cultures were cultivated in tryptic digest-casein-soy broth. The density was equilibrated visually with a 1% barium sulfate standard according to the method of Bauer et al. (1). Mueller-Hinton Agar (Difco) was prepared with rigid control of the temperature and duration of sterilization. The pH of the poured cooled medium was consistently 7.2. The melted agar was dispensed with an automatic device which allowed the depth of agar to be carefully regulated between 4 and 6 mm with a uniform depth throughout each plate. Plastic petri dishes 100 mm in diameter (Kimble Glass Div. Owens-Illinois Glass Co., no. 52700), 150-mm plastic petri dishes (Falcon Plastic, no. 1058), and 100-mm square plastic petri dishes (Kimble, no. 52900) were used.

Penicillin and chloramphenicol were selected as test antibiotics because of their rapid rate of diffusion. It was anticipated that these agents would be good indicators of factors that might interfere with diffusion and influence zone diameter. Penicillin discs containing 10 units and 30-μg chloramphenicol discs were obtained in one lot from a commercial source (BBB) and were stored at −20 C with desiccant. For daily use, these were stored with desiccant at 4 C. The discs were dispensed with two commercially available types of mechanical dispensers (dispenser 1 = BioQuest, no. 60456; dispenser 2 = BioQuest, no. 60458). Dispenser 2 was modified for use on the 150-mm plate through use of a plastic adaptor block which is provided by the manufacturer (BioQuest, no. 60473). We devised a similar plastic block for Dispenser 2 to provide a 3 × 3 distribution of discs on the 100-mm square
plate. The dispensers were adjusted as nearly as possible to drop the discs with the least amount of displacement. No manual repositioning of the discs was performed. Broth cultures were inoculated onto the plates with a cotton swab as described by Bauer et al. (1). The plates were incubated for 18 hr at 36 C. Plates were examined on an illuminated colony counter with 1.5 times magnification (Brunswick Scientific Co., no. C-110). The zone diameters were measured by use of a vernier caliper. The distance from the disc to the plate edge was also recorded.

**RESULTS**

Five hundred zone diameter measurements were collected on eight separate days (Table 1). An average of 25 determinations were made for any single combination of drug, culture, disc location, plate, and dispenser. The distance from the edge of the disc to the plate edge was also recorded. The greatest distance (15.6 mm) was measured on the 150-mm plate. The square plate dispenser dropped nine discs an average of 14 mm from the plate edge, whereas dispenser 1 placed six to eight discs 12.5 mm from the edge of the 100-mm round plate. Dispenser 2 distributed six to eight discs much closer to the edge (4.0 mm), and in some instances discs touched the edge of the plate.

Mean zone diameters from different plates. The mean zone diameters obtained on the various 100-mm plate-dispenser combinations were compared with those from the 150-mm plate. *Staphylococcus* penicillin and *Staphylococcus* chloramphenicol produced larger zones on the 100-mm round plate. The *E. coli* chloramphenicol combination produced a larger zone diameter on the 100-mm plate with dispenser 1, but dispenser 2 yielded a slightly smaller mean zone diameter than on the larger plate. *Staphylococcus* chloramphenicol was the only combination in which the difference was not significant by the *t* test (*P* < 0.050). There were no differences in mean zone diameters between the 100-mm square plate and the 150-mm plate for any culture-drug combination.

**Comparison of edge and center measurements.** When the mean diameter of all zones produced around the discs at the edges of the various plates was compared with the mean values obtained near the center, a difference of 0.4 to 1.2 mm was found. In each instance, regardless of culture-drug combination, the zones were smaller at the edge. Differences which exceeded 1 mm were significant by the *t* test (*P* < 0.050). Figures 1, 2, and 3 show a change in zone diameter when the diameter is correlated with the distance between the disc and the plate edge on plates of the

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**Fig. 1. Distribution of zone diameters for Staphylococcus aureus tested against penicillin by use of various plates and dispensers plotted against distance from disc edge to plate edge. Note that all values fall within ±10% of the overall mean, a level of precision generally accepted for this test in clinical laboratories.**
**Fig. 2.** Distribution of zone diameters for *Staphylococcus aureus* tested against chloramphenicol by use of various plates and dispensers plotted against distance from disc edge to plate edge.

**Fig. 3.** Distribution of zone diameters for *Escherichia coli* tested against chloramphenicol by use of various plates and dispensers plotted against distance from disc edge to plate edge.
### Table 1. Mean zone diameters in millimeters with range (±2 sd) and coefficient of variation (2 CV) obtained with various plates, dispensers, and disc locations

| Determination | 100-mm round plate | 100-mm square plate | 150-mm round plate | Univ. Wash. |
|---------------|---------------------|---------------------|--------------------|------------|
|               | Dispenser 1 | Dispenser 2 | Overall | Dispenser 2 + adaptor | Dispenser 2 + adaptor |                |
| S. aureus/penicillin | 32.9 | 32.3 | 32.7 | 32.8 | 31.4 | 32.4 | 31.7 | 31.7 | 32.4 | 31.9 | 31.7 |
| Range | 30.7-35.1 | 30.5-34.1 | 31.5-33.9 | 30.6-34.2 | 29.4-33.4 | 30.4-34.4 | 29.5-33.9 | 29.9-33.5 | 29.6-35.2 | 29.7-34.1 | 28.5-34.9 |
| Variation | 7 | 6 | 4 | 6 | 6 | 6 | 7 | 6 | 9 | 7 | 10 |
| S. aureus/chloramphenicol | 22.7 | 22.7 | 23.3 | 22.8 | 22.5 | — | 22.5 | 22 | 23 | 23.3 | 23 |
| Range | 20.7-24.7 | 20.7-24.7 | 21.3-25.3 | 20.8-24.8 | 20.1-24.9 | — | 20.3-24.7 | 20.0-24.0 | 20.6-25.4 | 19.9-24.7 | 19.8-26.2 |
| Variation | 9 | 9 | 9 | 9 | 10 | — | 10 | 9 | 10 | 10 | 13 |
| E. coli/chloramphenicol | 22.2 | 20.9 | 22.1 | 21.2 | 21.8 | 22.6 | 21.9 | 21.5 | 22.3 | 21.7 | 22.5 |
| Range | 21.0-23.4 | 18.7-23.1 | 20.3-23.9 | 18.8-23.6 | 20.0-23.6 | 21.0-24.2 | 20.1-23.7 | 19.5-23.5 | 20.9-23.7 | 19.9-23.5 | 19.5-25.5 |
| Variation | 5 | 11 | 8 | 11 | 8 | 7 | 8 | 9 | 6 | 8 | 13 |
| Disc to plate | Edge distance | 12.5 | 4.0 | 14.0 | 8.8-19.2 | 15.6 | 10.6-20.6 |
| Range | 7.9-17.1 | 0-10.4 | |

* University of Washington Laboratory control data are shown for comparison.
same type. No comparison of these locations was made on the 100-mm round plate with dispenser 1, because this dispenser did not allow positioning of discs near the center of the plate. A comparison of mean zone diameters from the edges of the three types of plates showed that these were larger on the 100-mm round plate except for E. coli/chloramphenicol with dispenser 2; the differences ranged from 0.3 to 0.7 mm, but these were not significant by the t test ($P < 0.050$). Little difference was observed between mean center values.

**Comparison with University of Washington data.** Mean zone diameters obtained in our study with the 150-mm plate were compared with the values previously observed with the same cultures in the University of Washington Laboratory. No difference was found when Staphylococcus was tested against penicillin. Mean zone diameters were 0.7 mm smaller in our laboratory when Staphylococcus was tested against chloramphenicol. Values obtained by us from the center of the plate were identical to the mean values from the University of Washington, but our edge values were 1 mm smaller. No observations were made for the center of the 100 mm square plate for Staphylococcus/chloramphenicol. Mean zone diameters for E. coli tested against chloramphenicol were 0.8 mm smaller in our laboratory. None of these differences was significant by the t test ($P < 0.050$).

**Precision.** The reproducibility of zone diameter measurements with different dispensers and plates and with different disc locations was expressed as two coefficients of variation as shown in Table 1. These ranged from 4 to 13%. The range of variability recorded at the University of Washington was 10 to 13% and was determined by repeated testing of control cultures.

**DISCUSSION**

In this study, we attempted to determine whether there is an important difference in zone diameter between the 100-mm round or square plate and the 150-mm round plate. Zones were slightly larger on the small plates, but the differences were 0.5 mm or less in all but one instance. There was no difference in results between the 100-mm square plate and the 150-mm plate.

Central area zones were consistently larger than those from the edge of the plate.

Unpublished observations and experience in numerous clinical laboratories with the procedure of Bauer et al. (1) have demonstrated that zone diameters of S. aureus cultures used for control purposes are reproducible within $\pm 10\%$ of a mean value; E. coli zone diameters are reproducible within $\pm 15\%$. Accepting these variations in zone diameter as analytical tolerance limits, all of the differences observed in this report would appear to be unimportant.

It is apparent that there is no difference in the precision of zone diameter measurement with any of the different plates, dispensers, or disc locations. In this study, only two cultures and two antibiotics were compared in these systems. Further investigation of differences in zone diameter with other combinations of antibiotics and cultures will be required. If the observations made in this report are borne out by such investigation, it should be apparent that zone diameters may be read with any of these plate-dispenser combinations by reference to values established by Bauer et al. (1) without producing erroneous reports.

It should be mentioned that zone diameters can be read more easily and quickly when discs are placed further from the plate edge, as is the case on the 150-mm plate. Most difficult and slow to read are zones produced on the 100-mm plate with dispenser 2. Although we achieved the same precision with all dispensers and plates, a busy clinical laboratory might not be able to achieve the same precision with the smaller plates.

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**LITERATURE CITED**

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