Advances in Forensic Haemogenetics 2
(c) Springer-Verlag Berlin Heidelberg

An approach to individualisation of micro-bloodstains using immunochemophoresis

S. Rand, A. Kohfahl, P. Ritter and B. Brinkmann

Institut für Rechtsmedizin der Westfälischen Wilhelms-Universität Münster, Von-Esmarch-Str. 86, 4400 Münster

INTRODUCTION

One important limiting factor for the individualisation of bloodstains is the size. When the size of the bloodstain is in the micro-litre range the choice of blood group systems to be tested becomes critical and for the traditional systems such as ABO, Gm, PGM etc. the size of the stain dictates which, and how many of these systems can be used. The choice depends, among other things, on the sensitivity of the system.

The application of polymorphic DNA-markers or "fingerprinting" would seem to be excluded from the investigation of these micro-stains because of the present requirements of stain examination. Gill (1985) has estimated 60 µl of blood are necessary and Baird (1986) approximately 100 µl although this figure will obviously be reduced with the advent of new detection methods.

By the application of immunochemophoresis to the detection of polymorphic protein systems such as Gc, a substantial increase in the sensitivity can be achieved and the inclusion of these systems in the repertoire for bloodstain grouping would be advantageous for individualisation.

The 8 polymorphic protein systems described here (fig. 1) have a combined discrimination value of 0,0002 or 99,98 % (1 in 5.000) and the most common phenotype combination occurs in 0,25 % of caucasians.

These proteins have all been extensively studied in serum samples but up till now only Gc is in general use for stain grouping. The other systems are at present undergoing the necessary stringent examination before acceptance can be possible.

METHOD

The term immunochemophoresis has been adopted to describe the combination of electrophoretic (IEF) separation and immunochemical detection. The latter necessitates the transfer of proteins to an inert carrier and in this case the transfer is carried out by semi-dry electro-blotting onto a nitro-cellulose membrane.

Advances in Forensic Haemogenetics 2
Edited by W. R. Mayr
© Springer-Verlag Berlin Heidelberg 1988

Advances in Forensic Haemogenetics 2
(c) Springer-Verlag Berlin Heidelberg

Extensive testing and comparative studies (Rand 1987) have revealed the considerable advantages of this method in - contrast to passive blotting methods for example.

An outline of the method is shown diagrammatically in fig. 2, and is preceded by an appropriate pretreatment or extraction process for stains depending on the protein tested.

Visualisation of the protein bands is carried out using a specific antibody, an enzyme-linked antibody-complex and substrate as outlined by Pflug (1986). The method is principally the same for all the systems except that the type of pretreatment, the optimal pH range, electrophoretic conditions and the antiserum dilutions are varied for optimal results within each system. A detailed description of the method for Gc (Rand 1987) has already been published. Methods for the other systems are in progress.

The maximum sensitivity of each system was investigated by doubling dilution series and the results are listed in fig. 3. A typical dilution series is shown in fig. 4 for Tf. The sensitivity of detection depends not only on the amount of protein present in blood but more important on the quality i.e. the avidity and affinity of the antisera.

As has been well documented the detection of a protein polymorphism in serum or plasma does not necessarily predispose its detection in blood stains. This depends mainly on the stability of the protein in the dried state or the ability to reconstitute or reverse any changes which have occured during this process.

The systems described can all be detected in stains and after appropriate pretreatment, or extraction, no appreciable differences to serum samples have yet been detected.

Studies are still being carried out, but Gc has been detected in laboratory-made stains in 1 1/2 year-old stains and FXIIIB, Tf and Pi in 6 month-old stains. Testing of the various systems under varying storage conditions (4°, 37°, humid chamber etc.) are still under examination but has as yet produced only a weakening of bands without the generation of anomalies.

DISCUSSION

The application of semi-dry electroblotting and immunochemical visualisation of proteins has increased the sensitivity and reliability of detection of many polymorphic protein systems so that they can now be considered for use in blood stain grouping. From the optimal dilution factors seen in fig. 3 it can be estimated that it would be possible to detect these systems in approximately 2 µl of serum. This in round figures would mean approximately 5-10 µl of blood stain. Quantitative experiments on stains are to be carried out to assess this value but it seems to be relatively realistic.

A micro-extraction procedure using micro-titre plates, as outlined in figure 5, has been tested for serum samples and is at

Advances in Forensic Haemogenetics 2
(c) Springer-Verlag Berlin Heidelberg

present being tested for stains. In practice, the different extraction conditions make the use of microtitre plates uneconomical and microtubes must be used for maximum efficiency.

Until the name DNA fingerprinting (Jeffries 1985) has been justified with respect to bloodstains and is equally as sensitive as these systems described here, it is felt that this approach offers an extremely reliable and flexible alternative.

	Phen	%	Nondisc.
Gc	1S	34	0·259
Tf	C1	57	0·406
PLG	1	51	0·388
α_2HS	1	43	0·395
FXIIIA	1	62	0·416
FXIIIB	1	54	0·408
Pi	M1	55	0·368
Hp	2FS	34	0·228
		0·25	0·0002

DI=0·9998

fig. 1. List of protein systems used, the frequency of the commonest phenotypes, and the individual and combined discrimination values.

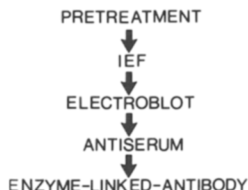

fig. 2. Outline of the immunochemophoresis technique.

Sensitivity

	Vol.(µl)	max.titr	Stain(m)
Gc	2	800	18
Tf	5	64	6
PLG	5	5	6
α_2HS	5	*	6
FXIIIA	2	16	*
FXIIIB	2	32	6
Pi	5	8	6
Hp	5	*	*

* still under investigation

fig. 3. Comparison of the sensitivity of each system listing the volume of sample applied, the maximum dilution obtained and the age of blood stains (months).

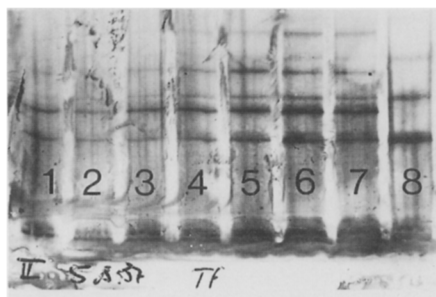

fig. 4. Titration series for Transferrin type C1-B. Sample 7 is undiluted serum followed by sequential doubling dilutions to sample 1, a 1/64 dilution. Sample 8 is TfC3-1.

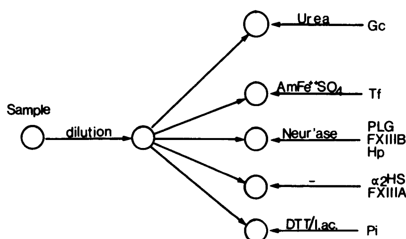

fig. 5. Scheme of extraction and pretreatment of samples using a micro-method.

Advances in Forensic Haemogenetics 2
(c) Springer-Verlag Berlin Heidelberg

LITERATURE

Baird M, Kanter E, Shalter R, Balazs I (1986) Application of DNA Polymorphisms to the forensic examination of dried blood stains. In: Brinkmann B and Henningsen K (eds): Advances in forensic haemogenetics 1. Springer-Verlag Berlin, Heidelberg, New York, Tokyo, p. 351-355.

Jeffreys AJ, Wilson V, Thein SL (1985) Individual-Specific "fingerprints" of human DNA. Nature 316; 4: 76-79.

Gill P, Jeffreys AJ, Werret DJ (1985). Forensic application of DNA "fingerprints". Nature 318, 12: 577-579.

Pflug W: Isoelectric focusing of Gc-Subtypes on reusable immunilized pH-gradient gels followed by detection with antibody conjugated alkaline phosphatase. In: Brinkmann B, Henningsen K (eds). Advances in forensic haemogenetics 1.Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, p. 372-377.

Rand S, Ritter P, Kohfahl A, Brinkmann B (1987) A comparative study of immuno-blotting techniques for the detection of Gc-subtypes after isoelectric focusing on agarose and polyacrylamide gels. Z Rechtsmed 98: 175-180