

chloride (bulk polymerization), vinyl ethyl ether-acrylonitrile, vinyl *n*-butyl ether-acrylonitrile, and vinyl isobutyl ether-acrylonitrile (prepared in acetone solution).

It seemed that the ideal conditions for precipitating and drying would be those wherein the copolymer would be precipitated with a large surface area, thus expediting complete solvent removal. For the acetone-soluble copolymers (those mentioned above), this was realized in the following manner: The copolymer solution was precipitated in four times its volume of methanol to remove the unreacted monomers. The methanol was then decanted and the copolymer taken up in acetone. The latter was added dropwise or in a very thin stream on a fast stream of water in the laboratory trough. The precipitated copolymer was caught on a screen placed at the end of the trough. This method of precipitation permitted complete removal of the acetone from the copolymer. The latter was then placed on paper toweling (without squeezing out the excess water) and dried for 48 hrs. After this time the copolymers were usually completely dry. If not, final drying was easily accomplished in a vacuum oven at 35° C.

## Automatic Masking of Lantern Slides

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In this laboratory we have developed a method for the automatic masking of lantern slides which may prove useful to scientists in many fields. The procedure follows:

The negative is projected to a suitable size, either by enlargement or reduction, upon the easel shown in Fig. 1.

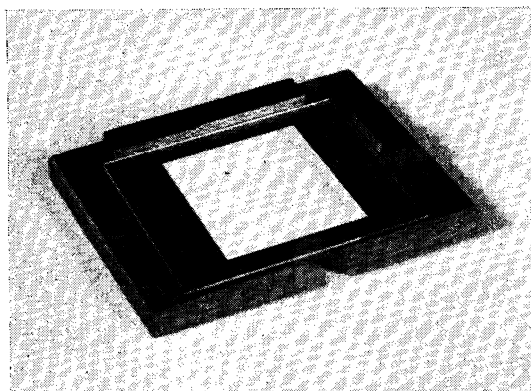


Fig. 1

On the easel is a lantern-slide cover glass, to the lower surface of which a square of white paper, backed with tin foil, has been glued. The subject material is focused and composed on the white paper.

A lantern-slide plate is then substituted and the exposure made in the usual manner. Before the plate is removed, the lantern-slide cover glass is placed on top of it with the tin foil in contact with the emulsion. After removing the negative carrier from the enlarger, the

border about the tin foil is then "burned" by double or triple the original exposure time.

On development, there is revealed a lantern slide that has been accurately composed and automatically masked to that composure. Three sizes of composing and masking shields, all of which have 1-cm top and bottom borders cover the range of most subjects. The lateral borders are 1, 2, and 2.5 cm, respectively. Almost any variation is possible. It should be kept in mind, however, that the binding tape accounts for about 0.5 cm, and any masking should exceed that dimension, if for no other reason than to allow space for thumb markers.

## A Simple Graphical Solution for Potency Calculations of Multidose Assays

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The necessity for calculation of the results of a number of multidose assays led to search for a simple method of solution. In the tests to be evaluated, samples were assayed at two or three dose levels, conditions varied as to preliminary estimates of potency of unknown, but the number of replicates per sample and standard at each dose were constant in any one experiment. The data from each test could thus easily be handled by the method of Bliss and Marks (1), but application of this technic is both tedious and time consuming for routine purposes.

A survey of the literature reveals that Knudsen (3) has described a graphical method which might be adapted to the problem in hand. However, her approach is limited by the necessity of drawing a network of radial lines for each dose interval employed as well as for every different assumed potency, and the axes must be rescaled for responses of different orders of magnitude. The nomograph provided for estimation of the error of the assay may be used only where rational basis for grouping of replicates exists; and even if litter mates were used in these tests, there is doubt whether they provide such a basis (2).

Sherwood (4), on the other hand, has reduced the calculation of potency from such data to relatively simple formulas, and these lend themselves readily to rearrangement which permits simple graphical solution. In the following, the same meanings are to be supplied to the symbols as those described by Sherwood. The case of the two-dose assay will be given in detail; similar reasoning leads to corresponding simplification for the cases of three- and four-dose assays.

The solution of the two-dose assay, as given by Sherwood, is:

$$\% \text{ Potency} = \text{Antilog} \left[ 2 \pm c + d \frac{(U_2 + U_1) - (S_2 + S_1)}{(U_2 + S_2) - (U_1 + S_1)} \right]. \quad (1)$$

If the data are substituted in the fraction within the

brackets and calculated as a decimal,  $P_2$ , then (1) may be written as

$$\% \text{ Potency} = \text{Antilog} [(2 \pm c) + d P_2] \quad (2)$$

or

$$\text{Log } (\% \text{ potency}) = (2 \pm c) + d P_2. \quad (3)$$

From (3) it follows that a straight line on semilog paper satisfies the equation for any assigned values of  $c$  and  $d$ . The entire information in Knudsen's chart is thus reduced from a network of 50 radial lines to one line on a semilog plot at the expense of requiring the assayer to calculate a simple ratio.

The work of preparing new graphical solutions for other values of  $c$  and  $d$  is vastly simplified. Following Sherwood further, the equations for three- and four-dose assays reduce to

$$\text{Log } (\% \text{ Potency}) = (2 \pm c) + \frac{4}{3} d P_3 \text{ and} \quad (4)$$

$$\text{Log } (\% \text{ Potency}) = (2 \pm c) + 5d P_4, \quad (5)$$

where  $P_3$  and  $P_4$  are the decimal equivalents of the appropriate fractions. These functions are as susceptible to simple graphical representation as Equation 3.

A family of lines for different values of  $c$ , at constant  $d$ , has been constructed for routine use here, and the average time of calculation of potency has been reduced to a matter of 2-3 min by use of this chart. Comparison with results obtained by the method of Bliss and Marks shows agreement within the limits of error of the interpolation (less than 1%).

#### References

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## A Method for Obtaining Massive Growth of Bacteria in Fluid Media

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A method has been developed which has been found useful for the preparation of *Brucella* antigen and for the collection of a large amount of bacterial sediment for agglutinin absorption.

A thoroughly washed cellulose sponge of coarse texture is mounted in the upper part of a 250-ml centrifuge bottle. The sponge is so cut and mounted that a pipette may be passed through a central hole to the bottom of the bottle. The dry sponge is readily cut to size and shape by a sharp knife or the moist sponge by scissors. The hole may be cut by a cork borer. When moistened and wrung nearly dry, the sponge may be forced into and out of the neck of the bottle. A glass tube of sufficient diameter to pass a pipette passes through the sponge and

rests on the bottom of the bottle, the sponge being supported at about 4 cm from the bottom by a bulb blown in the tubing (Fig. 2). The sponge may be cut square (about 35×35×55 mm) or cylindrical, but of such diameter that, when wet, an air space remains between the sponge and the sides of the bottle. Other methods of suspending the sponge have been tried but with less satisfactory results (see Fig. 1).

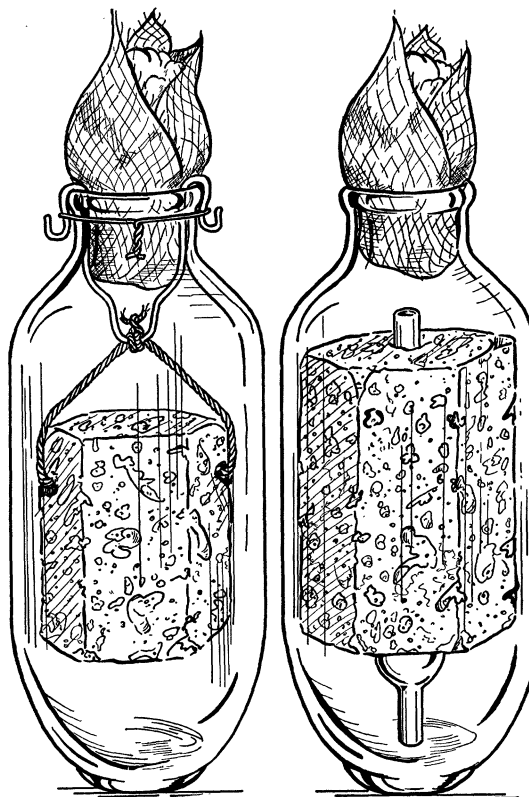


FIG. 1

FIG. 2

From 30 to 40 ml of broth is run through the central tube into the lower part of the bottle. This should reach a level not less than 2 cm below the sponge. The bottle is then plugged and autoclaved in the usual manner. The broth is inoculated by means of a pipette, after which the bottle is tilted to wet the sponge—a position which may be maintained for incubation.

To harvest the growth the bottle is centrifuged at low speed (about 500 rpm) for 5 min. A greater yield of organisms may be obtained by rewetting the sponge and recentrifuging two or three times. The turbid broth is pipetted out, transferred to a small centrifuge tube, and centrifuged at high speed to throw the sediment down.

This method may be used for many purposes where a maximum growth surface exposure is desirable or where the use of agar is to be avoided. The yields from a given amount of broth have been several times greater than those from the same amount of broth in bottles without the sponge.