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Method for Staining and Preserving Agar Gel Diffusion Plates

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An improved method for the preservation of agar gel precipitin lines has been developed.

Evaluation of the proteinaceous antigen-antibody reaction by precipitin formation in agar plates is one of the most common tools in immunology. It is often essential to keep records of the reaction or number of precipitin lines in a plate for comparative purposes. The most common methods of preservation are by sketching or photographing the precipitin lines. Both of these methods have disadvantages.

The former method is tedious and requires considerable practice to achieve accurate records. The second method is excellent for recording dense precipitin lines but requires considerable photographic technique for recording faint lines.

The following procedure provides a method of preserving the stained precipitin lines in the agar.

Immuno-diffusion plates were prepared by using Ionagar no. 2 (Colab Laboratories) in a phosphate buffer at *p*H 7.4. Sodium azide (0.2%) was added as a preservative. The agar was poured to a depth of 7 mm in petri dishes (85 mm diameter). The antigen and antisera were placed in wells in the agar, and the plates were incubated at 30 C in a sealed chamber for 3 to 7 days. After precipitin lines developed, the agar was removed from the petri dish with a flat-blade knife. The agar disc was placed between two paper towels, and the agar was allowed to dry at 25 to 37 C. After drying, the paper towels were wetted, and the dried agar disc was removed. The agar disc was placed in 500 ml of 0.9% NaCl solution and left for 36 hr at 20 to 25 C. The agar was removed from the NaCl solution, and the precipitin lines were stained by immersing the agar disc in the following stain solution for 2 hr: Woolfast Pink RL (American Hoecht Corp.), 1.0% (w/v); trichloroacetic acid, 5.0% (v/v); acetic acid (glacial), 1.0% (v/v); ethanol, 25.0% (v/v); tergitol anionic 4 (Carbide and Carbon Chemicals Co.), 0.01% w/liter. After staining, the agar disc was rinsed in tap water to remove excess dye, and was destained for 10 min by immersion in 70% isopropyl alcohol. Next, the agar disc was placed in 300 to 400 ml of hot (approximately 50 C) distilled water and allowed to remain 3 to 4 hr. Then, the distilled water was replaced with fresh distilled water, and the disc was destained 3 to 4 hr. The procedure was repeated until the excess dye was removed from the agar. After destaining of the agar, the agar disc was placed on a rectangular plate of glass (3.75 by 4 inch; Eastman Kodak Co.) slide and allowed to air dry. Plastic resin (Fitzgerld Enterprises, Oakland, Calif.) was applied by pouring over the stained agar disc on the glass side to a depth of approximately 3 mm. If the agar wrinkles during the drying, it may be wetted with tap water and smoothed. The plastic resin can be applied to the wet agar disc. (Note: The plastic resin is mixed according to the manufacturer's directions.)

The precipitin lines are stained deep red. The agar is usually a pale pink, but the intensity may vary with the amount of destaining. The plastic provides a permanent mount of the stained precipitin lines and can be stored indefinitely.

There are many methods for staining agar gel precipitin lines. Most methods rely on cationic stains which effectively stain proteinaceous precipitin lines, but also react with the agar medium. The agar-dye reaction does not present a serious problem when the agar thickness is shallow. However, when agar of 5- to 7-mm thickness is used, it is very difficult to remove the stain from the agar without destaining fine precipitin lines. Agar has an anionic characteristic, whereas proteinaceous precipitins are cationic in nature (1). Therefore, to selectively stain the precipitin line, it

is necessary to use an anionic dye which specifically stains protein and does not chemically unite with the agar. Woolfast Pink RL is an anionic dye which specifically stains protein and was chosen to fit the above parameters (2). Several other stains were tested, including crocein scarlet MOO, Acid fuchsin, imido schwartz, Ponceau S, and Congo red. None of the above stains tested could be effectively removed from the agar without removing some of the less dense precipitin lines. The above method will not stain polysaccharide type antigen and antibody precipitin lines.

To achieve plates with clear, distinctly stained precipitin lines, it is essential to extract the excess protein from the agar with the saline solution stated above.

The addition of acetic acid and trichloroacetic acid produce an acid *p*H which aids in staining and decreases the solubility of the precipitin line.

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