Photograms for Microbiological Assays

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The use of photograms provides a permanent record of microbiological assays in which diffusion of a substance in agar is measured. The accuracy of this procedure is comparable to direct measurement. This technique is inexpensive, does not require special photographic equipment, and is applicable to many tests commonly employed in clinical microbiology and immunology laboratories.

A method of permanently recording microbiological assays in which a substance diffusing in agar is measured can be useful in clinical and research microbiology laboratories (3). Although direct measurements of assay plates, such as those used to determine antibiotic drug levels or to quantitate immunoglobulins, are generally performed (1), permanent recordings of these assays provide data that can be measured at the convenience of the investigator and rechecked if necessary. Furthermore, these records permit documentation of results not noted at the time of assay in a form suitable for publication (4). Although cameras and photocopying machines have been used for recording assays, these techniques are expensive and require ready access to special equipment (3). Moreover, since both use optical lens systems which can cause image distortion, the accuracy of these procedures may be unacceptable in clinical and research settings. An inexpensive photogram technique was developed in which accuracy, when compared with direct measurement, was not compromised. A photogram is a form of contact printing in which an object is placed directly on photographic paper and exposed to light. A direct image of the object is reproduced. This process does not require special skills or photographic equipment (2).

The accuracy of direct measurement was compared with that of measurement from a photogram by using a standard microbiological antibiotic blood level assay in agar as a model (1). Petri plates (100-mm diameter) containing approximately 109 colony-forming units of Staphylococcus epidermidis per ml of agar were studied. Six wells, each 4 mm in diameter, were made in streptomycin assay agar (BBL Microbiology Systems) in 10 plates. Samples (20 μl each) of standard solutions of gentamicin (Schering Laboratories) containing 2.5, 5, and 10 μg/ml were placed in the wells. Plates were incubated for 18 h at 37°C. A single diameter, representing the zone of inhibition, was drawn on the bottom of the plate through the center of each well. The photogram was made as follows. In a darkened room these plates were centered on photographic paper (Kodak Kodabromide F4) and exposed to light for 2 to 3 s. The light source, a standard 7.5-W bulb, was placed 3 ft (ca. 91.00 cm) directly above the plates. The photographic paper was immediately processed according to the recommendations of the manufacturer. The diameter of each zone of inhibition on the plates and photograms was measured with a Vernier caliper to the nearest 0.1 mm. Best-fit lines, correlation coefficients, and slopes were derived by using an electronic calculator programmed for the method of least squares, and standard curves were plotted on semilogarithmic paper. To demonstrate the sensitivity of this technique, antibiotic assay plates were incubated for a shorter period, 4 h, at 37°C.

As shown in Fig. 1, distinct zones of inhibition occur around wells containing the three concentrations of gentamicin. Figure 1 also shows zones of inhibition around paper disks containing 20 μl of the standard gentamicin solutions. A comparison of mean zone sizes measured directly from the plates and photograms is shown in Fig. 2. The best-fit lines had slopes of 1.761 to 1.767, respectively, with correlation coefficients of 0.999. A slight magnification of zones (<5%) was seen on photograms. This magnification is the result of light striking an opaque material at a finite distance from the photographic paper. Since there is no distortion, the magnification does not affect the accuracy of this technique, and thus slopes and correlation coefficients are the same.

Clear zones of inhibition are depicted in a photogram of an assay plate incubated for 4 h (Fig. 3). The use of special photographic paper which enhanced contrast (data not shown) often made photograms easier to interpret than the original plate.

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FIG. 1. Representative photogram at 18 h containing both wells and antibiotic assay paper disks. Gentamicin concentrations used were as follows: (A) 10, (B) 5, and (C) 2.5 μg/ml.

FIG. 2. Comparison between mean zone sizes from 10 antibiotic assay plates by using direct measurement (X) and measurement of photograms (●).

The clarity of the print compares favorably with the original. The photogram can be made in most laboratories because it requires only a darkened enclosure, a light source, and photographic paper. The photogram must be developed in a darkroom; however, the exposed photographic paper may be stored at room temperature in an envelope designed to exclude light (available from Kodak) for subsequent transportation to a photography laboratory. This technique has many applications because it may be used in any assay system in which an opaque substance is incorporated into clear agar, such as radial immunodiffusion, Ouchterlony, immunodiffusion, and gel electrophoresis.

LITERATURE CITED