NEW GRID-REPLICA FOR PRECISE LOCALIZATION IN SLIDE CULTURES

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This report describes a simple technique for the production and use of Formvar film replicas of rulings, such as counting grids, etched on glass slides. The resulting thin, transparent films, when dried on cover slips, are an excellent tool for counting and for orientation in slide cultures (Taubeneck, 1957). The method is similar to that of Powell (1956) who employs a lattice-like ruled area on cellophane, obtained by engraving with a system of razor blades, but the latter method is limited to use with petri dishes.

Preparation of Formvar films. Two types of matrices appropriate for use in the production of replicas have been developed. Type I represents a small glass slide with four diamond-scratched lattice-like ruled areas, of 9 mm² each, similar to the grid system of the Thoma counting chamber for blood cells (figures 1 and 2). This type of matrix is manufactured by Feinoptische Werkstätten, Jena. Type II also bears four ruled areas each consisting of ten thousand numbered 60-µ squares (figure 3). This type of matrix has been produced with the aid of photographic techniques, and is manufactured by the Zeiss Works, Jena. Using the above-mentioned types of matrices, the following technique has proved particularly efficient in the preparation of the Formvar films. A conveniently bent glass rod is dipped into a solution of 1 per cent Formvar in dioxane, placed on one end of the matrix, and pushed over the glass surface in such a way that a film of fluid is formed which does not touch the edges of the slide. Before the dioxane has completely evaporated, the film is cut between the ruled areas into four parts. By dipping the matrix slowly into distilled water after complete evaporation of dioxane (after 1 to 2 min) four Formvar films are stripped from the surface of
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Figure 2. Slide culture of Mycobacterium tuberculosis (BCG) on Dubos agar medium after 5 days of incubation. Cover slip carries a replica of a matrix type I. 200:1, phase contrast (150X after reproduction).

Figure 3. Slide culture of Proteus mirabilis: (left) after inoculation; (right) after 4 hr incubation. Cover slip carries a replica of a matrix type II. 420:1, phase contrast.

Figure 4. Slide culture of Proteus mirabilis after 4 hr incubation. Cover slip carries a replica of a matrix type I. 1100:1, phase contrast.

The separation of the films becomes difficult when drying has gone too far. The floating films are fished up on well cleaned cover slips, and dried under conditions preventing contamination by dust and airborne germs. The films adhere firmly to the cover slip without forming wrinkles. Cover glasses prepared in this way may now be used with the film downward for covering the inoculated agar surface of slide cultures or other preparations. Slide cultures can be sealed with vaspar.

The mounted films may be sterilized by dry heat before cover slips are used, but it is not necessary in most cases because it is easy to manage all manipulations in such a manner that the resulting films are sterile. Use of an adequate hood is recommended for the prevention of contamination and to reduce possible poisonous effects of dioxane. In our experiments contamination occurred very rarely, even after prolonged periods of observation.

Variations in temperature during incubation and observation should be avoided in order to reduce the danger of displacing the inoculated agar block. It is advisable to use a thermostatically controlled warm hood with built-in microscope, containing an arrangement for storing the slide cultures.

Applications. Since the first application of phase contrast microscopy to the direct examination of developing bacteria on agar media in slide cultures (Knöll, 1944) the observation of living bacteria has grown to a remarkable extent. When distinct points in different slide cultures...
are to be examined at certain intervals, satisfactory means for orientation within each preparation are absolutely necessary. The equipment of every culture with the Formvar replica of a numbered grid offers a great advantage (figures 2 and 3), especially during simultaneous work with many cultures over a prolonged period of observation. It becomes easy to count or to take photographs successively of a fairly large series of connected squares, and, after having examined other cultures, to take additional pictures of precisely the same area as before. In this way some developmental processes, such as the rate of germination of spores, and the rate of conversion of Proteus rods into large bodies and L-forms under the influence of penicillin, have been followed quantitatively. In the control of BCG vaccine, Formvar replicas have been used for the determination of the proportion of viable forms during 90 hr incubation (Knöll, 1958).

Finally, the Formvar films of type II can be used to locate specific points in permanent preparations (tissue section, etc.) by writing the number of the particular square on the slide. It should be stressed that the films do not interfere either with the quality of the microscopic picture or with the processes of development. It is possible to use high magnifications (e.g., oil immersion) without loss of morphological or cytological detail (figure 4).

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REFERENCES