from the interclavicular and right abdominal air sacs, through No. 23 Yale needles into 2-cc. glass syringes, oiled and freed of air. Samples of the spirometer mixture are drawn similarly through the rubber tubing at R, after first rinsing some of the mixture through stopcocks D and E while valve U is closed and water flows from the bottle, L, into W. All samples are analyzed immediately for percentage of nitrogen, using Bayer's technique (*1*) with Krogh's pipette.

The respiratory volume (lungs, air sacs, and trachea) of the bird is calculated using equation (1). The figure 79.0 for the normal percentage of nitrogen in the air sacs has been found suitable for this calculation. The final VO_2 of the apparatus is measured accordingly if the points on the record, K, are slightly above or below their starting place.

Corrections for difference in temperature and the vapor pressure of water are added to the calculated respiratory volume. The dead space of the hood, X, is subtracted from the corrected volume. No correction has been made for the error, estimated at less than 2 per cent, due to the diffusion of nitrogen from the blood and tissues of the bird during a test.

Although normally the ventilation of the various air sacs differs greatly, samples taken from the air sacs during a test have been found to check, as a rule, within .5 per cent with those from the spirometer. The apparatus has been used with gulls, and with mallard, black, redhead, and wood ducks. Using mallards and wood ducks, we have tested the duplicability of observations with the apparatus and found the coefficient of variability of test runs made on the same duck, the same day, to be less than 6 per cent and that of runs made on the same duck on different days to be less than 8 per cent.

References

1. Bayer, Fritz. *Gasanalyse; neuere Methoden der Arbeitspraxis unter Berücksichtigung der physiologischen Wirkungen der Gase.* Stuttgart: F. Enke, 1938. Pp. 108–112.
2. Herrald, F. J. C., and McMichael, J. *Proc. roy. Soc. Lond.*, 1939, **126 B**, 491–501.
3. Peters, John P., and Van Slyke, Donald D. *Quantitative clinical chemistry.* (Vol. II.) Baltimore: Williams and Wilkins, 1932. Pp. 217–228.

A New Sterile Technic for Preparing Agar Cup-plates

John E. Christian and Milton L. Neuroth
Purdue University School of Pharmacy

The agar cup-plate method has found considerable application as a test for bacterial inhibitory properties of many substances. Examples of substances which have been tested by this method are liquid products, ointments, dusting powders, catgut, creams, and suppositories (*1*). The test, however, has been greatly limited by the difficulty encountered in preparing, in a sterile manner, suitable uniform cups in the agar. Two standard methods have been used: (1) Before the agar cools, a depression or cup is made in the medium by standing a sterile, flat-bottomed, glass tube in the liquified agar. When the agar is hard, the glass tube is removed by slightly twisting and pulling, and at the same time inserting a sterile wire down the side of the tube for the introduction of air in an attempt to prevent the cracking of the agar. (2) The agar is allowed to harden and then a disk is cut out of the agar by means of a suitably sized sterile cork borer. Both these methods are very cumbersome, and a uniform cup without cracked edges is difficult to obtain. Contamination of the plate is also very common in these procedures, since the lid of the plate must be removed. Still another operation is necessary in these methods—that of placing one or two drops of melted agar in the cup to seal cracks of crevices formed.

A simple new technic is proposed for the preparation of uniform agar cups in a sterile manner. Sterile, flat-bottomed, pyrex glass rods, the diameter of which determines the size of the agar cup, are placed in the liquid agar in the Petri dish, as is shown in Fig. 1. The pyrex glass rods are 10 mm. in length. The agar is allowed to harden, after which each glass rod is heated by means of a small heating element (see Fig. 1). The heating element is slipped over the end of the rod and is held in place by a small glass plunger operated with the forefinger. The agar melts evenly around the rod, which is then easily removed, leaving a uniform agar cup. The small amount of agar which melts adjacent to the rod flows down to the bottom of the cup and solidifies, forming an agar seal at that point. The agar cup-plate is then ready for use.

The heating element is simple in construction and operation. It consists of a 22-gauge chromel resistance wire, wound in a four-turn spiral, the ends of which are sealed with pyrex glass onto the outside of a glass tube with a 6-mm. O.D. and 14 cm. long. The ends of the resistance wire extending beyond the glass seal are attached to an ordinary extension cord held in place on the glass tubing by a tight wrapping of asbestos cord. The temperature of the wire spiral is controlled by plugging the extension cord into any variable resistance transformer; 8 to 10 volts produce a suitable temperature. The plunger consists of a 4-mm. pyrex glass rod which will slip inside the glass tubing. By manipulation with the forefinger the plunger is pressed against the side near the top of the glass rod standing in the agar. At the proper moment the glass rod may be lifted from the agar to form a uniform cup.

Four such cups are made in the agar of each Petri dish. Into one cup is placed a control preparation which contains no active material. Various dilutions of the preparation containing active material are placed in each of the other cups. In the case of liquid preparations 0.10 cc. is run into the cup by means of a sterile syringe or pipette; if an ointment is involved, 0.50 gram is placed in each cup. A pyrex glass tube, graduated and drawn down on one end to a 2-mm. opening, with a glass rod plunger, is used to introduce the solid or semisolid material into the cups. The graduated tube is filled by inserting the small end into the preparation, then carefully applying vacuum on the other end.

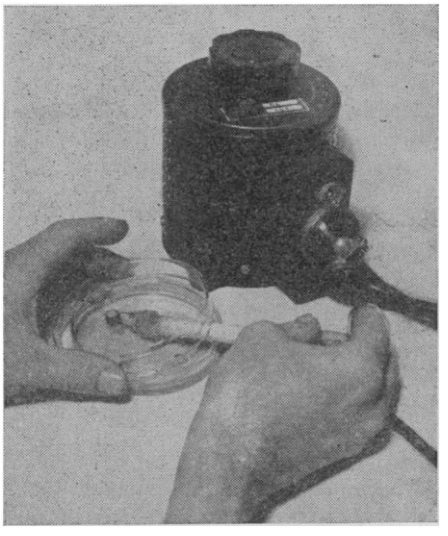

FIG. 1

Two methods are available for introduction of the test organism: (1) The base agar may be seeded before pouring into the Petri dish, the glass rods being set and removed as previously described. (2) The method adopted for routine testing of solid materials by the authors has been that of setting and removing the glass rods from sterile base agar. After the preparation has been placed in the cup, a layer of inoculated agar is poured over the base. Twenty cubic centimeters are used as the base layer, and 5 cc. of inoculated agar are poured on top. The inoculated agar consists of 2 cc. of a 16- to 18-hour beef broth culture of *S. aureus* placed in 100 cc. of melted agar.

The layer of inoculated agar, when solidified, seals the preparation in the cup. The Petri dish may then be placed in the incubation oven in an inverted position, thus eliminating any condensation of moisture. The plates are incubated at 37° C. for 16 to 18 hours. Fig. 2 shows the results of a test plate. The zones of inhibition are very uniform for any specific concentration and vary in size according to the degree of dilution. Table 1 indicates the uniform results obtained on a set of plates.

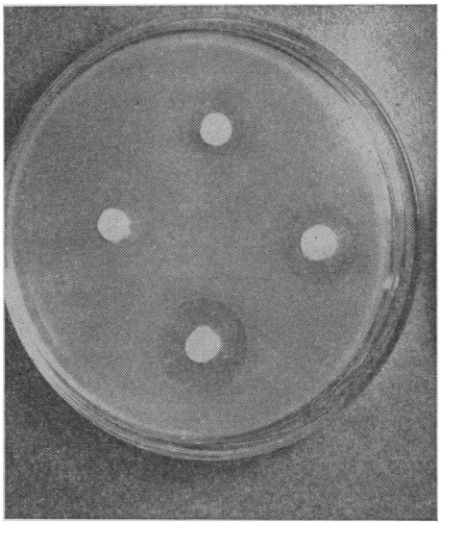

FIG. 2

TABLE 1

ZONES OF INHIBITION IN MILLIMETERS

Plate number	Ointment base	Base containing 1 unit penicillin/gram	Base containing ½ unit penicillin/gram	Base containing ¼ unit penicillin/gram
		mm.	*mm.*	*mm.*
1	no zone	20.50	17.50	13.50
2	"	20.50	17.50	12.50
3	"	20.75	17.50	14.00
4	"	20.75	17.75	13.50

The method described has been used with good success for the routine testing of ointments and creams containing penicillin and has been found to have many advantages over other technics used for this purpose. The heating element for this new technic is inexpensive and easy to manipulate.

Reference

1. ZINSSER, H., and BAYNE-JONES, S. *Textbook of bacteriology.* (8th ed.) New York: D. Appleton-Century, 1939. Pp. 956, 957.