The Use of Monoclonal Antibodies for the Detection of Red Cell Antigens in Stain Material

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INTRODUCTION

Where the grouping of stains for red cell antigens is used as a routine procedure in casework, considerable quantities of antisera are required and the availability of monoclonal reagents is a welcome development. Moreover the serologist may soon find that the most readily available reagents for our presently used techniques of inhibition and elution will be monoclonal antibodies.

Our previous experience with polyclonal reagents has shown that some antibodies are more suited than others for the detection of antigens in stains by elution and careful selection of the antisera has always been considered by us to be an essential preliminary to successful grouping. We considered monoclonal reagents could show variation in their suitability and in this study we have investigated the elution technique using mostly anti-A and anti-B reagents but also including tests with anti-M, anti-N and anti-D.

The elution technique used has been described elsewhere (Dodd and Lincoln 1982).

RESULTS

Preliminary experiments were made with a series of doubling dilutions of each monoclonal reagent to select a possible suitable dilution for successful grouping of bloodstains.

Example results, shown in Table 1 were obtained using a number of dilutions of a monoclonal anti-A, 3D3, kindly supplied to us by Dr D Voak of Cambridge. This reagent was considered to reach standards expected of normal anti-A licensed reagents. At a dilution of 1/16 and more slightly less strong reactions were obtained with the A_2 than A_1 bloodstains which were less than one month old. Also using the higher dilutions A_1 bloodstains produced stronger reactions than A_2 stains after the shorter incubation period. Positive reactions were obtained even when the reagent was diluted to 1/256. No false positive

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reactions were obtained even using the undiluted reagent.

A dilution of 1/4 was selected for further investigation. A dilution of 1/4 or 1/8 was selected for most ABO reagents used in this survey. This is the same level of dilution as normally used for polyclonal reagents.

Similar succcessful results were obtained using monoclonal anti-A 6D4 from Dr Voak and a seraclone reagent supplied by Biotest Ltd., when the stains ranged in age from 1 to 12 months. Results obtained using A_1 and A_2 bloodstains which were 3 years old indicated that not all monoclonal reagents would be equally suitable for detecting weaker A activity. Example results in Table 2 show how some reagents are less efficient for detecting the A antigen in stains of group A_2.

A critical factor for judging the suitability of anti-A reagents for normal grouping of fresh blood samples is their ability to detect samples of A_2B, therefore the various reagents were used to test A_2B bloodstains. Also the stains of various ages were used as an additional method of introducing stains with different antigen strength. The AB bloodstains were also tested with anti-B monoclonal reagents which had been shown to produce satisfactory results with fresh bloodstains of groups A, B and O.

Although most of the fresher stains could be successfully grouped using the monoclonal reagents, differences in suitability became particularly marked using A_2B stains which were 3 years old. Example results are shown in Table 3. It is interesting that all the monoclonal anti-B reagents used, satisfactorily detected the B antigen, so that the possibility of typing stains as group B which were really AB could be a problem.

It appears that some workers have found monoclonal ABO reagents totally unsatisfactory in their elution tests, particularly it seems the reagents offered by Ortho Diagnostics. Results obtained using three batches of Ortho anti-A and several examples of their anti-B reagents donated to us by workers at the FBI Research Centre in Quantico, produced satisfactory grouping reactions with bloodstains of groups A_1, A_2 and B and only with 3 year old A_2B stains did we clearly fail to produce satisfactory positives with these monoclonal reagents and clear cut positives with polyclonal reagents.

Table 4 shows example results obtained using monoclonal anti-M and anti-N reagents, Seraclone, supplied by Biotest Ltd. The difficulties experienced in the past with MN typing of bloodstains means that this systems seldom find itself a place in the repertoire of forensic serologists. It is interesting that the few results we have suggest that these reagents could produce far more satisfactory MN grouping of bloodstains than rabbit antisera of lectins.

Table 5 shows example results obtained using a monoclonal IgG1 anti-D produced by Dr Crawford in London. Various concentrations

of the reagent have been used for the detection of the D antigen in bloodstains. Success could be achieved using a carefully selected concentration. Higher concentrations of the anti-D produced positive reactions with the D negative stains as well as with the D positive stains.

It is interesting that this reagent is completely specific when used by a variety of techniques for normal agglutination tests on fresh blood samples, thus illustrating the need for careful re-standardisation of reagents before use by elution.

Monoclonal reagents are now available and hopefully will provide exciting potential for forensic serologists but we must get to know them well before we can rely on them and be confident in their use in casework. In spite of their high degree of specificity they must be carefully standardised and selected by the technique by which they are to be used.

Table 1 Example results of elution tests using various dilutions of anti-A 3D3

	Dilutions of anti-A 3D3						
Bloodstain	Neat	½	¼	1/16	1/64	1/128	1/256
A_1	C C	C C	C C	V V	+ ++	+ ++	+ ++
A_2	V C	C C	V C	++ ++	+ ++	- ++	- +
B	- -	- -	- -	- -	- -	- -	- -
O	- -	- -	- -	- -	- -	- -	- -

C = complete agglutination
V = visual agglutination
++ = strong microscopic agglutination
+ = medium microscopic agglutination
(+) = weak microscopic agglutination
- = no agglutination

Two results = first reading after 30 minutes incubation with indicator cells, second reading after 2 hours incubation.

Table 2 Tests with anti-A reagents in 3 year old bloodstains

	5138*	3D3**	6D4**	Seraclone**
A_1	C C	V C	C C	C C
A_1	C C	++ C	C C	C C
A_2	C C	- ++	+ V	C C
A_2	C C	W ++	+ V	C C
A_2	C C	- +	- -	V C
B	- -	- -	- -	- -
O	- -	- -	- -	- -

* polyclonal
** monoclonal

see also Key in Table 1

Table 3 Example results using 3 year old bloodstains with various anti-A and anti-B reagents

	anti-A						anti-B	
	3D3*	6D4*	Seraclone*	513**	5B2*	5A5*	Seraclone*	55441**
A_1	V C	V C	C C	C C	- -	- -	- -	- -
A_2	- ++	+ ++	C C	C C	- -	- -	- -	- -
A_2	W +	- -	++ ++	C C	- -	- -	- -	- -
B	- -	- -	- -	- -	C C	C C	C C	C C
O	- -	- -	- -	- -	- -	- -	- -	- -
A_2B	+ C	+ C	V C	C V	C C	C C	C C	C C
A_2B	- -	- -	- W	- W	C C	V C	C C	C C
A_2B	- -	- -	- ++	- V	V C	C C	C C	C C

* monoclonal reagent
** polyclonal reagent

see also Key in Tabe 1

Table 4 MN typing of blood stains using monoclonal reagents

Stain	anti-M diluted 1/8	anti-N diluted 1/8
MM	4+	-
MM	4+	-
MN	4+	4+
MN	4+	4+
NN	-	4+
NN	-	4+

Titre of original anti-M with MN cells = 128

Titre of original anti-N with MN cells - 64

Table 5 Example results from tests for the D typing of bloodstains using monoclonal anti-D UCH D4

Rh type of stain	Age of stain	Dilution of anti-D 1/8	1/16	1/32	1/64
CCDee	1 week	4+	2+	4+	4+
CcDEe	1 week	4+	4+	4+	4+
ccddee	1 week	2+	-	1+	-
ccddee	1 week	2+	-	-	-
CCDee	4 mths	4+	4+	4+	3+
ccdEE	4 mths	4+	4+	4+	4+
CcDEe	4 mths	4+	4+	4+	4+
ccddee	4 mths	2+	2+	1+	-

REFERENCES

Dodd Barbara E, Lincoln PJ (1982) The use of antigen antibody techniques in forensic serology. A Seminar on antigen-antibody reactions revisited. American Association of Blood Banks, p223.

IV. DNA Polymorphisms