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A Modified Method of Haemoglobin Typing From Bloodstains : An Eclectic Approach

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ABSTRACT Haemoglobin is the major protein of human red cells, comprising about 95 per cent of their dry weight. Hb is important both in anthropology and forensic serology as it exhibits a very large number of genetic variants, a few of which are comparatively common and conveniently detectable in fresh blood by electrophoresis. Of these, the most common variant is probably haemoglobin S. Differentiation of Hb A, F, S and C from dried blood stains in criminal investigations as well as from blood samples brought on filter paper strips for anthropological studies, is usually not possible beyond three months or so. Here, we describe a modified citrate agar gel electrophoresis method whereby it is possible to distinguish A, F and S from blood stains more than a year old. 590 samples belonging to Thoti tribe of Andhra Pradesh have been successfully typed by this method.

INTRODUCTION

The haemoglobin molecule and its genetic variation is not only one of the most studied subjects but also one of the most written about. The mammalian Hb molecule, like many other proteins, is a multi-chain unit, a tetramer of four monomeric peptide chains, two alpha chains and two non-alpha chains. In man, the non-alpha chains can be either epsilon, gamma, beta or delta depending on the stage of embryonic, foetal or adult development.

In normal adults, the major component, comprising about 97 per cent of the total haemoglobin, is Hb A. A minor component HbA2 accounts for 2.5 per cent and HbF, the main Hb in foetal life, is about 0.5 per cent.

Abnormal haemoglobins are Hb variants resulting from genetically determined abnormal synthesis of globin part of the Hb molecule. Homozygous states for Hb other than HbS such as HbD and E are almost symptomless or with mild degree of anaemia. The double heterozygote disorders show variable clinical manifestations. The fact that there are hundreds of different Hb variants shows that Hb is polymorphic. This enables us to differentiate not only between individuals but also between races. Although most haemoglobin variants are rare, it has been estimated that one in every 2000 persons carries a detectable Hb variant (Motulsky, 1974).

Numerous papers have been written on the identification of Hb variants in fresh blood, but only a few papers have been published on the differentiation of variants in bloodstains. The following describes a modified citrate agar gel electrophoresis method whereby it is possible to distinguish A, F, and S from bloodstains more than a year old.

MATERIALS AND METHODS

Blood samples were collected from 590 individuals of Thoti tribe of Adilabad District, Andhra Pradesh by finger prick method on filter paper strips and stored at room temperature in almirah.

Blood Stain Extraction

For the extraction of bloodstains a 2 mm x 2 mm piece of filter paper was cut and placed into a microcentrifuge tube and soaked in 10 ml each of haemolysing buffer and dithiothreitol (0.78 per cent). The stain was allowed to extract for at least 30 minutes whereafter the tubes were centrifuged at 3000 rpm for 2 minutes.

Reagents

1.	Stock Citrate buffer	0.5 M
	Sodium Citrate	147.08 g
	Citric acid (dihyhdrate)	4.30 g
	DW	Up to one litre
2.	Citric acid	30 g
	DW	Up to one litre
1.	Agar	
	Bacto agar	1.0 g
	DW	Up to 100 ml.

2.	Haemolysing reagent EDTA (tetrasodium salt)	0.1% (w/v)
	KCN	1 mM
\3.	Benzidine stain	
	Benzidine hydrochloride	200 mg
	Glacial acetic acid	0.5 ml
	DW	100 ml
	H_2O_2	3% (v/v)

Electrophoresis

(i) For electrophoresis method advocated by Division of Host Factors, Centre for Infectious Diseases, Atlanta (1984) was adopted with some modifications.

(ii) 5 cm x 7.5 cm clean glass slides were precoated with 1 per cent agar dissolved in distilled water (DW). This step may be performed even months before use.

(iii) The stock 0.5 M citrate buffer was diluted 1 : 10 with distilled water. 100 ml of this working buffer was set aside without adjusting the pH, while the remaining buffer was set to pH 6.0 - 6.2. The latter was used as tank buffer.

(iv) 1% Agar gel for the run was prepared in the 100 ml buffer set aside in step (iii).

(v) Samples were applied to the middle of the gel plate. For this, agar was not cut but was gently touched with the micropipette so that the samples are just on the surface. A double sheet of 3 mm Whatman filter paper strip was used for making buffer contacts on either side. The slide was placed, agar side down, on the filter paper bridges.

(vi) Electrophoretic run was performed at a constant current of 15-17 mA (70-90 volts) for 70 minutes.

(vii) After completing the electrophoresis procedure, agar plate was placed in staining tray agar side up.

(viii) Zones of activity were visible within 2 minutes.

RESULTS AND DISCUSSION

Electrophoresis procedures employing filter paper, starch, agar, agarose and polyacrylamide gels and cellulose acetate membrane as support media have been described. Isoelectric focusing techniques have also been used to separate Hb variants. Depending upon which haemoglobin variants are to be resolved, different buffer systems are used, for the haemoglobins which have the same electrophoretic mobility in one system may separate in another.

Pollack et al. (1958) first suggested the application of Hb typing in bloodstains. They used filter paper electrophoresis and could differentiate HbA and HbS.

In 1962, Huntsman and Lehman applied paper electrophoresis for the separation of HbA, S,C,D, and E in bloodstains a few days old. They claimed that HbD and HbF could be differentiated by alkaline denaturation. Culliford (1964) used the discontinuous buffer system of Poulik (1957) to separate HbA from S and C on Oxoid cellulose acetate membrance.

Thielmann and Moreira Aquino (1971) used a starch gel microelectrophoresis system for the separation of HbA and S in bloodstains stored on filter paper strips. In their study HbA behaved as one single band even after three months of storage.

In 1972, Wraxall reported that good A and F resolution could be obtained using barbital, pH 8.6 cathodic and Tris-EDTA-borate, pH 9.1 anodic tank buffers. Wilkins and Oepen (1977) confirmed Wraxsall's (1972) findings. The procedure devised by Wraxall was adopted later by MPFSL (1978) and Sartorious cellulose acetate memberanes were found to be the best.

Garrick et al. (1973) also used CAM electrophoresis as a screening procedure for sickle cell anaemia and other haemoglobinopathies but used a new haemolysing reagent for elution purposes : 0.1% (w/v) tetrasodium EDTA containing 1 mM KCN. Potassium cyanide is added to convert methaemoglobin to cyanohaemoglobin. Methaem oglobin from older samples could interfere with the interpretation. They reported that even a very small fraction of HbS was clearly resolved from HbE. With bloodstains from 'adults' (*i.e.* 4 months of age or older), satisfactory patterns could be obtained after storage at room temperature for upto one month. with stains from newborn infants, exposure at room temperature for longer than 3 weeks lead to difficulties. However, in samples stored in freezer, it was possible to obtain satisfactory results after three months of storage with 'adult' or six weeks with new born specimens.

Wiggins (1978) applied the citrate agarose

electrophoresis method for the separation of HbS from D and HbC from HbE in dried blood stains.

In 1979, Barnard and Grunbaum looked at a series of HbA, AS and AC stains upto 28 days old and noted that aqueous extracts of the bloodstains could exhibit altered electrophoretic mobility relative to the control haemolysates. The effect was reversible by extracting the bloodstains with (Cleland's reagent-Dithiothreitol). Removal of the observed effects by DTT strongly suggested that these alterations were based on sulphydryl effects of some kind (Gaensslen, 1983).

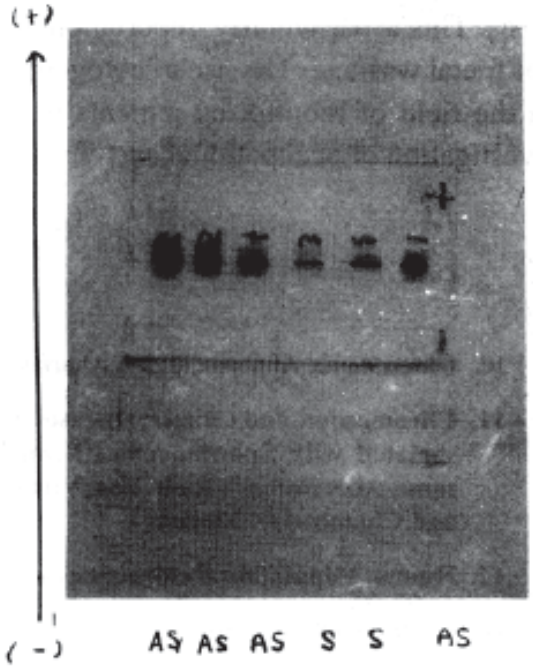

Fig. 1. Citrate agar gel electrophoretogram showing HbS and HbA obtained from one year old blood stains

In the present study, 590 blood samples stored at room temperature in steel almirah were analysed. It was possible to clearly differentiate A, S and F types in bloodstains more than a year old (Fig. 1). This was made possible by using an eclectic approach where the haemolysing reagent of Garrick et al. (1973) was combined with Dithiothreitol as advocated by Barnard and Grunbaum (1979). When the family data of 590 was randomised, out of 397 random samples, 45 were sickle cell positives (AS = 35 ; SS = 10) both by screening and electrophoresis methods.

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REFERENCES

Barnard, P.A. and Grunbaum, B.W. : Alteration of electrophoretic mobility of haemoglobin in bloodstains. *J. Forensic Sc.*, **24** : 384 - 388 (1979).

Culliford, B.J. : The identification of abnormal haemoglobins in bloodstains. *J. Forensic Sc. Soc.*, **4** : 155-157 (1964).

Gaensslen, R.E. : *Sourcebook in Forensic Serology Immunology and Biotechnology* U.S. Department of Justice, National Institute of Justice, Washington, D.C. (1983).

Garrick, M.D., Dembure B.S. and Guthrie, R. : Sickle-cell anaemia and other haemoglobinopathies : Procedure and strategy for screening employing spots of blood on filter paper as specimens. *New Eng. J. Med.*, **288** : 1265 (1973).

Huntsman, R.G. and Lehman, H : The detection of abnormal and foetal haemoglobin in bloodstains,. *Med. Sci. Law*, **3** : 59-64 (1962).

Metropolitan Police Forensic Science Laboratory : *Biology Methods Manual*. London (1978).

Motulsky, A.G. : Genetics of human haemoglobins : An overview. *Ann. N.Y. Acad Sci.*, **241** : 7-11 (1974).

Pollack, O.J., Barug, E.R., and Chubaty, W.G. : Toxicologic study of chemically altered haemoglobins and forensic study of bloodstains by paper electrophoresis. *J. Forensic Med.*, **5** : 200-207 (1958).

Thielmann K and Moreira Aquino, A : Whole blood samples dried and stored on filter paper as substrate for the electrophoretic separation of haemoglobin S from haemoglobin A : A screening procedure. *Clin. Chem. Acta*, **35** : 237-238 (1971).

U.S. Department of Health and Human Services : *Laboratory Methods for Detecting Haemoglobinopathies.* Public Health Service Centre for Disease Control. Atlanta, Georgia 30333 (1984).

Wiggins, K.G. : The identification of five haemoglobin variants in bloodstains. *J. Forensic Sc. Soc.*, **18** : 57-60 (1978).

Wilkins, R and Oepen, I : Nackweis non fetalen Haemoglobin in Blutspuren mit Hilfe der celluloseacetatfolien (CAF) - *Elektrophorese, Z rechtsmed.*, **79** : 79-80 (1977).

Wraxall, B. G.D. : The identification of foetal haemoglobin in bloodstains. *J. Forensic Sci. Soc.*, **12** : 457-458 (1978).