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# A Method for Routinely Producing High Resolution Black-and-White Journal Quality Photographs of Electrophoretic Gels

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**ABSTRACT:** A method is described for obtaining high resolution, black-and-white, journal quality photographs of electrophoretic protein patterns produced from a variety of stain systems. The photographic procedure employs a 35-mm single-lens reflex camera with an uncoupled, built-in light meter, Kodak Tech Pan Film, Kodak D-19 developer, and Kodak Grade 5 high-contrast paper. The procedure is applicable to a variety of protein stain systems which included Coomassie Brilliant Blue R250, bromophenol blue, silver, negative gold, tetrazolium dyes, and fluorescence.

**KEYWORDS:** forensic science, photography, electrophoresis, Coomassie Brilliant Blue R250, bromophenol blue, silver stain, negative gold stain, tetrazolium dye, fluorescence

Photography is an important part of data recording of electrophoretic protein patterns for scientific publications. However, photographic techniques for processing quality prints appear to cause many investigators considerable difficulty. This is due to the difficulty of obtaining reproducible, high resolution, black-and-white, journal quality photographs for the number of different staining systems used for detecting proteins in electrophoretic gels. Unfortunately, there are only a few reports known to these authors that address photography of protein patterns in gels [*1-3*] and only one brief reference dealing with black-and-white photography [*4*]. Allen et al. [*4*] suggested using a Mamiya RB 67 single-lens reflex camera with a 90-mm lens, a yellow filter, and positive/negative Polaroid #665 film to obtain good quality black-and-white photographs. Realizing that budgets can be a limiting factor, Allen et al. [*4*] alternatively proposed the use of a 35-mm single-lens reflex camera with an uncoupled, built-in light meter and a fine grain film. Following these suggestions, we have established a procedure that uses a 35-mm camera, standard equipment and supplies, and a darkroom. This method routinely yields high resolution, black-and-white, journal-quality photographs of electrophoretic protein patterns produced from a variety of staining systems.

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## Materials and Methods

Serum, plasma, or bloodstains were subjected to electrophoresis or isoelectric focusing and assayed for the identification of the genetic markers phosphoglucomutase-1 (PGM) [5], adenosine deaminase (ADA) [6], adenylate kinase (AK) [6], glyoxalase I (GLO) [7], erythrocyte acid phosphatase (EAP) [8], hemoglobin (Hb) [9], group-specific component (Gc) [10], transferrin (Tf) [11,12], and alpha 1-antitrypsin (Pi) [13] using previously described methods [5-13]. These genetic systems were used so that a variety of protein staining procedures would be used to demonstrate the efficacy and versatility of the photography method. The stains included the general protein stains Coomassie Brilliant Blue R250 [10,13], bromophenol blue [9], silver [11] and negative gold [12], the functional assay tetrazolium dyes [5-7], and a fluorescent assay [8].

Electropherograms were illuminated by transmitted fluorescent light except for EAP, which requires long-wave ultraviolet (UV) light illumination and Gc, which requires reflected light. A 35-mm single lens reflex camera (for example, Canon F1 with 50-mm macro lens or Pentax K-1000 with 50-mm lens) with an uncoupled, built-in light meter was used for photographing gels. The gels were photographed on Kodak Tech Pan Film with a lens equipped with a Tiffen #58 filter (green filter) except for EAP, which requires a Tiffen UV 15 filter. Exposure values were determined using the camera's meter with the filter in place. The meter readings were bracketed around ASA 100 film speed for all exposures. F-stop values (f 5.6 to 16) were selected and the exposures bracketed by shutter speed manipulation (see Table 1 for exposure settings). For all exposures the camera was secured to a copy stand to minimize vibrational effects.

The film was developed using Kodak D-19 developer at 68°C according to the instructions in Table 2 [14]. All prints were made on Kodak RC Grade 5 high-contrast paper according to the Kodak Manual [15].

## Results and Discussion

Figures 1 to 11 demonstrate that electrophoretic gel photographs with fine detail and good contrast can be obtained using the procedure described in this paper. With all photographs an almost white background with black bands (except for GLO, EAP, and negative gold staining, where the reverse applies) is achieved which facilitates photographic records. This

TABLE 1—*Exposure settings using a Canon F1 camera.*

| Genetic Marker | Stain | Lens Filter | Film Speed (ASA) | f-Stop | Shutter Speed, s |
|---|---|---|---|---|---|
| PGM | tetrazolium | Tiffen #58[a] | 100 | 13.0 | 1/4 |
| ADA | tetrazolium | Tiffen #58 | 100 | 13.0 | 1/4 |
| AK | tetrazolium | Tiffen #58 | 100 | 16.0 | 1/8 |
| GLO | tetrazolium | Tiffen #58 | 100 | 5.6 | 1/2 |
| EAP | fluorescence | Tiffen #15[b] | . . . | 8.0 | 10 |
| Hb | bromophenol blue | Tiffen #58 | 50 | 11.0 | 1/4 |
| Gc | Coomassie Brilliant Blue R250 | Tiffen #58 | 100 | 8.0 | 1/4 |
| Tf | silver stain | Tiffen #58 | 100 | 9.5 | 1/4 |
| Tf | negative gold | Tiffen #58 | 100 | 11.0 | 1/4 |
| Pi | Coomassie Brilliant Blue R250 | Tiffen #58 | 150 | 11.0 | 1/4 |

[a]Tiffen #58 filter is a green filter.
[b]Tiffen #15 filter is an UV filter.

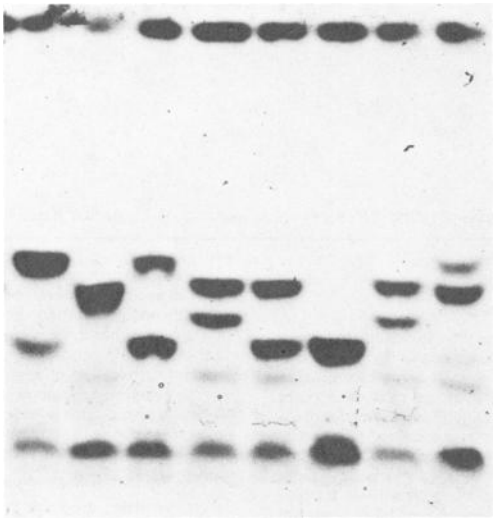


FIG. 1—*Isoelectric focusing gel for PGM using tetrazolium dye development. The phenotypes from left to right are 1−, 1+, 1−2+, 1+2−, 1+2+, 2+, 1+2− and 1+1−. The cathode is at the top.*

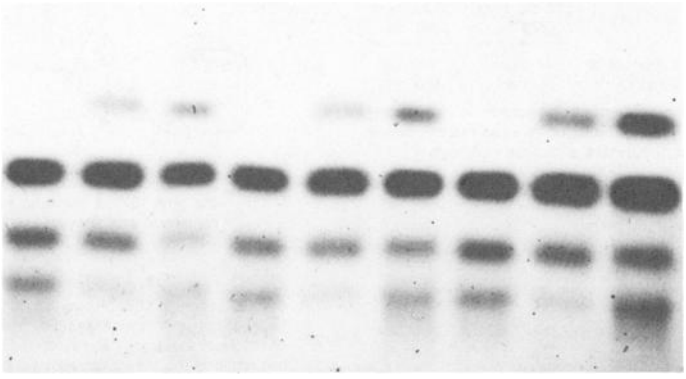


FIG. 2—*Conventional agarose gel for ADA using tetrazolium dye development. The phenotypes from left to right are 1, 2−1, 2−1, 1, 2−1, 2−1, 1, 2−1, and 2−1. The cathode is at the top.*

result of high contrast bands versus background is exceptional for some of the staining systems, particularly the tetrazolium dyes (Figs. 1 to 4) and the Gc immunoprint (Fig. 7), where the gel background is the same color as the bands. Previously reported photographs of these gels usually demonstrated low contrast black-and-white prints. Further, Casero et al. [*16*] have reported that negative gold stained gels are difficult to photograph as a result of high background problems. The photographic results of a negative gold stained Tf gel (Figs. 9 and 10) show a distinct black background with well-defined white bands.

The Kodak Tech Pan film allows enlargement of regions of interest on the negative without the loss of resolution from granular distortion (Fig. 10). The D-19 developer gives high

TABLE 2—*Film processing (reprinted courtesy of Eastman Kodak Company).*

## PROCESSING

### SMALL-TANK PROCESSING

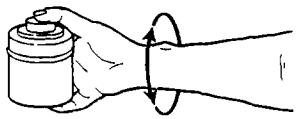
This film can be processed in small 16 oz. stainless steel or 20 oz. plastic tanks with spiral reels using the information given below. The same amount of dry chemicals is used in both cases. Since the 20 oz. mixture is more dilute, it requires a correspondingly longer time for proper development. Note that in some cases, particularly when processing with spiral reels, the 2415 Film may be susceptible to nonuniform processing effects if the technique is not perfected and carefully controlled. Nonuniform processing effects may be caused by pouring the developer on the dry film through the light trap in the tank top or by improper agitation techniques. Agitation should consist of rotating (inverting) the tank around a fixed point (your wrist); do not move the tank laterally. To avoid processing problems do not pour the developer through the top of the tank. Follow the agitation instructions carefully.

**To Prepare a Working Solution:**

| | 16 oz. tank 473 mL | 20 oz. tank 591 mL |
|---|---|---|
| 1. Start with this amount of water at 125°F (52°C): | 14 fl. oz. (414 mL) | 17 fl. oz. (502 mL) |
| 2. Slowly pour the contents of one packet into the water, and stir with enough agitation to keep the chemicals suspended until they are dissolved completely. | | |
| 3. Add water to bring the total volume to this amount: | 16 fl. oz. (473 mL) | 20 fl. oz. (591 mL) |
| 4. Stir until mixed completely. | | |
| 5. Cool to desired temperature and use immediately (within 24 hours). | | |

Overagitation caused by vigorous shaking within the first 30 seconds of development usually causes nonuniform development, especially adjacent to sprocket holes. On the other hand, insufficient agitation during this critical film-wetting period will also cause development nonuniformity because of uneven initial development. Because everyone's agitation style is slightly different, even when using the same directions, it is difficult to give definitive instructions. Bearing this in mind, we present the following instructions for reel-type developer tanks:

1. Fill the tank with developer.
2. In the dark, drop the loaded reel into the solution smoothly and without hesitation.
3. Attach the tank top to the tank. Then, to dislodge any air bells from the film, promptly tap the bottom of the tank against the table or other work surface from a height of approximately 1 inch.
4. Use initial agitation of up to 4 inversion cycles (down, up) depending upon your results. (Steps 2–4 will take from 7 to 20 seconds depending upon your type of tank.)



**Extend your arm and agitate the tank 180° at the wrist with no lateral arm movement.**

5. Let tank sit for the remainder of the first 30 seconds.
6. After 30 seconds, start 5-second inversion cycles at 30 second intervals. These agitation cycles should consist of 2 to 5 inversion cycles depending upon contrast desired and individual technique.

The above technique has produced acceptable results in a variety of tests performed in our laboratories. Other variations are possible and some people have had success with agitation every minute. Experimentation is in order. If your negatives are consistently too dense or contrasty, use less agitation or a shorter time; if they are too thin or flat, use more agitation or more time. Some limited testing indicates that rolling-tank agitation produced higher densities near the edges of the film. A water pre-soak was counterproductive and produced some unusual uniformity imperfections.

Before using these developers, make sure that the solution is free of small air bubbles which, if they adhere to the emulsion surface, may cause small undeveloped clear spots on the film—dark spots on the print. Bubbles may form more easily if cold and hot water are mixed together; if bubbles do form, let the developer stand until they dissipate. Attaching an aerator to the water supply will help form very large bubbles which will rise immediately to the liquid surface without remaining in solution.

**Develop** to the desired contrast index based on information in the exposure section and on the characteristic curves. The contrast index obtained depends primarily upon the developer, temperature, dilution, and development time chosen. It is affected to a lesser extent by exposure time (see Reciprocity Failure), specific processing techniques, and normal product variability. Therefore, the times given should be considered as starting points only.

For exposure meters marked for ISO (ASA/DIN), an exposure index (EI) of 25 may be used with the times and temperatures given below.

35 mm film—For one or two rolls in a full spiral reel tank, agitate 5 seconds every 30 seconds.

| **8 or 16 oz. stainless steel tank** | **10 or 20 oz. plastic tank** |
|---|---|
| 5 minutes at 68°F (20°C) | 15 minutes at 68°F (20°C) |
| 11 minutes at 77°F (25°C) | |
| 8 minutes at 86°F (30°C) | |

Tank size and design affect agitation and consequently film contrast and development time. Use these times as starting points for further experimentation to obtain desired results.

These processing instructions are for processing a single 135-36 roll or two 135-36 rolls simultaneously in a full tank. Do not process 2415 Film with developer that has been used previously

4 x 5-inch film—Use continuous interleaving-type agitation.

10 minutes at 68°F (20°C)

Process up to 8 sheets of film then discard developer.

**Rinse** at 65 to 70°F (18.5 to 21°C) in KODAK Indicator Stop Bath, KODAK Stop Bath SB-1a, or KODAK Stop Bath SB-5 for 15 to 30 seconds. Use running water for 30 seconds if no stop bath is used.

**Fix** at 65 to 70°F (18.5 to 21°C), with frequent agitation.

| | |
|---|---|
| KODAK Rapid Fixer | 1 1/2 to 3 minutes |
| KODAK Fixer | 2 to 4 minutes |
| KODAK Fixing Bath F-5 | 2 to 4 minutes |

**Wash** in clear, running water at 65 to 70°F (18.5 to 21°C) for 5 to 15 minutes, depending upon reduction of residual hypo needed.

To save time and conserve water, KODAK Hypo Clearing Agent can be used. Rinse the fixed film in running water for 15 seconds. Next bathe the film in KODAK Hypo Clearing Agent for 30 seconds with agitation. Then wash the film for 1 minute in running water at 65 to 70°F (18.5 to 21°C), allowing at least one change of water during this time.

**Dry** in a dust-free place. Heated forced air at 120 to 140°F (49 to 60°C) may be used to reduce drying time.

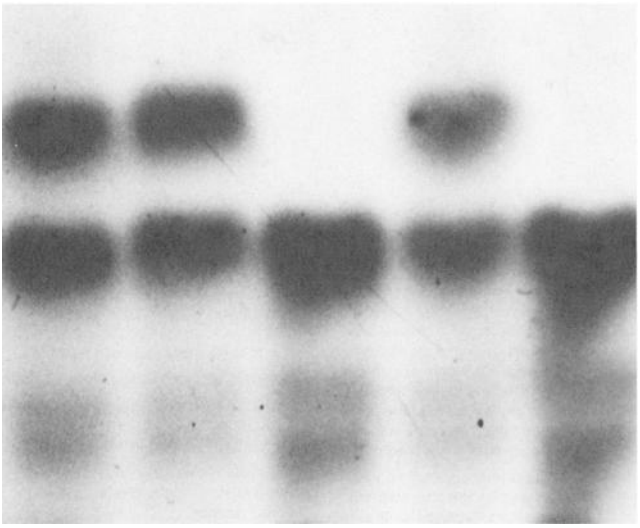


FIG. 3—*Conventional agarose gel for Ak using tetrazolium dye development. The phenotypes from left to right are 2−1, 2−1, 1, 2−1, and 1. The cathode is at the top.*

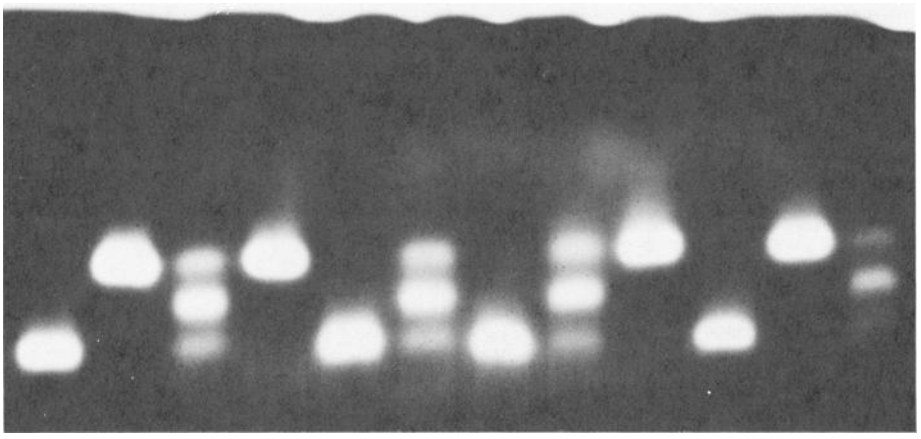


FIG. 4—*Conventional agarose gel for GLO using tetrazolium dye development. The phenotypes from left to right are 1, 2, 2−1, 2, 1, 2−1, 1, 2−1, 2, 1, 2, and 2−1. The cathode is at the bottom.*

contrast negatives and has the advantage of a short film processing time. Grade 5 black-and-white photographic paper provides high contrast black-and-white prints.

The combined use of these materials contributes to a photographic system that provides black-and-white prints that are highly detailed with good contrast, and it also provides the capability to enlarge photographs 25-fold without loss of resolution.[3]

The photographic system described here is based upon using a convenient and inexpensive 35-mm camera format. Larger photographic formats, such as Mamiya RB 67, can give bet-

[3]J. Schulman, Photography, FBI Academy, personal communication.

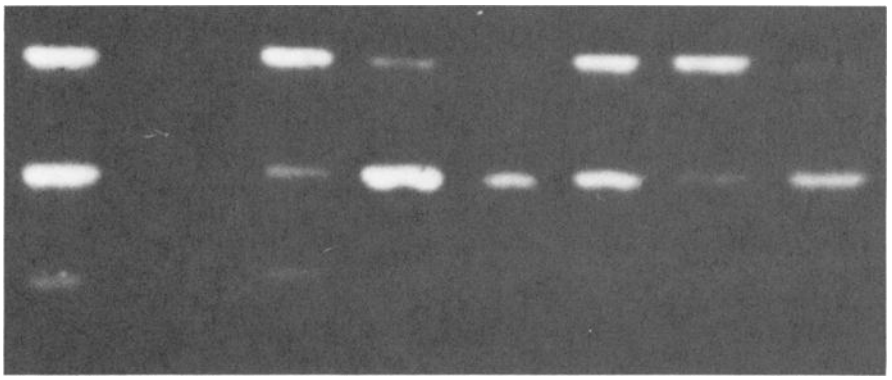


FIG. 5—*Isoelectric focusing gel for EAP using fluorescence. The phenotype from left to right are CB, negative, C, B, B, CB, C, and B. The cathode is at the top.*

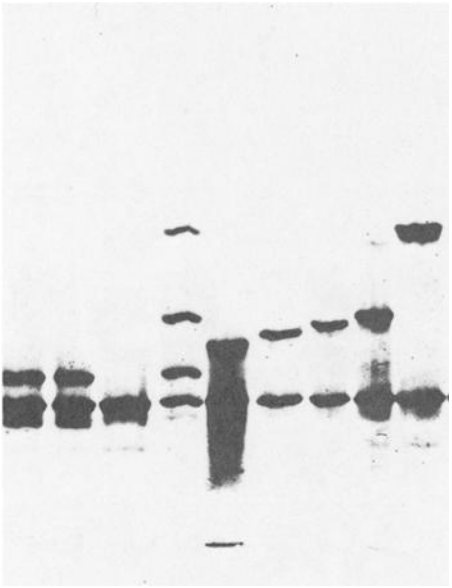


FIG. 6—*Isoelectric focusing gel for Hb using bromophenol blue. The phenotypes from left to right are AF, AF, A, AFSC (control), A-DEERPARK, A-G PHILADELPHIA, A-D PUNJAB, AS, and AC. The cathode is at the top.*

ter detail since the negative does not have to be enlarged as much to produce a print of the same size. However, larger format cameras are generally more expensive and more difficult to operate. Most laboratories have access to a 35-mm camera with an uncoupled light meter and darkroom equipment for processing the film and prints. Further, results with the less expensive Pentax camera and a normal 50-mm lens are comparable to that of the fivefold,

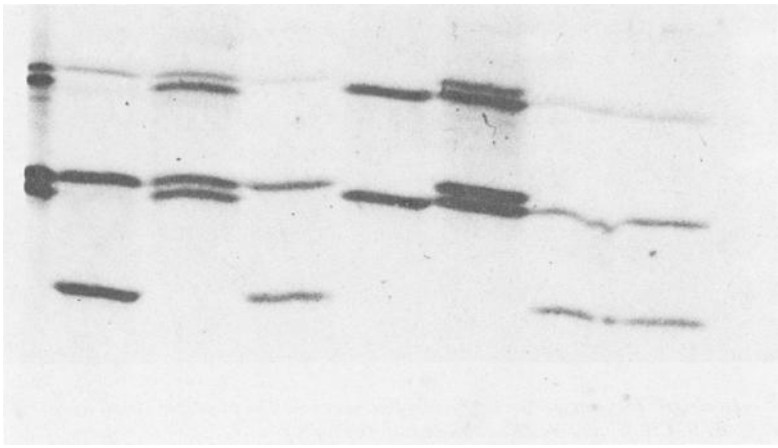


FIG. 7—*Immunoprint of Gc subtypes using Coomassie Brilliant Blue R250. The phenotypes from left to right are 2−1F, 1F−1S, 2−1F, 1S, 1F−1S, 2−1S, and 2−1S. The cathode is at the bottom.*

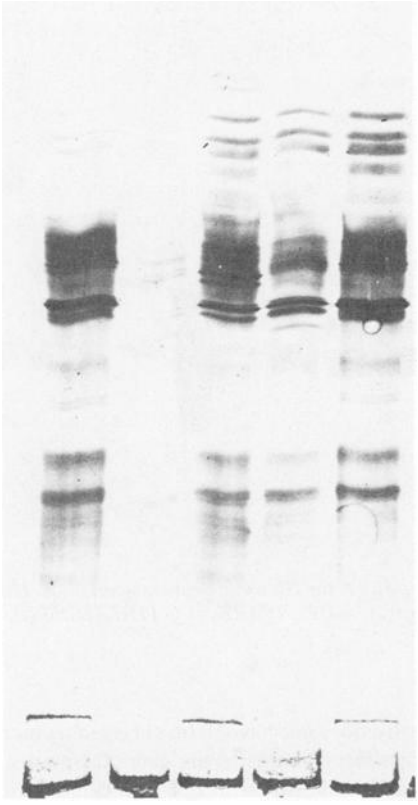


FIG. 8—*Isoelectric focusing gel (pH 5 to 7) with general protein profile developed by silver staining. The cathode is at the bottom.*

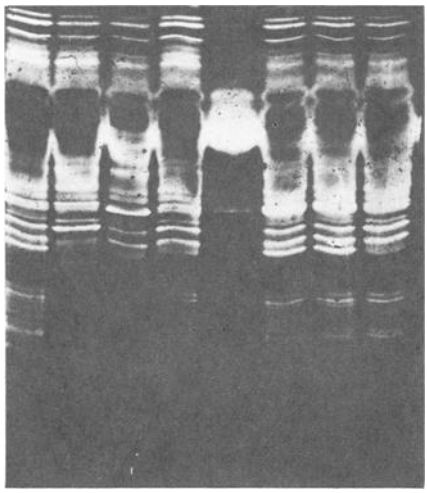


FIG. 9—*Isoelectric focusing gel for Tf using negative gold staining. The cathode is at the bottom.*

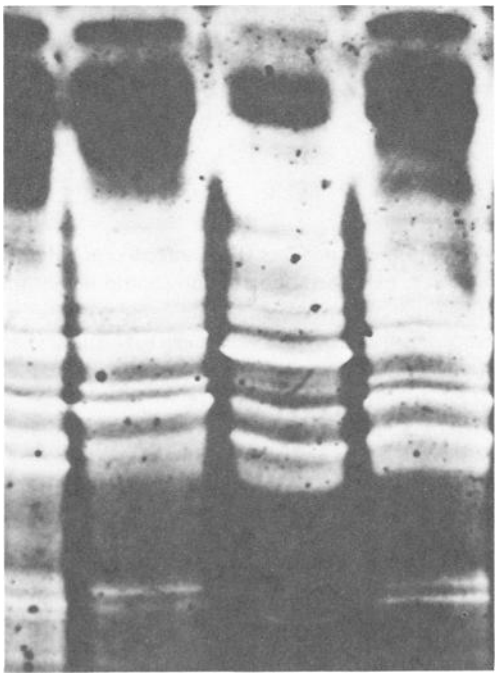


FIG. 10—*Enlargement of portion of Fig. 9. The cathode is at the bottom.*

more expensive Canon camera with a 50-mm macro lens (Fig. 11). Thus, less expensive camera equipment can be used.

The conditions expressed in this paper are only guidelines. Each laboratory needs to optimize its own photographic conditions. For example, variations in shutter speed among cameras can exist. Optimization of exposure can be achieved by setting the camera at different

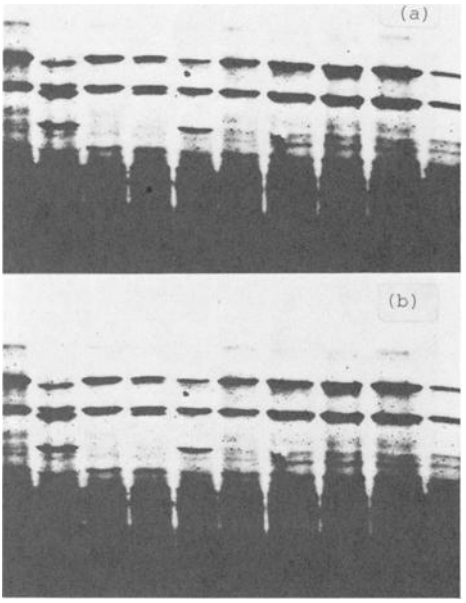



FIG. 11—*Isoelectric focusing gels for Pi using Coomassie Brilliant Blue R250. Photograph* (a) *was taken with a Pentax K-1000 camera; photograph* (b) *was taken with a Canon F1 camera. The cathode is at the bottom.*

ASA settings or by bracketing the exposures to obtain an equivalent range of film speeds. For greater ease and accuracy, exposure information should be obtained through the camera's light meter with the filter over the lens. If the camera's light meter is used, light is measured from the same position relative to the camera each time. Use of a hand-held meter is prone to position inconsistency. Of course, the battery for the camera should be checked periodically to ensure correct light meter readings.

In conclusion, a photographic procedure is described that is capable of producing journal quality black-and-white prints of a variety of stained electrophoretic gels. The procedure is reproducible, uses an inexpensive 35-mm camera format, and allows enlargement of negatives with virtually no grain distortion.

While this procedure yields very good quality photographs of electrophoretic gels, care should be exercised regarding the extent of use of these photographic reproductions of gels. The process described in this paper is specifically designed to enable the scientist to create good quality photographs reproducibly for journal publications and certainly could be used for monitoring internal quality control. However, no matter how good this procedure is at producing high resolution photographs, photographs at best are less than perfect copies of the actual results. The human eye still makes a better interpretation.

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