

# Technical methods

## Haemoglobin electrophoresis on cellulose acetate using whole blood samples

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A modification of the method of haemoglobin electrophoresis using cellulose acetate membrane described by Kohn (1969) enables whole blood to be applied direct to the membrane instead of a haemolysate. The method gives results which are indistinguishable from those obtained using a haemolysate. Normal protein stains are not recommended, because of interference from plasma proteins, but the method's speed and simplicity, coupled with the advantage that minute samples of blood are used, are ample compensations.

### Apparatus and Reagents

Celagram cellulose acetate membrane sheets, 78 × 150 mm (Shandon Scientific Co. Ltd).

An electrophoresis tank capable of taking narrow cellulose acetate membrane sheets. The Shandon

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Multimicroband outfit is suitable, but faster results have been obtained using a standard tank fitted with a perspex bridge, with a power pack capable of delivering 250 volts.

### BUFFER A (ANODE)

Tris (hydroxymethyl-aminomethane) ..	25.2 g
EDTA (ethylene diamine tetra-acetic acid) .. .. .	2.5 g
Boric acid .. .. .	1.9 g
Water to .. .. .	1,000 ml

### BUFFER B (CATHODE)

Sodium diethyl barbiturate .. ..	5.15 g
Diethyl barbituric acid .. .. .	0.92 g
Water to .. .. .	1,000 ml

### Method

The cellulose acetate membrane strip is soaked in a mixture of buffer A and buffer B together with saponin (0.1%). This is most easily prepared by adding 1 ml of 10% saponin to 100 ml of a 50 : 50 mixture of buffer A and buffer B. The strip is blotted and applied to the bridge of the tank. A *thin* line of whole blood is applied at the midpoint of the strip. Blood anticoagulated with EDTA is suitable. The sample is best applied with an artist's fine camel-hair

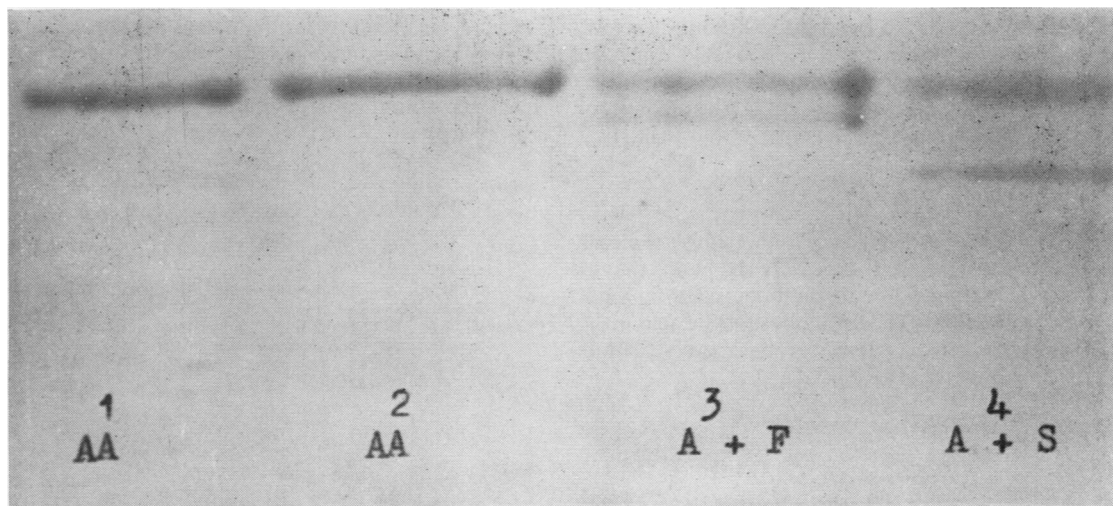


Fig. 1 Stained with benzidine. Samples 1 and 2 from normal patients: sample 3 from a neonate and sample 4 from a child with sickle cell trait.

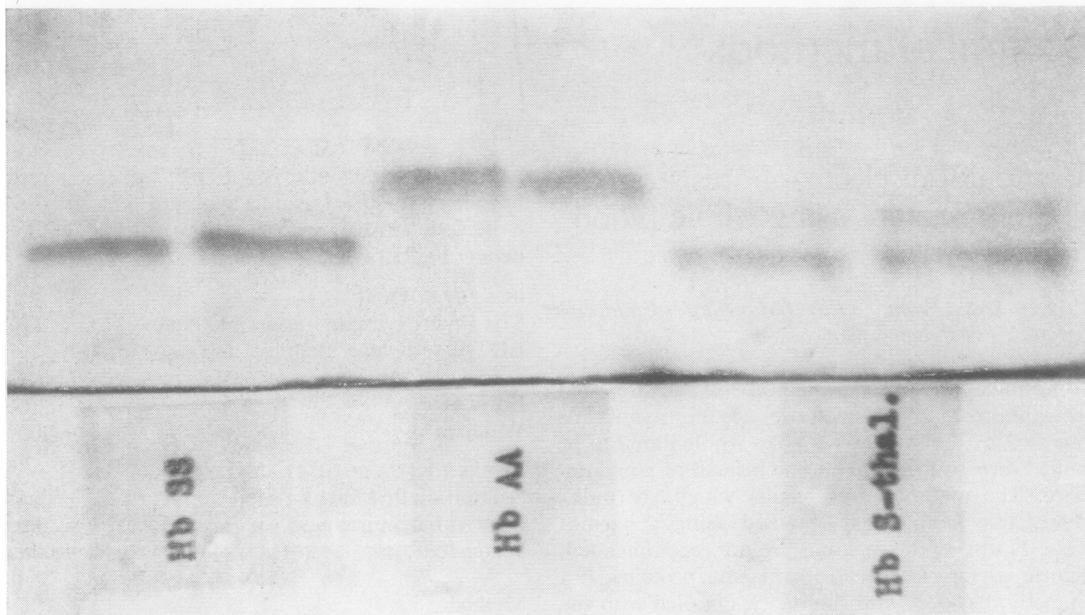


Fig. 2 Unstained strip. A haemolysate has been prepared from each sample and run next to the whole blood sample. No difference is seen.

brush; it is stressed that for good results only a trace of blood is needed. The lid is put on and the samples are allowed to soak into the cellulose acetate membrane strip. The red cells are lysed by saponin. After three to five minutes the current is switched on (9 to 14 mA at 200 V). The haemoglobin bands move to the cathode. Half an hour is enough to distinguish the presence of abnormal bands; and the run may be continued for up to two hours without deterioration. Higher voltages may be used to give a more rapid separation, as suggested by Kohn (1969). Best results are obtained with fresh blood, as older blood samples leave a precipitate at the origin (Fig. 1).

### Staining

For most purposes the haemoglobin bands are clearly seen without staining. If necessary the bands may be stained by the benzidine method described by Smith (1968) after fixation in 5% sulphosalicylic acid in 3% trichloroacetic acid, as described by Yawson, Huntsman, and Metters (1970).

### Comment

A rapid method of haemoglobin electrophoresis using whole blood was described by Giorgio (1970), but the author commented that the haemoglobin bands travelled more slowly than with a haemolysate, an obvious disadvantage. With the method described above there is no difference between bands separated from whole blood or from a haemolysate (Fig. 2). It is possible to distinguish HbF from HbA (see Fig. 1) and other Hb bands separate clearly. The method has been found most useful in screening for HbS before surgery.

### References

- Giorgio, A. J. (1970). A direct method of haemoglobin electrophoresis using capillary blood. *Clin. chim. Acta*, **27**, 362-363.
- Kohn, J. (1969). Separation of haemoglobins on cellulose acetate. *J. clin. Path.*, **22**, 109-111.
- Smith, I. (1968). In *Chromatographic and Electrophoretic Techniques*, 2nd ed., edited by I. Smith, vol. 2, p. 233. Heinemann, London.
- Yawson, G. I., Huntsman, R. G., and Metters, J. S. (1970). An assessment of techniques suitable for the diagnosis of sickle-cell disease and haemoglobin C disease in cord blood samples. *J. clin. Path.*, **23**, 533-537.