THE APPLICATION OF MONOCLONAL ANTIBODIES FOR THE DEFINITION OF GENETIC MARKERS OF HUMAN RED CELLS

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INTRODUCTION

Monoclonal antibodies have now been produced to the red cell antigens A, A_1, B, A and B, M, N, D, LW, e, H, I, Le^a, Le^b, P, P_1, K, k, LKE, Lu^b, T, T^n, Wr^b, and MER 2.

Selected stable cloned cell lines that produce high yields of useful monoclonal antibodies offer considerable advantages to reagent manufacturers by providing unlimited quantities of high quality reagents (Kohler and Milstein 1975)

The aims of this paper are to describe:-

(1) the serological behaviour of selected monoclonal antibodies to ABO, RhD and MN red cell antigens that have proved themselves at least equal or superior to conventional reagents.

(2) new types of problems encountered during the use and or development of these reagents that require special quality control techniques or a new approach to reagent production.

ABO MONOCLONAL ANTIBODY REAGENTS

ABO grouping reagents should meet FDA standards (Hoppe 1979) for speed of reaction and titre, and they must be stable and specific. Selected mouse monoclonal anti-As, anti-Bs, and anti-A,B antibodies achieve these criteria and are in widespread use as blood grouping reagents (Voak 1986). These IgM anti-A/anti-B antibodies are potent red cell haemolysins in the presence of the complement of fresh serum. Therefore, the reagents must be formulated with EDTA (0.01m) at pH 7.1-7.3 for use as typing reagents.

Anti-A Reagents

By 1984 superior monoclonal anti-As had been produced that exceeded conventional anti-A reagent specifications by reacting with A_x in addition to being superior with weak A_2B "A_3B" bloods (Voak 1986, Voak and Lennox 1986, Messeter et al 1984) and based on the recent (Paris) ISBT monoclonal antibody trials and personal contacts we now know of 10 monoclonal anti-As that see A_x.

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Advances in Forensic Haemogenetics 2
Edited by W.R. Mayr

The saline titres (Table 1) demonstrate the superior titres of the anti-A (MH04) compared to the monoclonal anti-A (3D3) that reacts about as well as a polyclonal FDA licensed serum anti-A reagent. (Lowe et al 1984)

Table 1. Saline Titres of Tissue Culture Supernatant Monoclonal Anti-A Reagents

	Anti-A Reagents		
3% Cells Saline RT	3D3 Neat	MH04 Neat	Group B serum Commercial X (USA)
A_1	1024	1024	512
A_2	512	512	256
A_1B	512	512	512
A_2B	64	256	64
A_2B Weak	4	256	8
A_3B	0	256	1
A_3	4	256	4
A_x	0	64	0
B(A) +++	0	32	0

Saline tests negative x B, O, A_m and A_{end} RBC

MH04 is the most powerful anti-A we have examined and it gave macroscopic reactions with 20 out of 24 examples of A_x. Absorption and heat (56°C) elution studies with MH04 confirmed it was an anti-A and not some form of anti-A,B antibody as it was not absorbed or eluted from B or control O cells. The strength of binding of MH04 absorbed by A_1 red cells is so strong that it is not eluted from A_1 cells, although it does elute from A_2, A_2B and A_x red cells. Thus the selection of monoclonal antibodies for blood grouping based on high avidity criteria produces reagents unsuitable for use by classical forensic elution techniques.

We suggest that forensic workers use these avid reagents by ELISA methods or select monoclonal antibodies to lower avidity criteria.

MH04 anti-A was used in thousands of automated and manual blood grouping tests using the UK sedimentation tube technique read after pipette transfer of the cell button to microscope slide, and no reactions were observed with B or O red cells. However, later in 1985 MH04 and 3D3 anti-As from Celltech were blended to produce Ortho's Bioclone Anti-A and after some months it became apparent that this reagent reacted weakly with a low incidence of "normal" group B red cells. Samples of some of these reactive group B bloods were referred to D. Voak by D. Davies (Ortho) and M. Beck (Kansas City) and we demonstrated that the reactive B red cells were reacting with

the MH04 anti-A. The agglutination with these B cells was extremely fragile and best observed by very gentle agitation (or tip and roll) spin-tube techniques, as shown in Table 2, and the reactions were enhanced by enzyme treatment of the red cells. We then demonstrated that the reaction with these B cells was an A reaction as it was inhibited by the A but not B substance of secretor salivas.

Table 2. The B(A) Phenomenon - Method Sensitivity of MH04 Anti-A Reaction with Group B(A) Red Cells

Test		Group B donor RBC 1	2	3	4	5	Reactive Group B 23509	21865	Group O Control
Spin	Saline	0	0	0	0	0	+	+++	0
	Papain	+	+	+	++	+++	++	+++	
Sed.	Saline	0	0	0	0	0	0	(+)/+	0
	Papain	W	0	W	0	0	W	++	0

The B(A) Phenomenon - B Transferase May Make Some A

Thus, all B cells have a little A that is not the product of an A gene. B cells reactive in saline tests were called B(A) red cells and the reaction with MH04 anti-A was called the B(A) phenomenon. We suggest that B(A) red cells have A levels approaching that of weak A_x cells while the majority of B red cells have a much smaller trace of A. One of us (D.V.) contacted Dr. Watkins with this news and she was delighted to inform me that our work had provided the serological confirmation that B transferases could make some A "in vivo" as suggested by their earlier "in vitro" immunochemical studies (Greenwell et al 1979).

Characteristics of B(A) Reactions

(For reviews see Voak et al 1987 and Treacy and Stroup 1987)

1. Shown by high concentrations of certain monoclonal anti-As that see A_x.

2. Also shown by anti-A of certain high titre group O sera after absorption of anti-B and anti-AB crossreacting antibodies.

3. Best detected by gently read spin-tube tests as the agglutinates are extremely fragile and method sensitive.

4. The reactions are enhanced (variable) by enzyme treatment of the red cells, but still fragile and method sensitive.

5. The incidence of B(A) reactions in saline tests is very low as routine manual or automated grouping methods involve sufficient agitation to destroy the B(A) fragile agglutinates. Very

careful screening with the first batch of Bioclone anti-A gave a variable incidence (> Negroes) from as high as 0.9% (Beck) to 0.1% (Mohn). Reproducibility of reactions was poor and many centres did not find any B(A) cases.

6. Occurs due to production of some A by elevated B transferase levels in group B persons.

Quality Control of Anti-A Reagents to Avoid B(A) Reactions

Most reagent manufacturers will soon have superior anti-A monoclonal reagents. Therefore it is important to realise that this type of reagent development is not prevented by the B(A) phenomenon, as B(A) reactions are a function of anti-A concentration that can be quality controlled with B(A) or papainised B red cells. The MHO4 anti-A diluted at 1/90 is still a superior anti-A reagent negative with the B(A) papainised B and O control cells by carefully read spin-tube tests (Table 3). The ability to detect weak A_x red cells is reduced but the 1/90 MHO4 reagent still detects more examples of A_x than the excellent BRIC 131 anti-A of Dr. Anstee and the FDA licensed conventional anti-A,B control reagent.

Table 3. The B(A) Phenomenon is Prevented by Dilution of MHO4 Anti-A and the Reagent Still Detects Many Weak A Variants

Spin Tests		Number of Variants Detected			
		Anti-A Reagents			Anti-A,B
Sub Group	Number Tested	Bioclone Ortho	MHO4* 1/90	BRIC 131 Sup.	Conventional
A_x	24	17	14	9	7
A_3	6	6	6	6	6 (1 Weak)
"A_3B"	8	8	8	8	8
A_x B	1	1	1	1	1
Control A in B					
Papain	B	1+	0	1+ weak	++++

*Also negative with B(A) that is ++ with Bioclone.

Anti-B Reagents

Monoclonal anti-B antibodies (Sacks and Lennox 1981, Voak et al 1983, Messetter et al 1984) are now in widespread use as typing reagents and careful blending of some of the examples shown in Table 4 has produced monoclonal anti-B reagents at least equal to conventional FDA licensed reagents.

None of these monoclonal anti-Bs detected acquired B and they are useful for studying acquired B cases in forensic work.

Table 4. Saline Titres of Tissue Culture Supernatant Monoclonal Anti-B Reagents

	Anti-B Reagents				
3% Cells Saline RT	NB1/19	3B4	5A5	5B2	Group A Serum Commercial X (USA)
A_1B	256	64	256	128	64
A_2B	512	128	512	256	64
B	512	128	512	256	128
B Cord	256	64	256	128	64
B Weak*	0	32	64	32	64
A_1	0	0	0	0	0
O	0	0	0	0	0

*Similar results obtained with 25 additional sub-groups of B

The detection of weak B variants in 25 samples kindly donated by Dr. Leong and Mr. Mak, Hong Kong, (not classified, as saliva not tested) by the anti-Bs 3B4, 5A5 and 5B2 but not by NB1/19, which has a similar titre with A_1B and B cord cells, suggests that weak B variants have a different B specificity to normal B cells.

Anti-A,B Reagents for the Detection of Weak Subgroups of A and B

Several monoclonal anti-A,B antibodies have now been described (Voak et al 1983, Messetter et al 1984 and Moore et al 1984). One example (ES 15) by Moore et al (1984) reacts well with all group A types down to and including A_x, but only reacts weakly with even strong group B cells. Thus it must be blended with a potent monoclonal anti-B to make an anti-A,B reagent. Anti-A,B reagents can also be made by blending a monoclonal anti-A (MH04) that sees A_x with a monoclonal anti-B (5A5) that sees weak B variants, as shown in Table 5.

Table 5. Monoclonal Reagents Agglutinating Both A and B Cells That May be Used Instead of Anti-A,B Serum Reagents

	Anti-A,B ES.15	Anti-A,B ES.15 + Anti-B 5A5	Anti-A MH04 + Anti-B 5A5	Conventional Commercial X (USA) O Serum
2% Cells				
A_1	V	V	C	C
A_2	V	V	C	C
B	(+)*	V	C	C
A_2B	V	V	C	C
A_3 (5439)	++	++	V	++
A_X	+/++	+/++	++	++
B_W	-	++	++	++
O Cells	-	-	-	-

*Weak reactions with B cells and negative with B weak cells

C	V (+++)	++	+	(+)	GW W
One Clump	Several Clumps	Smaller Clumps	Granules	Small Granules	Microscopic Cells/Clumps (8-12) (4-6)

Economy of Automated ABO Grouping (Technicon Autogrouper 16C)

MH04 anti-A at a 1,500 dilution of the supernatant detects all A subtypes down to "A_3B" (weak A_2B) but not A_x. A more concentrated 1:50 dilution of MH04 anti-A was used in an anti-A,B blend with a 1:50 dilution monoclonal anti-B (5B2) to detect weak subgroups of A and B. This anti-A,B and reverse grouping also confirms the ABO groups. This procedure enables us to identify A_x donors for research purposes as we did not want them to be labelled as group A donors. The monoclonal anti-B (5A5) was used at a 1:100 dilution.

Accurate results were obtained with these reagents and no false results were obtained in 113,703 tests with the anti-A, 135,039 tests with the anti-B and 47,259 tests with the anti-A,B. The economy of these selected monoclonal anti-A/B antibodies, enhanced by methyl cellulose (0.6-1.0%) and bromelin (0.25%) is at least five times better than with conventional reagents and offers complete reliability of donor blood typing.

Rh D MONOCLONAL ANTIBODY REAGENTS

The first monoclonal IgG anti-D was an IgG antibody produced by Crawford et al (1983). Most workers experienced difficulties in stabilising the anti-D secreting cell lines which were Epstein Barr

Virus (EBV) transformed human B lymphocytes. Monoclonal IgG anti-D antibodies have not yet offered advantages over polyclonal anti-D reagents, as the yields are not economic and they miss weak D(D^u) and some D variants (Voak 1986).

However, progress has been made on stabilising anti-D secreting cell lines by fusing them with a human-mouse heteromyeloma (Bron et al 1984) or a mouse myeloma (Thompson et al 1986), and Thompson et al have produced an excellent IgM anti-D that is suitable for reagent use. The tissue culture supernatant of this IgM anti-D (MAD-2) has a saline test spin-tube titre of at least 1,000 with red cells of all the common RhD positive phenotypes and it is sufficiently potent for use by slide or immediate spin-tube tests.

Reactions of Monoclonal Anti-Ds with Weak D and D Variants

The data in Table 6 shows the behaviour of various monoclonal anti-D antibodies with D variants and weak Ds. The IgM anti-D of Lowe et al, 1986, is not as potent as MAD-2, but is scientifically interesting as it misses a Category IV variant detected by MAD-2.

Table 6. Spin-Tube Test Reactions of IgM Anti-D Monoclonals with D Variants and Weak D (D^u) Red Cells

Conventional Anti-D								
% Positive		100	96	74	35	97		
Category		III	IV	V	VI	VII	$R_1{}^u r$	$R_2{}^u r$
Number tested		2	1	1	3	1	9	7
HD7 (IgM)	Saline	V	0	V	0	+	0/W	0/++
	Papain	C	0	C	++/C	+	+/V	++/C
MAD-2 (IgM)	Saline	V	C	+	0	0	0/++	0/V
	Papain	C	C	C	0	+	++/V	++/V
D4 (IgG1)	Papain	+++	+++	+++	0	+	0/++	0/++
GAD (IgG3)	Papain	+++	0/W	0/W	0	+	N/T	N/T

? Category IV is D^{abCD}, so HD7 detects D^A and/or D^B.

Monoclonal anti-Ds showing different patterns of reaction with the various categories of D variants (Tippett and Sanger 1962, Race and Sanger 1975) undoubtedly provide an avenue of research to help elucidate the fine structure of the D antigen complex.

The failure of IgM anti-Ds to agglutinate some weak D (D^u) and D variant types is a limitation of an IgM only type of reagent. MAD-2 gave 79 (0.5%) discrepant results out of 14,680 Rh D typing tests using a microplate method in our Cambridge pre-natal testing

laboratory in comparison to an albumin anti-D reagent. The activity of IgM anti-Ds can be enhanced by using 2-stage enzyme treated cells (Table 6) but this would mean setting up a second test and is time consuming.

A New Approach to Anti-D Reagent Production

MAD-2 IgM anti-D was shown to be able to agglutinate R_1r red cells, even though they were previously sensitised with high levels of IgG anti-D (Table 7). Thus MAD-2 with a saline titre of 1,024 is far more resistant to the blocking action of IgG anti-D than a polyclonal IgM anti-D that had a typical titre of only 16.

Table 7. Blocking of High Titre IgM Anti-D (MAD-2)
I. By Prior Sensitisation With IgG Anti-D

	2 Volumes IgG Anti-D : 1 Volume 3% R_1r RBC Level (IU/ml) of IgG Anti-D (GD110)				
	125	62	31	15	Saline Control
IgG Anti-D Sensitised R_1r	0	0	0	0	0
2nd Stage IgM Anti-D added*					
Polyclonal (2300)	0	Weak	$+^{W}$	$+^{W}$	++
Monoclonal (MAD-2)	0	+	++	+++	++++

*1 Volume IgM anti-D added to sensitised R_1r cell button after removal of IgG anti-D supernatant.

Therefore a new anti-D reagent approach was possible by blending the monoclonal MAD-2 IgM anti-D with a small amount of selected polyclonal IgG anti-D. This reagent blend was designed to provide a single anti-D reagent that could detect normal RhD positives by simple saline tests and only use an enhancement test, antiglobulin (AHG) test for the detection of weak D types in donor blood typing as we considered it unnecessary for patient Rh typing except in the case of discrepant results (Moore 1984, Voak 1986).

The final MAD-2 blend with IgG anti-D (IgG fraction) strongly agglutinated all normal Rh D phenotypes and most variants (except Category VI) by saline tests, even using rapid immediate spin techniques. The reagent had a saline titre (5 minute spin-tube) of 1,024 x R_1 (pool of 4) red cells and the results of saline and AHG tests with weak D (D^u) samples are shown in Table 8. The amount of IgG anti-D fraction added was just sufficient to give reliable AHG test detection of Weak D (D^u) red cells and did not reduce the saline test agglutination of the neat reagent with R_1r cells (weakest common Rh D positive phenotype). A minor blocking of weakly saline agglutinated D^u types occurred on incubation after 15 minutes at 37°C, but prolonged incubation does not reduce the saline agglutination with R_1r red cells.

Table 8. Tests for Weak D(D^u) With an IgM (MAD-2) + IgG Anti-D Blend*

	1 : 1 vols - Spin-Tube Tests X 3% RBC						
	Weak D(D^u) Samples						
	1	2	3	4	5≠	Controls	
Method	$R_1^u r$	$R_2^u r$	$R_2^u r$	$R_2^u r$	$R_1^u r$	R_1^r	rr
Immediate Spin							
Saline	wk	+	wk	+++	(+)	++++	0
15 min 37°C							
Saline	0	+	0	+++	wk	++++	0
AHG	+++	+++	+++	N/T	+++	N/T	0

* Saline titre 1,024 x R_1r (Pool of 4)

≠ Previously grouped as R_1r with an albumin anti-D.

Thus this new approach to anti-D reagents using a high titre IgM monoclonal anti-D represents a considerable improvement over conventional IgG anti-D reagents as it simplifies routine Rh D typing work. The expected incidence of discrepant types in the saline test phase of about 0.5% is regarded as acceptable and can be dealt with by using the antiglobulin phase in the same tube.

MN MONOCLONAL ANTIBODY REAGENTS

The production of mouse monoclonal anti-MN reagents by many workers (Frazer et al 1982, Sonneborn et al 1984, 1987, Wasniowska et al 1985, Nichols et al 1985, Fletcher et al 1986, Rubocki and Milgrom 1986) has overcome the problems of short supplies of MN reagents. However, red cell serologists must not assume that monoclonal anti-M/N reagents will behave as reliably as conventional reagents in physiological saline. Experience has shown that each monoclonal anti-M or anti-N must be carefully standardised to identify its optimum pH as well as the degree of crossreaction at various temperatures with the various MN phenotypes.

Monoclonal anti-M/N antibodies are mainly IgG types but some are IgM (Wasniowska et al 1985). The ability of IgG anti-M/N to cause agglutination in saline tests is due to the high number of M, N, "N", sites on red cells, as shown for the various phenotypes in Table 9 (calculated from data reviewed by Anstee 1983).

Table 9. MNSs Glycoproteins - structure and site density

Chain	α (Glycophorin A)			δ (Glycophorin B)
Antigens	M N			"N" S s
	Genotype			
No. of sites on each chain	MM NN	1,000,000		"N" 250,000 30% more if S+
	MN	500,000		
N + "N" α	NN MN	1,250,000 750,000	if S+	1,325,000 825,000

The M and N genes control the production of M and N on the α sialoglycoprotein, but an "N" (N-like) structure is also produced in the absence of an N gene on the δ sialoglycoprotein. This "N" structure is responsible for the "crossreaction" of anti-N reagents with the δ "N" on the red cells of people of the homozygous genotype MM. Furthermore MM people who are also S positive have more "N" than MMss types as S positive people have about 30% more δ (Dahr et al 1978).

The effect of trypsin and chymotrypsin is also useful in the characterisation of anti-M and anti-N reagents as they cleave the α and δ chains respectively (Dahr et al 1975).

Anti-N Reagents

Because anti-N antibodies detect δ "N" it is only possible to make anti-N reagents "specific" by dilution, as shown in Table 10. These reagents are only specific at selected dilutions for detecting the product of the N gene by appropriate agglutination tests. However, these reagents are not specific in absorption and elution tests as the antibodies still bind to "N" antigens. The reagent's specificity in agglutination tests is a function of the amount of antibody and the relative antigen site density. Thus, at a suitable dilution there is insufficient IgG anti-N to agglutinate red cells of relatively low site density such as MM with only δ "N" sites.

Table 10. Monoclonal Anti-N Reagents - "Specific" by Dilution

	Spin-Tube Titres RT at pH 8.5 Monoclonal Anti-N			
	BS 75 Ac.	BS 41 Ac.	NM 00286 Sup.	N3 Sup.
Source	Sonneborn		Moulds	Fletcher
Dilution "Specific"	4,000	500	32-64	3
3% RBC				
NN	128,000	4,000	256	16
MN	32,000	4,000	128	8
Controls*				
MMSS	1,024	256	16	1
MMSs	1,024	256	16	2
MMss	256	128	16	<1

*S+ show greater cross reaction - 30% more δ "N"

Three of the anti-N antibodies gave greater "N" reactions with S positive than S negative MM red cells. Therefore it is important to use S positive MM red cells as the negative control test for standardisation of the "specific" anti-N reagent dilution and also for N typing.

Chymotrypsin Effect on δ "N" is Reduced in ss Genotypes

The effect of chymotrypsin for destroying δ "N" reactions gave surprising results (Table 11) as some reactions were enhanced, presumably because enzyme enhanced agglutination occurs if the antigens involved are not destroyed by the enzyme.

Table 11. Ss Type Affects δ "N" Reaction With Chymotrypsin

	Titration Scores Spin-Tube RT at pH 8.5 Monoclonal Anti-N					
	BS 75		BS 41		NM 100286	
	Sal.	Chymo-trypsin	Sal.	Chymo-trypsin	Sal.	Chymo-trypsin
3% RBC						
MMss	69	74	40	31	22	40
MMSs*	83	33	48	0	23	16

*SS gave similar results

Thus, the δ "N" of MMss (S negative) red cells appears resistant to the action of chymotrypsin whereas it is reduced or destroyed in S positive red cells. It is possible that the development of S on δ causes unfolding of the sialoglycoprotein which reveals sites sensitive to chymotrypsin.

Anti-M Reagents

The M antigen on red cells is all the end product of the M gene. Therefore it is possible to have specific anti-M reagents that do not react at all with N. A good example of a specific anti-M antibody is BS57 (Table 12) which did not agglutinate and was not absorbed by NN red cells. Its failure to react with the M^C variant suggests glycine is an important part of the M antigen (Dahr 1977) recognised by this monoclonal anti-M. A specific anti-M monoclonal antibody that also detects M^C does exist (J. Moulds, personal communication).

Table 12. A Specific Monoclonal Anti-M (BS 57)

	Spin-Tube Titres		RT at pH 8.0
	Saline	Trypsin Destroyed	Chymotrypsin Enhanced
3% RBC			
MM	16,000	0	64,000
MN	16,000	0	64,000
M^VN	16,000	0	64,000
M^CN	0	0	0
Control			
NNSs	0	0	0

Amino acid sequence

Antigen	Position 1		Position 5
M	Serine	M^C	Glycine
N	Leucine		Glutamic acid

Other monoclonal anti-M antibodies that show some crossreaction with N but greater reactions with M, can, like anti-N reagents, be made specific by dilution, as shown by the example with BS 44 in Table 13. This "specific" anti-M reagent fails to detect M^C red cells which gave only the same reactivity as NN red cells. The reactions of BS 44 are enhanced with M-positive red cells suspended in 5% albumin. However, the reaction with NN red cells is not enhanced, perhaps because the crossreacting antigen site density is much lower than that of the M sites.

Table 13. Monoclonal Anti-M BS 44 - Specific by Dilution

	Spin-Tube Titres RT PH 8.0		
	Saline	5% Albumin	Trypsin
3% RBC			
MM	128,000	256,000	2,000
MN	64,000	256,000	2,000
M^VN	128,000	256,000	N/T
M^CN	2,000	4,000	N/T
NN (3)	1,000-4,000	4,000	128-1,024

Dilution to 8,000 - Negative with NN - Is also negative with M^C red cells.

The data in Table 14 shows a third type of anti-M (BS 38) that only behaves as a specific anti-M with neuraminidase treated red cells, while it gave greater reaction with N than M in other types of tests. This suggested that the N reaction was sialic acid dependent (Bird and Wingham 1970) on the α chain as it was not destroyed by chymotrypsin.

Table 14. A Monoclonal Anti-N>M (BS 38) - Which is an Anti-M With Neuraminidase Treated Red Cells

	Spin-tube titres RT at pH 5.0			
	Saline	Neuraminidase	i Chymotrypsin ii Trypsin	Chymotrypsin
3% RBC				
MM	4,000	512 - 1,000	128 - 4000	16,000
MN	8,000	128	8,000	16,000
M^V	N/T	128	N/T	N/T
M^CN	N/T	128	N/T	N/T
NN	16,000	0	16,000	32,000

Trypsin Sensitivity of MN Antigens (α chain)

The results of our studies confirmed the trypsin sensitivity of M reactions using the monoclonal anti-Ms, BS 57 and BS 44. However, with BS 38 the crossreactive anti-N>M antibody demonstrated trypsin resistant MN epitope(s) on the α chain. The possibility that these

reactions were due to crossreaction with "N" were excluded by using S positive red cells that were chymotrypsin treated prior to the trypsin treatment.

General Comments on MN Reagents

Correctly standardised monoclonal anti-M and anti-N reagents are excellent red cell typing reagents. Furthermore, we suggest that some monoclonal anti-M reagents eg BS 57 and BS 38 (with neuraminidase treated material) are sufficiently specific to be developed for forensic use, perhaps by ELISA techniques.

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