Hemoglobin Electrophoresis in Acrylamide Gel

By Thomas G. Ferris, Robert E. Easterling and Richard E. Budd

The various types of hemoglobins have been identified and reported by means of zone electrophoresis using supporting media such as filter paper, starch, agar, cellulose acetate, and combinations of these media. Recently a synthetic acrylamide gel (Cyanogum 41*) has been investigated and found to have characteristics which, in several respects, make it superior to other media for the electrophoretic separation of the serum proteins. Raymond mentioned that the gel was a satisfactory medium for the separation of hemoglobins. A technic and use of this acrylamide gel for the electrophoretic separation of hemoglobins in one hour is presented.

Methods and Materials

1. Polyacrylamide gelling agent (Cyanogum 41*).
2. DMAPN (beta-dimethylaminopropionitrile).*
3. Ammonium Persulfate, C.P.
4. Vertical electrophoresis cell.
5. Buffer: Tris-EDTA-Boric Acid, pH 8.7

Stock Solution: Tris (hydroxymethyl) aminomethane 30.25 Gm.
Ethylenediaminetetraacetic acid 3.90 Gm.
Boric acid 2.30 Gm.

Dissolve in distilled water and q.s. to 1 liter.

Working Solution: Dilute 1 volume of stock buffer to 12 volumes with distilled water.

6. Preparation of the gel: Dissolve 10.0 Gm. of Cyanogum 41 in approximately 150 ml. of the working buffer solution. Filter to remove insoluble particles. Bring the final volume to 200 ml. with working buffer solution. Carefully pipet 1.0 ml. of DMAPN into the solution, avoiding inhalation of vapor or contact of the liquid with the skin. Mix gently by rotation to prevent the formation of bubbles. Add 0.5 Gm. of ammonium persulfate and mix carefully as before.

Immediately pour the liquid carefully into the cell avoiding the formation of bubbles. Insert the plastic mold to form the sample slots and allow the gel to set. The gel must be formed in an air tight mold, otherwise a layer 3-5 mm. thick will remain liquid where exposed to the atmosphere. A slight opaqueness indicates the formation of the gel. It has been the authors' experience that with the gel prepared as above, the gelling is usually started within 5 minutes and completed in 15 minutes. Carefully remove the slot former after gelling is complete. Raise the cell to the vertical position and fill the electrode compartments with the working buffer solution.

7. Electrophoresis: A constant current of 400 volts is applied for 15 minutes to bring the gel and buffer into equilibrium. After preliminary equilibration, the current is turned off.
and 20 lambda (0.020 ml.) of hemoglobin hemolysate, which has been diluted 1:1 with working buffer solution immediately prior to use, is carefully pipetted into the preformed slots under the surface of the buffer. The specimen is allowed to settle in the sample slots for 2–3 minutes. The current (400 V) is then applied for one hour.

**RESULTS**

In specimens containing more than one hemoglobin type, separation is readily apparent in 20–30 minutes. Allowing the run to proceed for 60 minutes produces optimum separation of the hemoglobin types. During a 60 minute run, hemoglobin “A” will migrate approximately 4.0 cm. from the origin. Typical patterns obtained by this method are shown in figure 1. The presence of hemoglobin “S” was confirmed by solubility studies. This technic separates the hemoglobins into bands that are clear and distinct with a minimum of tailing.

A narrow band migrating ahead of hemoglobin “A” is apparent in figure 1 and has been noted previously in some of our hemoglobin studies. This component seems to correspond to that described by Pearson et al. This band does not fluoresce under ultraviolet light but does stain with Amido-black and benzidine. The band is not apparent in carefully and freshly prepared specimens taken from umbilical cords of newborn children. On the basis of these observations it is concluded that this rapidly migrating artefactual band is probably red cell stroma combined with a buffer front.

![Fig. 1.—Typical hemoglobin patterns obtained by electrophoresis for one hour in acrylamide gel. Mobility is from top to bottom.](image-url)
HEMOGLOBIN ELECTROPHORESIS

DISCUSSION

Cyanogum 41 gel has several advantageous properties which make it an ideal supporting medium for hemoglobin electrophoresis. The gel is readily formed at room temperature. The gelling time can be controlled by gel concentration, pH, temperature, and the amount and type of catalyst used. If the ammonium persulfate is allowed to deteriorate due to exposure to atmosphere and/or moisture, the gelling time will be extended. The gel has a relatively high tensil strength in concentrations of 3 per cent or greater and is unaffected by water or other ordinary solvents. The transparency of the gel permits quantitation of the fractions by scanning in a densitometer.5,7

SUMMARY

A method for hemoglobin electrophoresis is described, using acrylamide gel as the supporting media. The advantages and characteristics of this gel are mentioned. Clear and distinct separations of hemoglobin types obtained after 60 minutes electrophoresis in a vertical cell are illustrated. It is felt that the use of this gel affords a simple and rapid method for the detection and quantitation of hemoglobin abnormalities.

SUMMARIO IN INTERLINGUA

Es describite un metodo pro le electrophorese de hemoglobina, in le qual gel a acrylamido es empleate como le medio supportante. Le avantages e le caracteristicas de iste gel es mentionate. Es illustrate le clar e distincte separation de typos de hemoglobina obtenite post 60 minutas de electrophorese in un cellula vertical. Es opinate que le uso de iste gel provide un simple e rapide methodo pro le detection e le quantification de anormalitates de hemoglobina.

REFERENCES

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