Lid Method for Evaluating Zone of Inhibition of Treated Fabrics

I. S. PRADIP, L. SHU, AND P. R. CURTIS

Department of Biology, The American University, Washington, D.C. 20016

Received for publication 2 October 1969

Stainless-steel wire cloth lids were found to be advantageous in the evaluation of zones of inhibition with germicide-treated cotton fabrics.

There has been increasing use of germicides in floor mats, rugs, tool covers, hospital uniforms, surgical gowns, bed sheets, toothbrushes, paints, etc., to reduce bacterial contamination. Compounds incorporated with such merchandise may have either bacteriostatic or bactericidal effect and when applied to fabrics, this self-sanitizing activity may be retained for variable time periods. It is difficult to measure quantitatively bacteriostatic activity of treated loose natural or synthetic fabrics because an irregular zone is seen. In our laboratory a method has been developed which eliminates this irregular zone and can be used qualitatively as well as quantitatively. This is a modification of the filter paper disc method as described by Vincent and Vincent (1).

In this method lids were made of stainless-steel wire, plain weave cloth [mesh 150 by 150; thickness 0.0026 inch (0.0066 cm)] obtained from Cambridge Wire Cloth Co., Cambridge, Md. Circles, 30 mm in diameter, were die-cut and then formed into shallow lids or cups, 20 mm in diameter and 5 mm in depth. The lids were formed in a steel punch press fabricated by the machine shop of the Physics Department of The American University (Fig. 1). The disassembled apparatus is shown in Fig. 2.

The test organism used was Staphylococcus aureus (ATCC 6538). The culture was grown in Synthetic Broth AOAC (Difco) containing 1% glucose on a rotary shaker (New Brunswick Scientific Co., New Brunswick, N.J.) at 200 strokes per min for 18 to 24 hr at 37 C. After incubation, the cells were centrifuged, washed, resuspended in saline, and standardized to 0.4 optical density units by means of the Spectronic 20 spectrometer (Bausch & Lomb, Inc., Rochester, N.Y.) set at 650 nm. This suspension contained 3 × 10⁸ cells per ml by direct microscope count. A 0.1-ml amount of this standardized cell suspension was used as inoculum for each 25 ml of melted nutrient agar at 43 C. This amount of agar gives a height of 5 mm in disposable

---

**Fig. 1.** Steel punch press used to form wire cloth lids, lateral view.

**Fig. 2.** Components of steel punch used to form wire cloth lids. The disassembled press is shown in parts A–D. Part E shows the 30-mm diameter wire cloth circle before formation of the 20-mm lid (F).
satisfactory for loose fibers. Quantitative estimations can be computed as zone of inhibition versus concentration of the germicide used. A standard curve requires a constant weight of

plastic dishes (100 by 15 mm); if a larger petri dish is used, the amount of nutrient agar is adjusted accordingly to give the same height.

Floor mats or cotton fibers were obtained from Kex National Association, College Park, Md. In the case of the mats, circles were die-cut (20 mm diameter), weighed, fixed into the stainless-steel wire lids, and autoclaved at 121 C and 15 lb of pressure for 15 min. When loose fibers were used, the fibers were cut into smaller pieces with scissors, weighed, packed in the lid by hand, and autoclaved. A measured amount of phenyl mercuric acetate (PMA) dissolved in 1 ml of sterile distilled water was placed evenly on the fibers or mat circles.

Seeded agar was poured in the plates, and the lids containing samples were placed while the agar was still in liquid form; in this way lids were completely embedded in agar. After the agar was solidified, plates were incubated in inverted position at 37 C for 18 to 24 hr, and the zones of inhibition were measured with the aid of a magnifying glass.

It is apparent that stainless-steel wire cloth lids do not hinder the diffusion rates. Comparison of samples with and without lids at different concentrations of PMA showed no significant difference in size of zone of inhibition. This was confirmed statistically by the use of the t test. The controls shown in Fig. 3 indicate that stainless steel alone had no oligodynamic action on the test organism used in this investigation.

A standard curve for various amounts of PMA is presented in Fig. 4 and Fig. 5.

It appears from the results that evaluation of zone of inhibition by the lid method is quite

FIG. 3. Controls. A, stainless steel wire cloth; B, untreated fibers with lid; C, untreated fibers.

FIG. 4. Staphylococcus aureus lid method assay for phenyl mercuric acetate representing standard curve with fibers (220 mg) saturated with solutions of 20, 40, 80, and 100 μg. The arrow indicates the lowest concentration with increasing amounts counter-clockwise. Circles of black paper, the same diameter as the zone of inhibition, were used on the bottom of the plate for photographic purposes.

FIG. 5. Standard curve showing graphically the results of the experiment in Fig. 4.
fibers to be tested with varying amounts of germicide. Unknown samples must have the same weight as the standard-curve samples. Results can then be expressed as activity in terms of zone of inhibition per unit weight of fabrics. However, this technique will be suitable only for germicides which are water-soluble and can diffuse readily in agar.

Stainless-steel lids can be made of any size, and finer mesh or thinner wire could be used. Such lids are easy to clean and sterilize, and mass-scale manufacturing of such lids would seem to be feasible. Although phenyl mercuric acetate appeared to have no effect on the stainless steel, it is possible that other disinfectants could react with the steel wire to cause an oligodynamic action. In such cases, similar lids could possibly be prepared from some type of synthetic fiber mesh which may not have an oligodynamic action in the test system.

We are grateful to John R. Plunkett of The American University Physics Department for assistance in making the punch press.

This investigation was supported by the Kex National Association, College Park, Md.

LITERATURE CITED
1. Vincent, J. G., and H. W. Vincent. 1944. Filter paper disc modification of the Oxford cup penicillin determination. Proc. Soc. Exp. Biol. Med. 55:162-164.