IMPROVED MATRIX FOR MICROIMMUNODIFFUSION

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Both micro- and macrotechniques for double-diffusion tests in agar are currently in use, and many modifications of the micromethod have been described, including one in which a Lucite template supported by tape is used on top of a microscope slide, 25 by 75 mm (Crowle, *Immunodiffusion*, Academic Press, Inc., New York, 1961). Shortcomings of this technique include jarring of the template while loading it with molten agar solutions and the possibility of substances from the tape diffusing into the adjoining agar and precipitating antiserum.

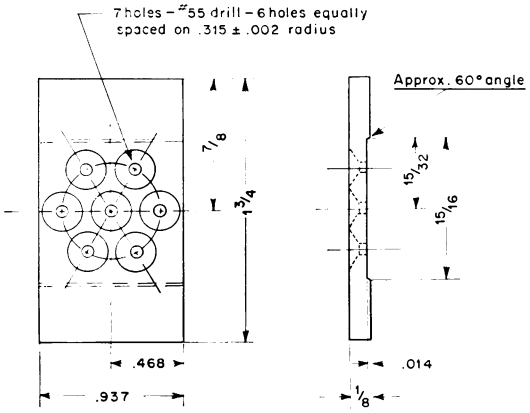

FIG. 1. *Plans for a matrix for microimmunodiffusion. Unit is constructed of Lucite with a polished finish. Dimensions are in inches.*

To avoid these problems, a template was designed (Fig. 1) with a built-in loading trough. The 60° angle at the sides of the trough was essential to prevent tearing of the agar when the matrix was removed. The template was placed on a clean, agar-coated microscope slide, 25 by 75 mm (Fig. 2), and molten agar was allowed to flow under the central trough until the entire area under the wells was filled. After allowing the agar to set for 15 min at room temperature, the unit was ready for use.

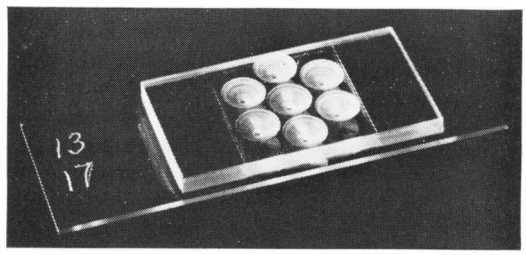

FIG. 2. *Complete unit with Lucite matrix placed on a glass slide and loaded with agar.*

In this laboratory, diffusion was allowed to proceed for 3 to 4 days in a humidifying chamber at 4 to 6 C. At the end of this time, the Lucite matrix was removed by simply lifting up on it. The agar layer adhered to the slide. The slide then could be photographed directly or else washed free of nonspecific protein, stained, dried, and protected with a cover slip for future reference.

USE OF URANYL ION IN MEMBRANE TRANSPORT STUDIES

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Membrane transport mechanisms can be studied by a variety of methods. Procedures which utilize chemical or radioisotope techniques to measure intracellular levels of transported substrate must be corrected for substrate trapped in the extracellular space, if unwashed

packed cells are analyzed, or the cells must be washed before analysis. Washing with ice-cold, substrate-free medium is most common, and is usually done either on a membrane filter or by centrifugation. For most systems, this is an adequate procedure, since efflux into substrate-free medium of intracellular substrates is very slow at ice-bath temperatures. Cold-washing is the preferred procedure in the study of systems such as sugar transport in baker's yeast, which does not result in accumulation against a concentration difference, since, in such cases, the concentration of substrate in the extracellular space may be greater than that intracellularly. Cold-washing in such systems has proved quite efficient, with little loss of intracellular substrate. However, the washing procedure may be either inconvenient or impractical when several samples are wanted over a relatively short time interval. It would be desirable to be able to stop transport immediately at any predetermined interval, and to carry out the washing after all the samples from a given experiment have been collected. This has been accomplished for sugar transport in baker's yeast by the use of uranyl nitrate, a nonpenetrating transport inhibitor, which inhibits both sugar influx and efflux (Cirillo, Trans. N.Y. Acad. Sci. **23**:725, 1961).

In the experiment shown in Fig. 1, 2-ml samples of 5% baker's yeast in 5% sorbose (a nonmetabolized sugar) were transferred at 1-min intervals to tubes containing 1 ml of 3×10^{-3} M uranyl nitrate (pH 4.0), immediately mixed, and left standing at room temperature until all samples were collected. When the last sample was collected, all tubes were washed three times by centrifugation in 10^{-3} M uranyl nitrate at pH 4.0. Analyses of hot-water extracts of the washed cells (*see* Cirillo) are shown. The results are identical to those obtained when transport was stopped by immediate cold-washing. It was found that the cells could be kept for more than 1 hr in 10^{-3} M uranyl nitrate at room temperature without sugar loss.

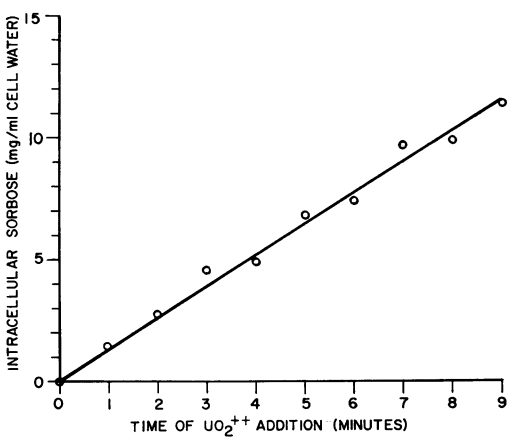

FIG. 1. *Analyses of hot-water extracts of washed cells.*

TABLE 1. *Prevention of sugar loss from the psychrophilic yeast Candida No. 5 by cold-washing in 10^{-3} M uranyl nitrate (pH 4.0)**

Wash medium	*Saccharomyces cerevisiae*	*Candida* No. 5
NaCl	35.5†	28.8
Uranyl nitrate	35.0	38.4

* The cells were equilibrated with 5% sorbose for 2 hr at room temperature before washing.

† Results are expressed as milligrams of intracellular sorbose per milliliter of cell water.

In recent studies on sugar transport by a psychrophilic yeast (Cirillo, Wilkins, and Anton, Bacteriol. Proc., p. 100, 1963), considerable sugar loss was noted during cold-washing, under conditions which resulted in negligible loss from baker's yeast. Substitution of uranyl nitrate for the NaCl wash medium eliminated this loss (Table 1).

Uranyl ion has been reported to inhibit sugar (Wilkins and O'Kane, Bacteriol. Proc., p. 189, 1961) and amino acid (Gale, Symp. Soc. Exptl. Biol. **8**:242, 1954) uptake in bacteria, sugar utilization in *Neurospora* (Cochran and Tull, Phytopathology **48**:623, 1958), and sugar as well as urea and glycerol uptake in yeast (Sols, *personal communication*). It should, therefore, be of general applicability in microbial transport studies. The fact that uranyl ion inhibition is readily reversible (Rothstein, Symp. Soc. Exptl. Biol. **8**:165, 1954) adds to the advantages of its use.