# IMPROVEMENTS IN THE CYLINDER-PLATE METHOD FOR PENICILLIN ASSAY[1]

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## INTRODUCTION

The most widely used technique for penicillin assay is the so-called cylinder-plate method of Abraham *et al.* (1941), with various modifications such as those described by Foster and Woodruff (1944) and by Schmidt and Moyer (1944). In using this method for large numbers of assays we have found it advantageous from the standpoint of time involved and reproducibility of the method to use a $4\frac{1}{2}$ x $11\frac{1}{2}$ inch, rectangular, culture plate on which 40 cylinders can be placed.

As compared with conventional petri plates, these culture plates increase efficiency in two ways, namely, by decreasing the proportion of cylinders devoted to standards and by reducing the time required to pour, flood, and wash the plates needed for a given number of assays. In addition to increased efficiency, increased reproducibility is obtained by using culture plates with flat bottoms and pouring them on a carefully leveled surface. This insures a degree of uniformity of depth of culture medium not attainable in petri dishes because of irregularities in their bottoms. Such irregularities are of sufficient magnitude to constitute a real source of error.

## EQUIPMENT

Culture dishes are made of stainless steel rims placed on 6 x 13 inch sheets of single strength window glass. A second sheet of glass of like dimensions is used as a lid. The rims are made of 24-gauge, stainless steel strips bent to form $\frac{1}{4}$ x $\frac{3}{4}$ inch angles. From these angle strips rectangular rims $\frac{3}{4}$ inches high, $4\frac{1}{2}$ inches wide, and $11\frac{1}{2}$ inches long are made with the $\frac{1}{4}$-inch flange extending outward from the bottom edge. Such rims are illustrated in figures 1 and 2. They are attached at their ends to the bottom plate with 1-inch, gummed paper tape strips. Leakage of the medium under the rim is prevented by running 2 medicine droppers of liquid agar medium under the rim and allowing this to set as a seal. Plates are poured on leveled plate glass sheets.

To facilitate placing cylinders on inoculated plates a leucite guide, as illustrated in figure 2, is used. This is constructed of two sheets of $\frac{3}{8}$-inch leucite or similar plastic bolted together in parallel fashion in such a way as to allow a stainless steel slide to move freely between them and be removed from one side. Through the two sheets of leucite four rows of ten holes are drilled one inch apart each way to act as guides for the cylinders. These holes must be properly aligned

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and of such size as to guide the cylinders accurately while allowing them to fall freely as the slide is removed. The holes in the lower plate are slightly enlarged on the top side with a taper reamer. Those on the top plate are beveled with a countersink to facilitate placing cylinders in them.

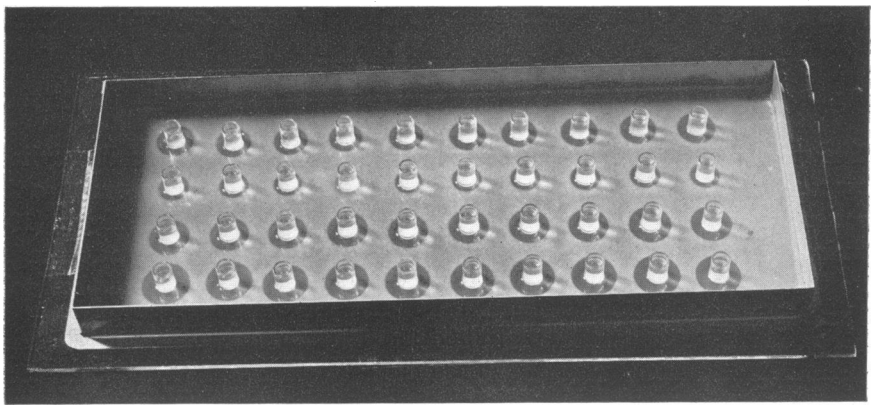

FIG. 1. VIEW OF CULTURE PLATE SHOWING ARRANGEMENT OF CYLINDERS

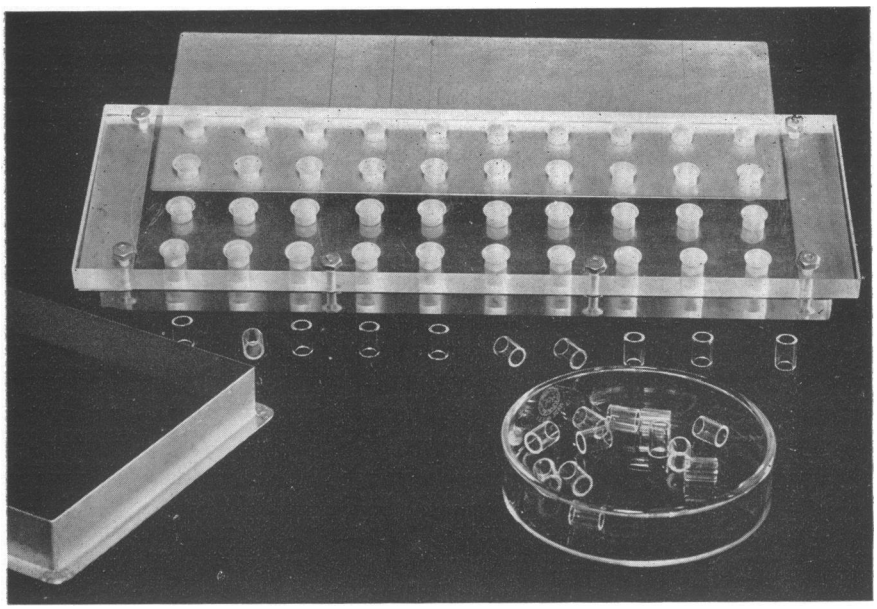

FIG. 2. VIEW SHOWING LEUCITE GUIDE, CYLINDERS, AND METAL RIM

Guides are used by loading the top plate with cylinders, placing the guide over the culture plate, and allowing the cylinders to drop into place by removing the slide. If one end of the guide is held in place on the plate while the other is lifted, cylinders are not moved on removal of the guide. Guides are cleaned daily with 70 per cent alcohol and stored in a dust-free cabinet.

Cylinders are made of standard pyrex tubing selected for an outside diameter of 7.9 ± 0.1 mm and cut into sections one cm long. The ends are ground flat on a carborundum wheel and then fire-polished just enough to produce a glaze but not enough to round the cylinder edges.

## ASSAY PROCEDURE

With respect to test organisms, media, buffer, dilutions, time, and temperature of incubation, etc., we follow the recommendations of Schmidt and Moyer as given in their 1944 paper and as subsequently modified by them. We recommend that investigators making extensive use of penicillin assay methods communicate with the United States Department of Agriculture, Northern Regional Research Laboratory, Peoria, Illinois, Dr. R. O. Coghill, for the current recommendations of the Peoria group.

In a further effort to increase reproducibility, we attempt to keep time intervals constant between successive steps in the assay procedure. Plates are poured (83 ml per plate), allowed to set, and stored in an incubator at 44 C for 3 to 4 hours. Using a rapidly draining pipette, the plates are flooded with 17 ml of inoculated medium. The agar is then allowed to solidify and the cylinders dropped into place. Following an interval of 15 minutes, test solutions are pipetted into the cylinders, and the plates placed in a 37 C incubator over night (ca. 16 hours).

Inhibition zones are measured by means of a light box containing a daylight fluorescent tube under two glass plates. Between these plates is placed a celluloid sheet on which circles differing in diameter by one millimeter increments are drawn. In routine assays diameters are read to the nearest half millimeter.

On the completion of readings, the plates are flooded with lysol and subsequently dismantled for cleaning. After this they are reassembled, wrapped in paper, and sterilized by autoclaving. They are dried by exhausting the autoclave and then allowing it to develop a vacuum for 15 or 20 minutes.

## ERROR VARIANCE

To obtain an indication of the magnitude of unavoidable errors of the method an analysis of variance was made on a series of replicated runs made at levels near 1.0 unit penicillin per ml with inhibition zones read to the nearest 0.25 mm. In eight different determinations, each based on from six to nine individual tests, 75 measurements were made. The "within-series" variance was 0.1 mm in zone diameter. This corresponds to a standard error of about 0.3 mm. At a level of one unit per ml this represents approximately 0.07 unit penicillin per mm, or 7 per cent. Expressed in another way, at a level of 1 unit, about two-thirds of the individual determinations would fall within 0.07 unit of the true value. Duplicates and higher replicates would, of course, reduce the standard error of a determination inversely as the square of the number of cylinders used per determination.

The above measurement of variance does not include errors of dilution or those due to variations in standards. Neither does it include interplate varia-

tion. In our experience the plate-to-plate variation is less than that encountered with the use of petri plates.

SUMMARY

A $4\frac{1}{2}$ x $11\frac{1}{2}$ inch rectangular culture plate for use in the cylinder-plate method is described. A guide is used for dropping cylinders upon the inoculated culture plate. The use of this equipment saves time in large-scale assay work and permits a higher degree of standardization than does the conventional petri plate technique.

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