Performance Comparison of the Corning Thin-Film Agarose and the Hyland Thick-Film Agar Methods for Immunoelectrophoresis

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Many clinical laboratories do immunoelectrophoresis for qualitative assessment of proteins in biological fluids. Commercial kits are available that supply some or all of the necessary components, but the nature of these components varies. Seeking a reliable method for most easily preserving the original immunoelectrophoresis pattern as a permanent record, we compared a thick-film agar method to a thin-film agarose method in immunoelectrophoresis of a total of 70 serum and urine samples. For each sample, either method resulted in the same interpretation. The thin-film agarose method not only yields a stained, permanent record in about the same time that the thick film agar is ready for interpretation but not preservation, but also requires less antiserum, a smaller sample, and may not require purchase of additional hardware.

Additional Keyphrases: intermethod comparison · method for the small laboratory

Immunoelectrophoresis is being used widely by clinical laboratories for qualitative assessment of proteins in serum, plasma, and urine.

The basic components of an immunoelectrophoresis system include a support medium, electrophoresis apparatus, buffers, and antisera. Staining of the completed immunoelectrophoresis pattern before it is interpreted is preferred by some. A photograph may be made, or the original pattern preserved for record keeping.

Commercially available kits contain some or all of the required components, but the nature of each component supplied may differ from one kit supplier to another.

We were seeking a reliable method that would be suitable for permanent record keeping. The primary requirement was that the original pattern must be preserved easily for filing and retrieval. We considered technical acceptability and turnaround time as well.

The Agar Gel IEP System (Hyland, Costa Mesa, Calif. 92626) was compared to a method in which the Corning Universal Electrophoresis Film (Corning Medical, Palo Alto, Calif. 94306) was used. In the former method, which already was in use in our laboratory, a thick agar gel mounted on a rigid backing is used. Our usual practice was to interpret the unstained pattern and discard the gel. The Universal Electrophoresis Film is a thin agarose gel mounted on a flexible backing. Immunoelectrophoresis on thin-film agarose was attractive because our experience with this gel for serum protein electrophoresis had shown it to be well suited for preservation after staining.

We undertook to assess the relative performance of the two methods, because the Corning method would better suit our needs if equal sensitivity and comparable performance could be demonstrated.

Materials and Methods

We examined by the two methods each of 70 consecutive serum and urine samples submitted to the laboratory for immunoelectrophoresis. Each pattern was qualitatively interpreted by more than one observer without knowledge of patient identity, corresponding pattern by the other method, or opinions of other observer(s). Interpretations were either designated as "normal pattern" or were descriptive. Sensitivity was considered to be equal if interpretations of each pattern for each sample were substantially the same. The same lyophilized control material was used in each method (Quantitrol; Kallestad, Chaska, Minn. 55318).

The Hyland method consisted of the Agar Gel IEP System (Hyland, Costa Mesa, Calif. 92626) used with Hyland antisera (IEP Antisera, Hyland) (1). A 4-μl sample of serum or control material was introduced into alternate wells of the precut thick-film agar gel, which then was electrophoresed at pH 8.6 (barbital buffer, Hyland) for 35 min at 32 mA (Electrophoresis Power Supply, Hyland). Then a 60-μl volume of an appropriate antiserum was introduced into each precut trough and diffusion was allowed to take place for 20 to 24 h. Antiserum to human IgG, IgA, IgM, bound and free kappa light chains, bound and free lambda light chains, and whole human serum were used. The patterns were interpreted unstained at the end of the incubation, under indirect light and with use of a magnifying viewer (Calibrating Viewer; Transdyne General Corp., Ann Arbor, Mich. 48106).

The Corning method consisted of Corning’s Universal Electrophoresis Film (Corning Medical, Palo Alto, Calif. 94306) used with antisera supplied by Corning Medical (Corning Medical, Medfield, Mass. 02052). A 1-μl sample of serum or control material was introduced into alternate precut wells of the thin-film agarose gel, which was then electrophoresed at pH 8.6 (Special Barbital Buffer; Corning Medical, Palo Alto) 35 min at 20 mA (Electrophoresis Power Supply; Corning Medical, Palo Alto). Then a 20-μl aliquot of an appropriate antiserum was introduced into each precut trough and diffusion was allowed to take place for 20 to 24 h. The same antiseras as mentioned above were used. After diffusion, the gel was covered with two pieces of Sta-Moist paper (Corning Medical, Palo Alto) and glass (15 x 15 x 0.75 cm, with polished edges). A 1-kg weight (water in a plastic container) then was placed on top of the glass for 10 min, and the gel was then placed into a bath of isotonic saline (9 g of NaCl per liter) for 10 min. The 10-min press was repeated with use of dry Sta-Moist paper. For staining, we used 20 ml of an amido black stain (Amido Black 10B; Corning Medical, Palo Alto)

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Fig. 1. Dried, stained gel on its clear, flexible backing attached to a previously prepared white paper template. Information printed on the template facilitates interpreting and filing the pattern pipetted onto the gel surface. The stain was removed at the end of 5 min by immersing the gel in a solution of acetic acid (50 ml/liter) for 30 s. The gel was dried for 20 min at 60 °C in an oven, then cleared by placing it into a bath of dilute acetic acid (25 ml/liter) until the background was clear and distinct stained precipitin arcs were visible. Final drying was at 60 °C for 5 min.

Paper templates printed with labels corresponding to the contents of each well and trough were prepared by photocopying a master. The dry gel on its flexible clear backing was attached to the template (Figure 1).

Results and Discussion

In all cases, the interpretation of each pair of immunoelectrophoresis patterns by each observer was the same; no observer rendered a decision on a pattern prepared by one method that differed substantially from that by the other.

The thin-film method fulfilled our primary requirement: the stained original immunoelectrophoresis pattern becomes a permanent record that can be maintained for inspection and comparison to subsequent samples, and it gives results of quality equal to that for the thick-film method. The stained patterns are easy to photograph, photocopy, or project on an overhead or opaque projector.

We have demonstrated equal sensitivity in comparing interpretation of unstained thick-film agar to stained thin-film agarose plates. The procedure for drying and staining the thick-film agar requires a 48-h soaking in isotonic saline, for which reason we declined to consider it (I).

Agar was compared to the agarose method because the Agar Gel IEP System was in use in our laboratory at the time we initiated the search for an improved method. Agarose may give better resolution than agar in this application, although our comparison of unstained to stained gels does not explore this point. Thick-film agarose gels are available commercially. We did not compare the relative performance of thin- vs. thick-film agarose as we were satisfied with the performance and advantages of thin-film agarose. The titer of the antiserum is important in using thin-film agarose. Use of an antiserum of inferior quality might result in poor sensitivity.

Other requirements were met as well, although the thin-film method is more demanding. The narrow precut Universal Electrophoresis Film troughs are more difficult to fill than those of the thick-film Agar Gel IEP System. After brief experience (three or four plates), each technologist accepted the Corning method as being, overall, as easy to perform as the Hyland method. The pressing and staining adds 1 h to the procedure; otherwise the two methods take about the same time to perform when the same incubation periods are used. The extra time has not delayed reports on patients, as compared to the thick-film method.

No special viewer or indirect lighting is required for the Corning method. The use of prepared templates facilitates interpretation of labeled arcs against a white background. The unstained patterns of the thin-film method can be interpreted in indirect lighting, although not as easily as the stained pattern.

An additional and unanticipated benefit was that the Corning method requires less antiserum (20 μl) than does the Hyland method (60 μl), which can be a significant cost savings.

Because serum protein electrophoresis was being performed on the Universal Electrophoresis Film with use of the same buffer, power supply, and stain as described above for immunoelectrophoresis, the same inventory is maintained for the two procedures. Thus a smaller laboratory that does electrophoresis but has less demand for immunoelectrophoresis could still provide it by purchasing in addition only a stock of antisera, if the hardware is appropriate.

Reference