Simple Technique for Dark-Field Photography of Immunodiffusion Bands

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A simple dark-field photographic technique was developed which enables laboratory personnel with minimal photographic training to easily record antigen-antibody patterns on immunodiffusion plates.

There are many reports in the literature describing techniques for photographing immunodiffusion plates. The methodology, equipment, and results vary. In some instances, a single light source may be sufficient for dark-field illumination (1, 9, 11). A nonreflecting black background is generally used to afford maximal contrast (2, 4). Diffusion of the light source may eliminate undesirable reflections but may cause a loss in definition of the bands (6, 7). When photographing circular immunodiffusion plates, a uniform circular light source is preferable (3).

There are several methods for preparing adequate dark-field photographs of immunodiffusion plates. Reed (10) uses an X-ray view-box, the center of which is covered by a disc of blackened cane board. On top of the box is a heating tripod on which the agar plate is placed. The results are adequate with well-defined immunodiffusion bands, but the technique does not afford sufficient contrast to clearly differentiate less well-defined bands from the background. Engel (4) describes a general diagram for dark-field photography of rectangular agar-gel plates, but the procedure is rather complicated and awkward. Whitley (12) describes a box 19 by 19 by 3 inches with lights and glass plates. This apparatus is large and, therefore, also satisfactory for photographing anatomical specimens, although results with agar diffusion are variable. Murchio (8) uses several pieces of general laboratory equipment with very good results. The difficulty with this procedure is that the camera and equipment must be realigned each time to attain a good photograph. Thus, the results are variable, usually requiring personnel trained in photography to attain the best results especially when less well-defined bands are to be photographed. Gilder (5) uses a box about 1 foot in width, length, and height with two built-in fluorescent lights. The nature of this arrangement is not adequate for photographing circular plates.

In general, the preceding methods of photographing immunodiffusion precipitin bands have several deficiencies. First, most of the previous photographic equipment is large and bulky as compared to the size of the object photographed. Second, the equipment is either built especially for this single purpose, or consists of several pieces which have to be arranged each time a photograph is to be taken. In the latter case, reproducible results are extremely difficult to attain. Finally, as a result of these limitations, personnel would have to be trained in the basics of photography to produce even minimally satisfactory results.

An improved method of photographing immunodiffusion plates is based on the use of two fiber optic units (Bausch & Lomb, Inc., Rochester, N.Y.) and a circular Lucite form. The Lucite form is 3.5 inches in diameter and 1.4 inches high, painted flat black (Fig. 1), and holds two four-lead Bausch & Lomb fiber optic units, illumination being provided by two Bausch & Lomb stage lights. A heating tripod is used to hold the Lucite form. With the exception of the Lucite form, the other equipment is readily available. The Lucite form presented in this paper was designed to hold the standard small petri dish. The forms can be designed for any size plate, the only critical factor being the angle at which the retaining holes for the fiber optic heads are drilled (20° in the present form). This angle should be adjusted so that the beam of light strikes the opposite corner of the agar plate. The Lucite form was made in the Medical Center machine shop according to our specifications (angle of holes, petri dish diameter and height, and inside diameter). Most universities or medical centers have an interdepartmental facility capable of making this form. A wooden form would be just as effective. No black background is required. Figure 2 shows a plate photographed with two types of Polaroid film. Although the high-contrast picture (Fig. 2 B) is aesthetically more pleasing,
use of the normal-contrast type 57 film results in a more informative record.

The present photographic technique has several advantages over previous procedures. (i) A full circle of concentrated light permits the observation and the photographing of minimally defined immunodiffusion precipitin bands. (ii) The present method is rapid, efficient, and reliable. (iii) No measurements are needed as all the components are standardized in their positions relative to each other. (iv) The procedure is ideal in terms of size and convenience since all components can be used for other purposes with the exception of the Lucite form (Fig. 3). (v) All personnel are able to achieve reproducible results with minimal photographic training.

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LITERATURE CITED


FIG. 1. Schematic diagram showing the construction of the Lucite form used to retain the agar-diffusion plate and light sources.

FIG. 2. (A) Photograph of an agar diffusion plate with the use of the present equipment. The photograph was made with Polaroid type 57 3000 speed film. The contents of the wells of the immunodiffusion plate are: center well (C), rat kidney; well 1, anti-serum against yolk sac; well 2, anti-serum against rat kidney; well 3, rat kidney; well 4, anti-serum against yolk sac; well 5, yolk sac; well 6, anti-serum against rat kidney. (B) Photograph of the same plate made with Polaroid Type 51 high-contrast film.

FIG. 3. Photograph showing the unit ready for photographing immunodiffusion plates.