Technical methods

A method for permanent preservation of antigen-antibody precipitation lines in agar

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Permanent preservation of antigen-antibody reactions, demonstrated by the Ouchterlony (1948) agar diffusion technique, is frequently of use. A method described by Uriel and Scheidegger (1955) appears to be commonly employed for the purpose. This technique, in which the agar is dried at 37°C. by successive applications of filter papers, was originally designed for electrophoretic studies where the thickness of agar and crystallization of residual salts led otherwise to cracking, distortion, and lack of

homogeneity in the preparation. A further method is given by Grabar (1960) using 15 to 20% glycerol as a desiccant: in this case the agar may be peeled off the underlying glass and stored as a thin pliable sheet. Both these methods are time consuming, and, for much of the agar diffusion work at present in progress, quite unnecessary. The present method depends on the apparently little known fact that sections of salt-free agar 2 to 3 mm. thick may be dried rapidly at 60°C. without difficulty.

METHOD

The specimen to be preserved must first be cleared of as much free protein and salts as possible. It is therefore thoroughly washed in its petri dish overnight in cold running tap water. In some cases a preliminary washing with changes of normal saline may be necessary to remove euglobulins and sometimes washing in tap water for up to

FIG. 1.
Photographic
prints of
antigen-
antibody pre-
cipitation lines,
stained with
amido black.
A, contact
print.
B, enlargement
× 3.
C, enlargement
× 24 of area
within the
rectangle
marked in B.
Orientation of
lines is the same
for each print.

48 hours improves the quality of the finished product. After washing, the unwanted parts of the agar are cut out and discarded and the remaining specimen flooded with freshly boiled (gas-free) distilled water. This prevents liberation of dissolved air during drying. Where tap water has a high mineral content it may be necessary to equilibrate the specimen for some hours against distilled water.

With freshly boiled water almost to the brim of the petri dish, the specimen is floated gently loose from the glass with a clean, grease-free scalpel and with slight rocking of the petri dish. The latter is then tilted and the specimen floated on to a grease-free glass plate such as a lantern slide, using the fingers as a guide. It is essential that the slide is absolutely clean and grease-free (flaming it over a bunsen burner for about 30 seconds gives the best results). A little experience and care is required in dealing with sheets of agar larger than about 2 in. square; using plenty of water for support is the secret.

When the agar is in position on a lantern slide it is trimmed and all bubbles trapped between agar and glass are removed with excess boiled water. Excess water is drawn off by tilting slightly and applying blotting paper to the agar edges. The upper agar surface must not be blotted. The preparation is then put in a horizontal position to dry in an incubator or oven at 55 to 60°C. Any tendency for the agar to creep out of position in the first few minutes of drying is due to excess water and/or tilting of the specimen. The process of drying takes 1 to 3 hours depending on the size of the specimen and after this period it is transparent and is firmly adherent to the glass. Staining with protein stains such as amido (naphthalene) black can then be done with little risk of detaching the agar. Sometimes, although not experienced personally, a preparation fails to take the stain, and Norris (1960) finds that a previous rapid wash with water removes the difficulty. Should the agar become partially or completely detached it may often be replaced on the slide; on re-drying it will be found adherent once more.

After staining and drying the preparations have similar properties to photographic plates. They may be stored between paper sheets; contact prints, enlargements, or lantern slides may be made for direct projection. The latter are often of excellent quality and superior to photographs of the same material. Fig. 1 shows a contact print (A), and enlargements $\times 3$ (B), and $\times 24$ (C) of the same material. Note that the definition of lines at the highest magnification is still good and the picture is devoid of 'graininess' which is usually apparent in prints from photographic negatives at this degree of enlargement.

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A micro method for agar-gel precipitin reactions

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The technique described here was devised with the object of conserving scarce reagents (especially sera) used in agar-gel precipitin tests. Although it is possible to use for this purpose a 'miniature Ouchterlony plate', in which small holes are cut in agar coated on to a microscope slide, in our experience it is difficult both to cut the holes cleanly and to ensure precision in their spacing. In these circumstances repeatability of results is often unsatisfactory. In the method to be described an agar layer on a microscope slide is used; but, instead of introducing the reagents into holes cut in this agar, they are placed in holes drilled in a perspex block applied to its surface. In effect this provides a series of hollow cylinders resting on the agar, into which the reagents are free to diffuse. The capacity of the holes can be controlled accurately by selection of an appropriate thickness of perspex and a suitable diameter of drill. Similarly a high degree of precision can be obtained in positioning the holes.

PERSPEX BLOCKS

Figs. 1 and 2 show the apparatus assembled. The thickness of the perspex and diameter and spacing of the holes will depend on individual requirements, but for general purposes we have found that perspex 3 mm. thick and holes made with a number 35 drill are satisfactory. The capacity of these holes is 0.02 ml. The spacing of the

FIG. 1. Diagram of the apparatus.

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