Preparation of Agar Wells for Antibiotic Assay

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A variety of devices such as stainless-steel or ceramic cylinders, single or multiple in design, and paper discs of several sizes are commonly used for antibiotic assays performed by the agar diffusion method. Holes cut in agar (agar wells) are wells with melted agar is often necessary to obtain uniform inhibition zones (2).

This note describes a method for the preparation of agar-well plates which obviates the disadvantages. In our laboratory, agar-well assays occasionally useful for the assay of such materials as serum, milk, eggs, or viscous materials. The routine use of agar wells is hampered by the tedious of cutting and removing the agar plugs without disrupting the agar layer (1). Sealing the reduce contamination problems with milk and distortion of inhibition zones with eggs. Greater sensitivity is achieved with some antibiotics, erythromycin and neomycin, for example.

Agar wells are cut from seeded agar plates by a
cylindrical metal tube attached through a trap to a vacuum source. A section of a no. 4 cork borer is a convenient cutter. A most important addition is a 1.5-mm diameter hole in the side of the cutter, about 10 mm from the cutting edge, which acts as a vacuum bypass and prevents tearing the agar from the bottom of the plate. In our laboratory, the bench vacuum is about 550 mm of mercury and the cutter assembly with bypass open operates well in the range of 375 mm of mercury. Figure 1 illustrates the cutter in use. The agar plate is placed in the template on which are marked six evenly spaced locations for the agar wells. The operator cuts the agar with rapid strokes, holding the cutter vertical to assure even contact with the base of the plate. Cutting is greatly simplified if the plates are refrigerated at least 30 min prior to cutting. The firmness of cold agar is very helpful. An agar layer 5 to 6 mm thick makes convenient-size wells.

Practice in the use of the equipment is necessary to insure holding the cutter in the proper position and to determine the amount of vacuum required to readily remove the cut agar plugs. An experienced technician can easily cut 100 usable plates (600 wells) in 30 min.

**Literature Cited**
