Guidelines for the Blood Transfusion Services

11.2: Specifications, performance evaluation and quality control of blood grouping reagents

http://www.transfusionguidelines.org/red-book/chapter-11-reagent-manufacture/11-2-specifications-performance-evaluation-and-quality-control-of-blood-grouping-reagents

11.2: Specifications, performance evaluation and quality control of blood grouping reagents

11.2.1: Blood typing antisera

11.2.1.1: General requirements

- It is essential that blood grouping reagents are prepared using reliable manufacturing procedures that are consistently capable of producing safe and efficacious products. The products must comply with requirements of the EU Directive (98/79/EC) on *in vitro* diagnostic medical devices and other relevant international standards detailed in section 11.3.
- The term weak D is used in these guidelines to indicate a weakened expression of a normal D antigen. The term partial D is used in these recommendations to indicate the expression of only a part of the normal D antigen. The reactivity of RhD blood grouping reagents against partial D red cells is determined by the nature of the D variant, the anti-D reagent and the technique used.
- Red cell samples with partial antigen expression (e.g. partial D) or weak antigen expression (e.g. A_X) may not react with some reagents and, where this is known to be true, must be stated in the limitations.
- The blood grouping reagent is satisfactory if an unequivocal positive result is obtained with all the red cell samples having the antigen corresponding to the blood grouping reagent being assessed, by all the methods recommended for use by the manufacturer.
- If reactivity is claimed by the manufacturer against weak variants or subgroups of a particular antigen, red cells from at least two confirmed/reference samples should be tested (see Table 11.3).

The grading system shown in Table 11.2 is used throughout these guidelines for manual tube/microplate serological testing.

Table 11.2 Grading system for serological tests

Reaction grade	Description
Grade 5	Cell button remains in one clump or dislodges into a few large clumps
Grade 4	Cell button dislodges into numerous large clumps
Grade 3	Cell button dislodges into many small clumps
Grade 2	Cell button dislodges into finely granular but definite, small clumps
Grade 1	Cell button dislodges into fine granules
Grade 0	Negative result
Unless otherwise stated, an unequivocal manual tube reaction is defined as a grade 3 or greater and for column tests as 1+ or greater	

11.2.1.2: Performance evaluation

Performance evaluation should be undertaken in accordance with:

- BS EN 13612:2002 Performance Evaluation of *In Vitro* Diagnostic Medical Devices.
- Reagents listed in Annex II, List A, of the EU *In Vitro* Diagnostic Medical Devices Directive must also comply with the Common Technical Specifications for *In Vitro* Diagnostic Medical Devices (2009/108 /EC).

Stability testing should be performed in accordance with BS EN ISO 23640 *In vitro* diagnostic medical devices. Evaluation of stability of *in vitro* diagnostic reagents.

Where appropriate, the following requirements should also be included in performance evaluation:

- In the case of polyclonal antibodies, contaminating antibodies to antigens having a prevalence of greater than 99% in the general population of the UK should be excluded. Negative results in tests using samples of red cells from four different individuals who lack the antigen corresponding to the antibody specificity under test. Tests for the presence of contaminating ABO antibodies should be performed with red cells from a minimum of two individuals of group A1 and two of group B who lack the antigen corresponding to the antibody specificity of the reagent but have the antigens to the potential contaminating antibodies should be obtained.
- If tests using all methods recommended for use by the manufacturer do not exclude the presence of antibodies to the following antigens, these antibody specificities should be stated in the package insert2 as not having been excluded in specificity testing:
    - Xg^a, Do^a, Yt^b, Co^b, Wr^a and V^w.

- Blood grouping reagents which are chemically modified, and/or contain in their formulation a potentiator of agglutination, or require the user to add a potentiator, shall be tested, by all methods recommended by the manufacturer, with red cells lacking the antigen corresponding to the antibody specificity under test but sensitised with an IgG antibody to effect a grade 5 reaction in the anti-human globulin technique.

- Potentiated blood grouping reagents producing agglutination by those methods recommended by the manufacturer, should be supplied with a reagent control that has been shown to effect a degree of non-specific reaction with IgG-coated red cells similar to the corresponding blood grouping reagent.

- Blood grouping reagents recommended for use by a direct agglutination method should not contain antibodies reactive against red cells coated with IgG when used by direct agglutination methods recommended by the manufacturer.

11.2.1.3: Batch release testing requirements

Specificity tests

- The manufacturer must provide a certificate of analysis to customers once evidence has been obtained by the manufacturer that the product achieves the specificity and reactivity claimed by the manufacturer for each method recommended by the manufacturer, Assurance of Specificity should be determined in accordance with the requirements in Table 11.3. The certificate of analysis should also ensure that the potency of the material meets the requirements of the final bullet point on Potency below.

- If a range of incubation times or incubation temperatures is recommended by the manufacturer, the range(s) should be used in these test procedures.

Requirements

- Blood grouping reagents should not produce a positive reaction when tested with red cells lacking the antigen corresponding to the antibody specificity under test, by any method recommended for use by the manufacturer. Should reactivity to a low-frequency antigen be observed with subsequent batches of a reagent, this fact should be brought to the attention of all primary consignees of that reagent.

- Rouleaux formation, prozone or haemolysis should not occur in tests using any of the methods recommended by the manufacturer.

Potency tests – tube or microplate methods

- Potency titrations should be performed in accordance with the manufacturer's recommended method of use using an appropriate diluent.

- Manufacturers should compare the potency titre of each batch of reagent with an appropriate reference preparation (see section 11.3).

- Potency titrations for each batch tested should equal or exceed any existing British or International reference preparations.

Table 11.3 Requirements for conventional blood typing reagents

		Performance evaluation	Batch release testing	
			Specificity	Potency

Antibody specificity	Specification	(as a minimum, two examples of the following reference cells should be included if available)*	Positive reactors		Negative reactors			
			Cell type	No.	Cell type	No.	Cell type	No.
anti-A	Normally blue coloured Should equal or exceed potency of reference preparation (s) Should detect variants and subgroups as detailed in the manufacturer's instructions for use	A_X,A_3	A_1	2	B	2	See insert of reference preparation(s)	
		A cord cells	A_2B	2	O	2		
			A_X*					
anti-B	Normally yellow coloured Should equal or exceed potency of reference preparation (s) Should detect variants and subgroups as detailed in the manufacturer's instructions for use	B_X, B_3, B_v	B	2	A_1	2	See insert of reference preparation(s)	
		B cord cells	A_1B	2	O	2		
anti-A, B	Normally clear coloured Should equal or exceed potency of reference preparation (s) Should detect variants and subgroups as detailed in the manufacturer's instructions for use	A_1, A_2, B, A_1B, A_2B	A_1	1	O	4	See insert of reference preparation(s)	
		A_X A_3	A_2	2				
		B_x B_3	B	2				
		A and B cord cells	A_X	2				

anti-A1	Normally clear coloured Should detect variants and subgroups as detailed in the manufacturer's instructions for use		A_1	2	A_2	2	A_1	2
			A_1B	2	A_2B	2		
					B	2		
					O	2		
anti-D	Normally clear coloured Should equal or exceed potency of reference preparation (s) Should detect variants and subgroups as detailed in the manufacturer's instructions for use	Weak D (500 sites /cell)	R_1r	2	r'r	1	See insert of reference preparation(s)	
			R_2r	2	r''r	1		
		D^{VI} type 1, D^{VI} type 3, D^{IV}, D^{V}, D^{VII}, DFR, DBT, R_0^{Har}	Weak D*	2	rr	1		
anti-C	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	C^w, C^x, r'^S	R_1r	1	R_2 R_2	1	R_1r	2
		R_2R_Z	R_1R_2 R_1R_Z	1 1	r''r	1		
			r'r	1	rr	1		
anti-E	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in manufacturer's instructions for use	R_1R_Z	R_2r	1	R_1R_1	1	R_2r	2
		E^w	R_1R_2	2	r'r	1		

	Should detect variants and subgroups as detailed in the manufacturer's instructions for use		r''r	1	rr	1		
anti-c	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	R_1R_Z, $R_1{}^wR_1$	R_1r	2	R_1R_1	3	R_1r	2
			R_1R_2	1				
			r'r	1				
anti-e	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	R_2R_Z	R_2r	2	R_2R_2	3	R_2r	2
			R_1R_2	1				
			r''r	1				
anti-C^w	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use	$R_1{}^wR_1$,r'^{w}r, $R_1{}^wr$	$R_1{}^wr$ or	2	R_1r	1	$R_1{}^wr$	2
			$R_1{}^wR_2$	2	R_1R_1	1		
			r'^{w}r	1	r'r	1		

anti-K	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	K+k+ Kp (a+b+)	K+k+	4	K–k+	4	K+k+	2
		K+k+ Kp (a–b+)						
anti-k	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	K+k+ Kp(a+)	K+k+	4	K+k–	4	K+k+	2
			Kp(a+b+)	2				
			K+k+ Kp(a–)	2				
anti-Fy^{a}	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Fy(a+b+)	4	Fy(a–)	4	Fy(a+b+)	2

anti-Fy^b	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	Fy^x	Fy(a+b+)	4	Fy(b–)	4	Fy(a+b+)	2
anti-Jk^a	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Jk(a+b+)	4	Jk(a–)	4	Jk(a+b+)	2
anti-Jk^b	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Jk(a+b+)	4	Jk(b–)	4	Jk(a+b+)	2

anti-S	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	S+s–, S+s+, S–s+	S+s+	4	S–s+	4	S+s+	2
anti-s	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		S+s+	4	S+s–	4	S+s+	2
anti-M	Normally clear coloured Potency titre greater than 2 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	M–N+ He+	M+N+	4	M–N+	4	M+N+	2

anti-N	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		M+N+	4	M+N–	4	M+N+	2
anti-P1	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		P1+ strong	4	P1–	4	P1+	2
			P1+ weak	4				
anti-Le^a	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Le(a+b–)	4	Le(a–)	4	Le(a+)	2

anti-Le^b	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	A_1B Le(a–b+)	A_1B Le(a–b+)	4	Le(b–)	4	Le(b+)	2
'Other'	Normally clear coloured Potency titre greater than 2 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Heterozygous positive	4	Antigen negative	4	Heterozygous positive	2
* For reagents where reactivity against the antigen is claimed								

For batch acceptance testing the user must ensure that the typing reagent reacts with the weakest available antigen expressing cells (refer to batch release positive reactors in table 11.3) and does not produce false positives with cells negative for the antigen.

11.2.2: Anti-human globulin reagents

11.2.2.1: Introduction

Monoclonal antibodies have been developed which necessitate revision of the optimal composition of anti-human globulin reagents. For example, because of the limitations imposed by the presence of C3d on normal red cells, particularly in stored blood, conventional polyclonal anti-complement reagents rely on anti-C3c to detect *in vitro* bound complement and limited amounts of anti-C3d to detect *in vivo* bound complement. However, some monoclonal IgM anti-C3d reagents can be used at concentrations adequate to detect both *in vitro* and *in vivo* bound complement without causing unwanted positive reactions with normal red cells and fresh, inert, group-compatible serum in routine tests.

11.2.2.2: General requirements

- anti-IgG is the essential component since the majority of red cell alloantibodies are non-complement binding IgG.
- anti-complement should be present in reagents recommended for use with serum test samples.
- anti-light chain activity is desirable in reagents recommended for use with plasma test samples in order to detect IgM antibodies at levels unable to be detected in direct agglutination tests, especially with washed red cells.
- anti-C4d must be avoided. It is accepted that very low titres of anti-C4c may occur in reagents of animal origin.
- Reagents should be tested for the presence of heterospecific antibodies which can cause haemolysis or agglutination of unsensitised red cells in the indirect antiglobulin test and for the presence of unwanted positive reactions.

11.2.2.3: Performance evaluation

Performance evaluation should be undertaken in accordance with:

- BS EN 13612:2002 Performance Evaluation of *In Vitro* Diagnostic Medical Devices.
- Reagents listed in Annex II, List A, of the EU *In Vitro* Diagnostic Medical Devices Directive must also comply with the Common Technical Specifications for *In Vitro* Diagnostic Medical Devices (2009/108 /EC).

Stability testing should be performed in accordance with:

- BS EN ISO 23640 *In vitro* diagnostic medical devices. Evaluation of stability of *in vitro* diagnostic Reagents.

11.2.2.4: Batch release testing requirements

Specificity testing

Tests for IgM or IgG red cell heterospecific antibodies

- Heterospecific antibodies can cause haemolysis or agglutination of unsensitised red cells in the indirect antiglobulin test. Details of tests for heterospecific antibodies are outlined in section 11.4.

Requirements

- The anti-human globulin reagent should not agglutinate or haemolyse washed unsensitised red cells from two individuals of group A1 RhD positive, two individuals of group B RhD positive and two individuals of group O RhD positive, whether or not treated with proteolytic enzyme (e.g. papain, bromelin or ficin).

Tests for unwanted positive reactions

- These test for excess anti-C3d and anti-C3c, which can cause unwanted positive reactions in the indirect antiglobulin test, and for the presence of any undesirable antibodies in the reagent. Details of tests are outlined in section 11.4.

Requirements

- All reactions should be negative on macroscopic examination.

anti-IgG potency: polyspecific anti-human globulin and anti-IgG reagents for use in tube or microplate techniques

- The anti-human globulin reference reagent should be tested in parallel with the test reagent, each being titrated against red cells sensitised with potent IgG anti-D antibody.

Requirements

- The potency titre of the test anti-human globulin or anti-IgG reagent should be at least equal to that of the reference reagent.

Potency tests

anti-IgG potency by chequerboard titration studies with red cells sensitised with weak IgG antibodies (anti-D, anti-K and anti-Fy^{a})

- Test anti-human globulin or anti-IgG reagents against a selection of weak antibodies to determine the optimum potency. Antibody preparations should not be diluted and the use of single-donor antibody preparations is preferred. Antibodies should include:
    - an IgG anti-D to give an anti-human globulin potency titre of 8–32 using a pool of group O R_1r red cells from four individuals
    - an IgG to give an anti-human globulin potency titre of 8–32 using Kk red cells
    - an IgG anti-Fy^{a}, to give an anti-human globulin potency titre of 8–32 using Fy(a+b+) red cells.

Details of tests are outlined in section 11.4.

Requirements

- The anti-human globulin reagent or anti-IgG reagent is satisfactory if the reaction grade at all dilutions attains or exceeds that of the reference reagent without significant prozone, against red cells sensitised with all dilutions of the anti-D, anti-K and anti-Fy^{a}. In this context, a significant prozone is more than one grade difference between the reaction of the anti-human globulin reagent undiluted and 1 in 2.

anti-complement potency; polyspecific anti-human globulin reagents for use in tube tests

- Test anti-human globulin or anti-complement reagents against a selection of complement-coated red cells to determine the optimum potency. C3 and C4 complement-coated red cells should be prepared as described in section 11.4. In addition, anti-complement activity may be evaluated by tests with complement-fixing antibodies, such as anti-Jk$^{a.}$

Requirements

- The anti-human globulin reagent should have an anti-C4c titre of 1 in 2 or less.

- The anti-human globulin reagent should not affect a macroscopic reaction with EC4d red cells.

- The reagent should attain the potency titre of the reference reagent.

- Conventional (polyclonal) anti-human globulin or anti-human globulin containing monoclonal IgG anti-C3d that attain adequate reactivity with an optimal incubation period different from that recommended for the detection of IgG antibody, should state in the instructions for use the

appropriate incubation period required for the optimum detection of red cell bound C3c/d complement components.

Tests for unwanted positive reactions

- These test for excess anti-C3d and anti-C3c, which can cause unwanted positive reactions in the indirect antiglobulin test, and for the presence of any undesirable antibodies in the reagent. Details of tests are outlined in section 11.4.
- All test results should be negative as defined by the manufacturer in the ‘instructions for use’.

Instructions for use

The instructions for use for anti-human globulin reagents used in tube and microplate tests should also include a statement that:

- Inadequate washing of red cells in the anti-human globulin test may result in neutralisation of the anti-human globulin reagent.
- Following completion of the wash phase in the anti-human globulin test, excess residual saline may dilute the anti-human globulin reagent, when added, beyond that in the manufacturer’s assessment.
- No single test is capable of detecting all clinically significant antibodies.
- For each batch of antibody screening being undertaken by an anti-human globulin test, a positive and negative control should be included. The positive control should be a weak anti-D (not more than 0.1 IU/mL); the negative control an inert serum, tested against the antibody screening cells being used.

11.2.3: Reagent red cells

11.2.3.1: Introduction

Reagent red cells prepared from human blood are essential in ensuring safe transfusion practice. They are used in the determination of ABO blood groups, in the control of blood grouping reagents and of the anti-human globulin technique, and in the detection and identification of atypical red cell alloantibodies.

11.2.3.2: General guidelines for reagent red cell manufacture

- When testing reagent red cells, in order to confirm the presence or absence of antigens listed in the antigen profile, a sample from each individual should be tested wherever possible, with a minimum of two antisera for each specificity prepared from different donors/cell lines.
- Where such testing produces conflicting results, repeat and further testing with at least one additional example of the relevant antibody(ies) should be undertaken to confirm the antigenic status of that cell.
- Where such testing has been performed with only one example of any blood grouping reagent, this information should be stated in the antigen profile included within the package insert.
- Reagent red cells should be shown not to produce unwanted positive reactions by the methods recommended for use by the manufacturer.

- Except for IgG-sensitised and C3-sensitised red cells, reagent red cells should be negative in the direct anti-human globulin technique with anti-IgG and polyspecific anti-human globulin reagents.
- With the exception of umbilical cord blood, alloabsorption and quantification cells, red cells used to test a patient's samples for atypical antibodies should not be pooled.
- Reagent red cells should be processed by a method and suspended in a medium that consistently ensures stability of the antigens specified in the antigen profile included within the package insert.
- With the exception of controls for automated systems representing whole blood, all red cell reagents should be free of ABH-specific blood group substances and blood group antibodies, including anti-A and anti-B, demonstrable by the manufacturer's recommended methods of use.
- The method of manufacture should ensure that white cells are removed from donations of red cells before the white cells lyse and release enzymes, which may adversely affect the properties of the red cells.

11.2.3.3: Immediate container label and/or instructions for use sheet

The immediate container and instructions for use sheet for reagent red cells should also meet the following criteria:

- Include a statement regarding the use of 'pooled cells', if cells are prepared from pooled material.
- Where reagent red cells are intended for use in ABO grouping or control of ABO or D blood grouping reagents, only the ABO and D group need be stated.
- When the reagent red cells are a multi-container product such as a red cell panel, the label on the immediate containers and packaging should be assigned the same identifying batch reference and carry a number or symbol to distinguish one container from another. This number or symbol should also appear in the antigenic profile.
- The date of expiry of reagent red cells should be stated on the antigenic profile.
- Where reagent red cells are provided suspended in preservative medium, the components of the medium should be stated in the instructions for use.
- The concentration and limits of the red cell suspension should be stated in the instructions for use.
- For enzyme-treated reagent red cells, information should be given in the instructions for use concerning those antigens which are rendered inactive or less active by the enzyme treatment used.

11.2.3.4: Reagent red cells for use in ABO and RhD grouping

- Reagent red cells should be groups A_1 and B. In addition, A_2 or O red cells may be included.
- At least one of the set should be RhD positive and one RhD negative.

11.2.3.5: Reagent red cells for use in antibody screening

The detection of atypical antibodies in the serum of a patient is of greater clinical significance than if such antibodies are detected in blood donors. Reagent red cells of a lesser specification may be used when performing antibody screening tests on blood donor samples.

In general the following should apply:

- Reagent red cells for use in antibody screening should be confirmed as group O by an ABO blood grouping procedure that is capable of demonstrating the A*x* phenotype.
- Where practicable, reagent red cells known to express antigens having a frequency of less than 1% in the general population of the UK should not be included in reagent red cells for antibody screening.
- Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA antibodies should not be used as reagent red cells for antibody screening of patients.

11.2.3.6: Reagent red cells for use in antibody screening of patient samples

- As a minimum the following antigens should be expressed on the reagent red cells for antibody screening:

  C, c, D, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P_1, Le^a and Le^b

- As a minimum, reagent red cells from two individuals should be provided. These red cells should not be pooled. One reagent red cell should be R_2R_2; the other R_1R_1 (or $R_1{}^wR_1$).
- Apparent homozygous expression of the following antigens is desirable:

  Fy^a, Fy^b, Jk^a, Jk^b, S and s

11.2.3.7: Reagent red cells for use in antibody screening of patient samples who have received prophylactic anti-D

- For pregnant patients who have received prophylactic anti-D, as a minimum the following antigens should be expressed:

  c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P_1, Le^a and Le^b

- The cells must be RhD negative
- As a minimum, reagent red cells from two individuals should be provided. These red cells should not be pooled.
- Apparent homozygous expression of the following antigens is desirable:

  Fy^a, Fy^b, Jk^a, Jk^b, S and s

11.2.3.8: Reagent red cells for use in antibody screening of donor samples

- Reagent red cells may be:
  - provided unpooled from a minimum of two individuals OR
  - as a pool of red cells in equal proportions from no more than two donors OR
  - red cells from a single donor.
- Pooled reagent red cells for antibody screening should be used only for testing samples from blood donors, not samples from patients.
- As a minimum the following antigens should be expressed:

  D, C, c, E, e and K.

- To enhance the antigens of these screening cells they may be treated by proteolytic enzymes.

11.2.3.9: Reagent red cells for use in antibody identification

- Reagent red cells for use in the identification of atypical antibodies should be confirmed as group O by an ABO blood grouping procedure which is capable of demonstrating the A*x* phenotype.

- Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA antibodies should not be used in reagent red cells for antibody identification.

- The antigen profile of reagent red cells for antibody identification should permit the identification of frequently encountered antibodies (e.g. anti-D, anti-E, anti-K and anti-Fya), and of commonly encountered alloantibody mixtures (e.g. anti-D+K).

- A red cell antibody identification panel comprises cells from eight or more individuals which should between them express the following antigens:

  C, C^w, c, D, E, e, K, k, Kp^a, Fy^a, Fy^b, Jk^a, Jk^b, S, s, Le^a, Le^b, M, N, P_1 and Lu^a.

- Red cells from one individual should be R_1R_1 and from another $R_1{}^WR_1$ and between them should express the antigens:

  K, k, Fya, Fyb, Jka, Jkb, S and s.

- Red cells from one individual should be R2R2, another r''r and those from another r'r.

- Red cells from a minimum of three individuals should lack the Rh antigens C, E and D. One of these three individuals should be K positive. Between them, red cells from these individuals should exhibit apparent homozygous expression of the antigens:

  c, k, Fy^a, Fy^b, Jk^a, Jk^b, S and s.

11.2.3.10: Reagent red cells (IgG-coated) for use in the control of the anti-human globulin technique

- To ensure that the anti-IgG activity in negative antiglobulin tests has not been fully or partially neutralised, control red cells 'sensitised' with IgG antibody are added to negative tests.

- Group O RhD positive red cells are sensitised with sufficient anti-D to render an indirect antiglobulin test negative when a volume of these sensitised red cells and a volume of serum diluted 1 in 1000 are added, but remains positive if a volume of saline instead of diluted serum is added.

11.2.3.11: Reagent red cells for use in antibody in antibody strength determination (other than anti-D and anti-c)

Reagent red cells for use in the identification of atypical antibodies should be confirmed as group O by an ABO blood grouping procedure which is capable of demonstrating the A_X phenotype.

- As a minimum, reagent red cells from two individuals should be provided

- These red cells should not be pooled

- One D+ C+ E+ c- e+ (R_1R_Z)

- One D+ C+ E+ c+ e+ (R_1R_2)
- Between them will show heterozygous expression of the following antigens: M, N, S, s, K, k, Fy^a, Fy^b, Jk^a and Jk^b
- They will be negative for Wr^a

11.2.3.12: Reagent red cells for use in patients with pan-reactive autoantibodies to determine the presence of underlying alloantibodies

Reagent red cells

- may be provided un-pooled OR as a pool of red cells from more than one donor
- should be selected to ensure the ID of underlying clinically significant antibodies to the following specificities;
    - C, C^W, c, D, E, K, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N
- an R_2R_2 cell may be included to exclude the presence of an underlying allo anti-e in e negative patients
- Rh (D, C, E, c, e), K, Jk^a and Jk^b phenotypes must be provided for the users as a minimum.
- The cells may be enzyme treated which would result in them being negative for the following red cell antigens:
    - M, N, S, s, Fy^a and Fy^b

11.2.3.13: Reagent red cells for quantification of anti-D and anti-c antibody strength

Reagent red cells for use in quantification techniques should be confirmed as group O by an ABO blood grouping procedure which is capable of demonstrating the A_X phenotype.

Reagent red cells

- may be provided unpooled OR as a pool of red cells from more than one donor
- For anti-D quantification have the following phenotype: D+ C+ E– c– e+ (R_1R_1)
- For anti-c quantification have the following phenotype: D– C– E– c+ e+ (rr)
- The cells should be negative for C^W and K antigens
- The cells should be enzyme treated to enhance the antibody-antigen reaction»

11.2.3.14: Other reagent red cells

These reagent red cells should be manufactured in accordance with the general guidelines in section 11.2.3.2.

11.2.4: Miscellaneous reagents

11.2.4.1: Fetal calf serum and bovine serum albumin

When used in the formulation of reagents, fetal calf serum and bovine serum albumin should be obtained from a closed herd in the female line since 1980, in which no animal has been clinically suspected of having bovine spongiform encephalopathy (BSE), and which has not been fed rations containing ruminant-derived protein during that period.

Bovine albumin, usually supplied as a 20% or 30% solution, can be used as a constituent of a diluent for use in automated blood grouping antibody detection machines, for antibody quantification or as a potentiator in antisera, monoclonal reagents and anti-human globulin. When diluted and used in the system prescribed it should not cause:

- red cells to become T/Tk etc. transformed
- inhibition of antigen:antibody reactions
- false-positive reactions or rouleaux.

11.2.4.2: Proteolytic enzyme preparations

The activity of each batch of proteolytic enzyme should be assessed to ensure batch-to-batch consistency using a biochemical assay (e.g. azo-albumin technique).[5]

For manual antibody detection techniques, red blood cells treated with the enzyme should achieve activity comparable to that of the reference enzyme preparation 92/658 used with an anti-D of 2.5 to 3.5 IU/mL.

For automated antibody detection techniques for patient pre-transfusion samples red blood cells treated with the enzyme should readily detect a weak anti-D of no more than 0.1 IU/mL (e.g. NIBSC anti-D standard for assessing operator and test performance as described at www.nibsc.org).

For automated antibody detection techniques for donation testing the red blood cells treated with the enzyme should readily detect a weak anti-D of 0.5 IU/mL.

11.2.4.3: Water

The quality of water used in the production of a reagent should be adequate for that reagent. Ionic and non-ionic contaminants of water may interfere with components of reagents or may result in a conductivity or osmolality other than that intended. Water should have a conductivity of 1.0 µS/cm or less or a resistivity of 1.0 Mohm/cm or greater.

11.2.4.4: Saline

Saline is an isotonic solution containing 8.5 to 9.0 g/L NaCl (0.145–0.154 M) and should contain sufficient buffer to maintain pH 7.0 ±0.2 at 22 ±1°C during its shelf life.

11.2.4.5: Low ionic strength solution

The term low ionic strength solution (LISS) should not be used to denote a low ionic strength formulation other than that described by Moore and Mollison.6 LISS should not be used in place of preparations designed for a particular technology. LISS has the following properties:

- pH 6.5–7.0 at 22 ±1°C
- conductivity 3.4–4.0 mS/cm
- osmolality 285–305 mOsmol/kg.

The reactions obtained by an indirect antiglobulin test (IAT) with a weak anti-D and D positive cells suspended in LISS should be equal to, or better than, those obtained with the same cells suspended in saline and incubated at 37°C for 15 minutes.

11.2.4.6: Weak antibodies for use as controls in antibody investigation techniques

Weak antibodies, such as anti-D, -K, -Fy^a can be used to control antibody detection techniques using indirect antiglobulin methods.

To act as a wash control the weak anti-D positive control could be diluted in serum or plasma. If the diluent is saline/bovine serum albumin, the control test could be positive, even though the cell washing was sub-optimal and this should be noted in the package insert.

These weak antibodies should:

- when used undiluted give a grade 2–4 reaction with red cells with homozygous antigen expression and have a mean IAT titre of 4 with the same cells.

For weak anti-D the antibody activity should be expressed in IU/mL.

11.2.4.7: Antibodies and cells representative of patient samples i.e. whole blood controls to control automated systems

As a minimum two vials containing red cells and plasma combined:

- One sample to be RhD positive, the other RhD negative with anti-D in the RhD negative plasma
- Red cells of the same ABO/D group may be pooled
- The red cells must give an unequivocal positive reaction with the appropriate ABO/Rh D grouping reagents
- One sample will contain anti-K or other non-Rh antibody (the red cells for this sample must be antigen negative for the corresponding antibody)
- Anti-D and anti-K (or other non-Rh antibody) must give an unequivocal positive reaction with antigen positive red cells by IAT at 37°C
- The plasma component of the whole blood controls should be free of other blood group antibodies unless stated in the instructions for use

11.2.4.8: AB Serum

The reagent should be:

- prepared from a pool of human group AB plasma or serum
- IAT antibody screen negative
- Negative for rouleaux inducing properties by direct agglutination at room temperature and by IAT and enzyme techniques at 37°C

11.2.4.9: Dithiothreitol (DTT)

DTT can be used to alter the red cell membrane and/ or reduce the disulphide bonds of IgM molecules and can be supplied at different concentrations to treat red cells and plasma samples or reagents.

11.2.4.10: Reagents for use in assessing the amount of D positive red cells in a suspected fetomaternal haemorrhage (FMH) by flow cytometry

Fluorescently labelled monoclonal antibodies used as a group of reagents to accurately determine the size of an RhD positive fetal bleed in an RhD negative person.