Micro Diffusion Agar Precipitin Technique
Convenient for Viewing and Recording

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Micro gel diffusion reactions, though used extensively in recent years, still present problems in viewing and recording. Direct photographs of these reactions, that usually must be enlarged, are not always satisfactory. Better results are obtained by using the dried, stained film as a negative and printing directly from this. Microscope slides are most often used, but glasses (2 by 2 inches) such as those used for binding photographic slides are more handy and have several advantages. They can be bound like 35-mm slides and projected for evaluation, lecture presentation, or similar purposes, and may be used as a negative in the enlarger for printing positives.

The most successful technique (Fig. 1 and 2) employed in this laboratory when working with weak antigen-antibody systems has been the use of ultra-thin glass plates in conjunction with a template and agar (1/4 inch thick). Templates were made slightly smaller than the glass (13/4 by 13/4 inches) from 1/4-inch-thick Plexiglas, with funnel-shaped wells (A. J. Crowle, J. Lab. Clin. Med. 52:784, 1958) to allow continuous feeding of the reactants. Well centers were set 7 mm apart and arranged with six satellite wells around a center well. A. J. Crowle (J. Lab. Clin. Med. 52: 784, 1958) suggests 4-mm distances and the use of thinner agar and smaller wells. Holes 1/16 inch in diameter were first bored through the Plexiglas. Each hole was then rebored most of the way through with a 3/4-inch bit to produce the funnel-shaped well. Well volume was 0.025 to 0.03 ml.

In most reactions, 1% Ionagar no. 2 in barbital buffer (pH 7.4) was used (A. J. Crowle, Immunodiffusion, p. 302, Academic Press, Inc., New York, 1961). Two 1/4-inch wide glass strips cut from another ultra-thin glass plate were placed on two sides of a warmed glass plate as spacers. Approximately 2 ml of the agar at 60 to 65°C was pipetted onto the plate. The template, previously coated on the underside with Desicote (Beckman Instruments, Inc., Fullerton, Calif.) and with the wells covered on top with lightly sticking tape, was also warmed and gently lowered onto the agar starting at one side. The weight of the template extruded the excess agar through the open sides. When the agar had set, it was trimmed from the two sides and the “sandwich” was carefully placed in a petri dish with moistened filter paper in the bottom. After 15 min, the tape was carefully removed and the reactants were added with fine-drawn disposable pipettes. The cover was placed on the petri dish, and the reaction was allowed to proceed at room temperature for the necessary period (3 days in this case).

At the end of the reacting period, the template was removed carefully and the unreacted material in the agar was leached out with several changes of the barbital buffer (pH 7.4) over a period of approximately 24 hr. It did not matter

FIG. 1. Direct print from stained gel diffusion reaction on 2 by 2 inch slide on which rabbit anti-Trichomonas foetus sera were reacted with soluble T. foetus antigens. Magnification is approximately ×4.
if the agar came loose from the slide; in fact, it facilitated rinsing. The buffer was then washed out with distilled water, and the agar was reori-

![Image](image-url)

**Fig. 2. Direct print from stained gel diffusion reactions on 2 by 2 inch slide.** For better comparison, agar from two reactions after washing was trimmed, oriented on a single slide, and completed. Magnification is approximately x 4.

mented on the plate and allowed to air-dry at room temperature in a protected place.

The plate was stained for 15 min with Amidoschwartz 10B as recommended by A. J. Crowle (*Immunodiffusion*, p. 304, Academic Press, Inc., New York, 1961). It was then differentiated in 2% acetic acid until no more stain could be removed, was rinsed in distilled water, and again allowed to dry.

The slide was then mounted with another clean slide, separated by 127 superslide foil masks, and was bound in 2 by 2 inch aluminum slide binders (masks, slides, and binders such as supplied by the Emde Products, Inc., Los Angeles, Calif.).

The slides were now ready for projection and printing and could be kept and used for long periods without change.

Agar preparation, agar thickness, well distances, sizes, and arrangement and staining procedures may be varied according to materials being used. These details are not original but are reported here as one workable system that may be used with the 2 by 2 inch glass plate and template.