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Article

Alloantibody Identification: The Importance of Temperature, Strength Reaction and Enzymes—A Pratical Approach

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Abstract: Red blood cell (RBC) alloimmunization and antibodies formation against non-self-antigen on red cells may occur after blood transfusion, pregnancies or other exposures. The RBC alloimmunization rates varies from 2% to 6% according to recent studies. The antibody screen is performed to identify or confirm the presence of antibodies in patient's serum or plasma, as a preoperative or pretransfusion tests. The antibody identification process and major crossmatch are provided to improve the safety of red blood transfusion administration. I report three examples associated with clinically significant single and multiple alloantibodies. Ultimately, I summarize key points for a clinical transfusion practice.

Keywords: red blood cell alloimmunization; antibody identification; transfusion safety

1. Introduction

Blood group antigens are sugars or proteins attached to red blood cell (RBC) membrane. Antibodies are immunoglobins produced by lymphocytes and plasma cells following antigen stimulation and found in serum or in body fluids. They can be demonstrated, serologically. Antibodies against red blood cells can be natural (e.g. Anti-A, Anti-B) or immune immunoglobulins (e.g. Anti-D or Anti-Fya). The former are IgM class with large molecular weight, capable of binding complement cascade and causing intravascular hemolysis, the latter are immunoglobulin IgG, capable of crossing the placenta and causing hemolytic disease of the fetus and newborn (HDFN) [1,2].

Some of blood group antibodies are agglutinins, whereas others are hemolysins. Many factors influence antigen-antibody reaction, such as temperature, incubation time, pH, ionic strength of environment, zeta potential between red cells, concentrations of antigen and antibody, effect of centrifugation [1,2].

Hemoagglutination occurs when IgM react with their corresponding red cell antigens, sensitization occurs when IgG react with their red cell antigens. The latter is not an observable reaction without potentiators such as Antihuman globulin (AHG) which contains IgG and complement fractions. Hemolysis is the result of antigen-antibody reaction that utilizes the complement proteins to mediate red cell membran attack and lysis [1,2].

The indirect antiglobulin (Coombs) test is used to determine whether serum or plasma contains IgG antibodies (antibody screen) and their identification. The sensitization of red cells is observed in vitro by incubating the red cells with the corresponding antibody from patient serum or plasma at 37 °C for 30 minutes, with or without potentiators (eg enzymes, low-ionic-strength solution) [1–4].

Antibody formation against non-self-antigen on red blood cells may occur after transfusions, pregnancies or other exposures. Multiple triggers for red blood cell alloimmunization include donor and recipient factors (eg ethnicity, clinical conditions) and red blood cell factors (eg antigen variants, immunogenicity) [5–9].

The RBC alloimmunization rates varies from 2% to 6% according to recent studies. The Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) which included 319,177 recipients, recognized that older age, female sex, Rh (D) negative status, hemoglobinopathies and autoimmune disorders were risk factors for alloimmunization [10].

RBC transfusion is a key component of therapy for medical and surgery patients and for chronic treatment of hematologic malignancies and hemoglobinopathies. After exposure to non-self-protein or non-self-carbohydrate of RBCs, B cells are activated from primary to secondary immune response. The former is T-cell independent, slow and weak response and the latter is T-cell dependent, strong and rapid response [9].

Considering the complex interaction between donor and recipient, genetics of blood group antigens and clinical conditions further studies are necessary to better analyze the risk factors for alloimmunization and improve the ability to supply compatible red blood units [6].

2. Materials and Methods

A type and screen sample, ethylenediaminetetraacetic acid (EDTA) anticoagulant, is commonly submitted to the blood bank with a request of units of red blood cells.

The antibody screen is performed by mixing patient's plasma or serum with three reagent red cells of blood type O, to detect non-ABO antibodies. These reactions are incubated, centrifuged and interpreted based on the degree of agglutination (from 0 to 4+, no reaction versus strong reaction).

A negative antibody screen suggests absence of antibodies or that they are undetectable. In case of positive antibody screen, an antibody panel of 11 reagent red cells of blood type O is provided to detect non-ABO antibodies. Assigning presumptive antibody specificity is useful to select appropriate red blood units and determine compatibility of donor red cells with patient's serum or plasma (major crossmatch).

The antibody identification is a multistep process [4,12]. Firstly, is important temperature evaluation of antigen-antibody reaction (for example, reaction at 37°C suggests clinically significant IgG antibody), strength reaction (for example, different scores of hemoagglutination may be due to multiple antibodies or antibody with dosage) and second, cross-out antibody properly.

More specifically, an exclusion procedure is based on observation of antigens present on RBC reagent of the panel with which patient serum or plasma did not react. For antibodies which demonstrate dosage (Rh excluding D, Kidd, Duffy, MNS blood groups) caution should be exercised when ruling out panel cells that have homozygous antigen expression.

Furthermore, cross-out antibody to the following antigen D, C, E, c, k, M, N, S, s, Fya, Fyb, Jka, Jkb is based on a minimum of two non-reactive cells with homozygous antigen expression, while exclusion of anti-K is based on one non-reactive cells with homozygous antigen expression or two non-reactive cells with heterozygous antigen expression [4,12].

Ultimately, it is important detect three antigen-positive cells to be reactive and three antigen negative cells to be non-reactive to assign presumptive antibody specificity ("Rule of Three"), match the pattern of reaction and confirm correlation with patient's phenotype (Figure 1) [4,12].

Autocontrol tests the patient's serum or plasma with own red cells to exclude autoantibodies, drug interaction, transfused cells sensitized with antibodies [4,12].

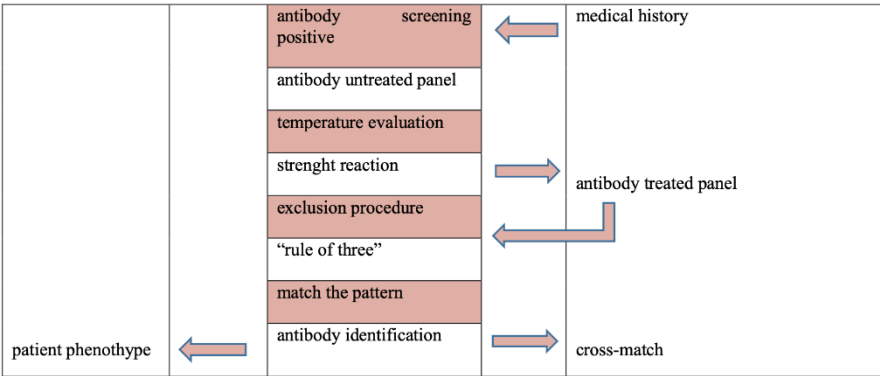


Figure 1. Antibody identification process.

3. Results

Example 1

Table 1 represents ABO/Rh type. Forward type of the patient’s sample using gel method (microtube filled with gel particles) and reagent Ant-A, Ant-B e Anti-A, B indicates that the patient’s group is A. Reverse type of the sample (using A1 cells, B cells and O cells) confirms that anti-B isoantibodies are detected in the plasma. Testing with reagent anti- D recognizes that the Rh(D) is present on the patient’s red cells. Therefore, patient’s group is A Rh-positive.

Table 2 summarizes antibody screen using gel method. Alternatively, the reaction may be performed using tube test or solid phase technology.

Table 3 summarizes antibody identification panel using gel test with untreated RBC reagents. *Anti-M antibody* can be identified and other clinically significant alloantibodies can be ruled out [11,12]. This antibody is active at 37°C and expresses a dosage effect. In fact, the reaction is stronger with homozygous M antigen cells than with eterozygous M antigen cells (M+N- reagent red cells versus M+N+ reagent red cells). Most antibodies to M antigen are cold reacting and they are not considered clinically significant. Dithiothreitol (DTT) is a reducing agent used to differentiate IgM and IgG reactivity of the antibody. This case, suggests a warm reacting alloantibody anti-M [13–16].

RBC units can be provided compatible through indirect antiglobulin test (IAT). In addition, serologic phenotype may confirms identification of the antibody. In case of pregnancy, IgG alloanti-M should be confirmed using different method such as tube test or solid phase technology and anti-M titer should be monitored for prevention of hemolytic disease of the fetus and newborn [17].

Table 1: AB0/Rh

ABO/Rh (gel method)			
Patient RBCs (forward typing)			
Anti-A	Anti-B	Anti-A,B	Anti-D
4+	0	4+	4+
Patient plasma (reverse typing)			
A1 cells	B cells	0 cells	
0	4+	0	

Reaction scale =0 (no reaction) to 4+ (strong reaction)

Table 2: Antibody screen

Cell	D	C	E	c	e	f	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P l	M	N	S	s	Gel
1	+	+	0	0	+	0	+	+	0	+	+	0	0	+	+	0	+	0	+	0
2	+	0	+	+	0	0	0	+	+	0	+	+	0	+	+	+	0	+	0	2+
3	0	0	0	+	+	+	0	+	+	+	0	+	+	0	+	+	+	0	0	0

Reaction scale=0 (no reaction) to 4+ (strong reaction), +s: strong reaction

Table 3: Antibody identification panel with untreated RBCs

Cell	D	C	E	c	e	f	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P l	M	N	S	s	Gel
1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	+	+	0	+	+	2+
2	+	+	0	0	+	0	0	+	0	+	+	+	+	0	+	+	+	+	0	0
3	+	0	+	+	0	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0
4	+	0	0	+	+	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0
5	0	+	0	+	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0
6	0	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0
7	0	0	0	+	+	+	+	+	0	+	+	+	0	+	0	+	0	0	+	2+
8	0	0	0	+	+	+	0	+	+	0	+	+	+	0	+	+	0	+	0	2+
9	0	0	0	+	+	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0
10	0	0	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0
11	+	+	0	0	+	0	+	+	0	+	0	0	0	+	+	+	0	+	0	1+
Patient cells	+	+	0	+	+											0	+	0	+	

Reaction scale=0 (no reaction) to 4+ (strong reactio

Example 2

The patient’s group is A, Rh (D) pos using gel method (Table 3). Table 4 represents antibody screen. Table 5 and Table 6 summarize antibody identification panel using gel test with two different untreated RBCs.

Alloantibodies against the Rh (D) is confirmed in gel panel. *Anti-c and Anti-E* can be identified and all other clinically significant alloantibodies can be ruled out (Table 5). 12,13 Rh blood group system antibodies are commonly observed after transfusion or pregnancy. They show specific characteristics; most are IgG, bind at 37°C indirect antiglobulin test (IAT). Some Rh antibodies may be found in individual who never pregnant (Anti-Cw) or never undergone transfusion (Anti-E). After alloimmunization, antibodies may persist for several years [18–20].

Furthermore, the Rh(c) antigenic is more immunogenic than Rh(E) antigen and they may cause severe HDFN. Interestingly, a variety of strength reactions may suggest the presence of multiple antibodies [12].

A low titer of anti- E and Anti-c were recognized using gel method. Potentiators such as enzymes or more sensitive method of antibody identification may be used in case of weak reactions. Ultimately, c-negative and E-negative blood unit should be provided and crossmatched [12].

Table 4: ABO/Rh

ABO/Rh			
Patient RBCs (forward typing)			
Anti-A	Anti-B	Anti-A,B	Anti-D
4+	0	4+	4+
Patient plasma (reverse typing)			
A1 cells	B cells	0 cells	
0	4+	0	

Reaction scale =0 (no reaction) to 4+ (strong reaction)

Table 5: Antibody screen

Cell	D	C	E	c	e	f	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Gel
1	+	+	0	0	+	0	0	+	+	0	+	+	+	0	+s	+	0	+	0	0
2	+	0	+	+	0	0	+	+	+	+	0	+	0	0	+	+	0	+	+	0.5/1+
3	0	0	0	+	+	+	0	+	0	+	+	0	+	+	+s	0	+	0	+	1+

Reaction scale=0 (no reaction) to 4+ (strong reaction), +s: strong reaction

Table 6: Antibody identification panel with untreated RBCs

Cell	D	C	E	c	e	f	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Gel
1	+	+	0	0	+	0	0	+	+	+	+	+	0	+	+	+	+	+	+	0
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	0
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	1+
4	+	0	0	+	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	2+
5	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	+	0	+	0	0
6	0	0	+	+	+	+	0	+	+	+	+	+	0	+	+	0	+	0	+	2+
7	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	0	+	0	+	1+
8	0	0	0	+	+	+	0	+	+	0	0	+	+	0	0	+	+	+	+	2+
9	0	0	0	+	+	+	0	+	+	0	+	0	0	0	+	+	0	+	0	1+
10	0	0	0	+	+	+	+	+	+	+	0	+	0	+	+	0	+	+	+	1+
11	+	0	0	0	+	0	+	+	0	+	+	+	+	0	+	+	+	0	+	0
Patient cells	+	+	0	0	+		0	+												

Reaction scale=0 (no reaction) to 4+ (strong reaction)

Table 7: Antibody identification panel with untreated RBCs

Cell	D	C	E	c	e	f	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Gel
12	0	0	0	+	+	+	+	+	+	0	+	0	0	0	+	0	+	0	+	1+
13	0	0	0	+	+	+	0	+	+	+	0	+	0	+	+s	+	0	+	0	1+
14	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	1+
15	+	0	+	+	0	0	0	+	+	+	+	0	0	0	+	+	0	+	0	1+
16	+	0	+	+	0	0	0	+	0	+	0	+	0	0	0	0	+	0	+	1+
17	+	0	+	+	0	0	0	+	+	0	+	+	0	0	0	+	+	0	+	1+
18	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0
19	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+s	0	+	0	+	0
20	+	+	+	0	+	0	0	+	+	+	0	+	0	0	0	0	+	+	0	0
21	+	0	+	+	+	+	0	+	+	0	0	+	0	0	+s	0	+	0	+	2+
22	0	+	0	+	+	+	+	0	+	+	+	0	+	0	+	+	+	0	+	0.5+/1+
Patient cells	+	+	0	0	+		0	+												

Reaction scale=0 (no reaction) to 4+ (strong reaction), +s: strong reaction

The patient’s group is B, Rh (D) pos using gel method (Table 7). Table 8 represents antibody screen. Table 9 and Table 10 summarizes antibody identification panel using gel test with untreated RBCs and enzyme (ficin) treated red blood cells.

Multiple antibodies need a selected reagent panel red blood cells and additional techniques such as enzymes. More specifically, antibody reactions are enhanced using enzymes in Rh, P1, I, Kidd, Lewis blood groups. Antigens destroyed by enzymes are M, N, Duffy. The Kell blood group is unaffected by enzyme (ficin) reagent. S and s antigen present a variable effects on enzymes [11,12].

Alloantibodies against the Fya and s are confirmed in gel panel and all other clinically significant alloantibodies are rule out (Table 6)[12]. Duffy antibodies are observed after acute and delayed transfusion reactions and are uncommon cause of HDFN. Anti-Fya is IgG, it usually does not usually bind the complement proteins. The antibody is classically non-reactive with ficin-treated RBC reagent because the enzyme degrades the antigen. Anti-s, is clinically significant IgG antibody that can induce hemolysis and HDFN. Anti- s shows a variable effect after ficin-treated red blood cells [4,21].

Fya-negative and s-negative red blood units should be provided and crossmatched for trasfusion request. Matching the pattern is more difficult when more than one antibody specificity exists [12].

Table 8: AB0/Rh

ABO/Rh			
Patient RBCs (forward typing)			
Anti-A	Anti-B	Anti-A,B	Anti-D
0	4+	4+	4+
Patient plasma (reverse typing)			
A1 cells	B cells	0 cells	
4+	0	0	

Reaction scale =0 (no reaction) to 4+ (strong reaction)

Table 9: Antibody screen

Cell	D	C	E	c	e	f	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Gel
1	+	+	0	0	+	0	0	+	+	+	+	+	0	+	0	+	0	+	0	0
2	+	0	+	+	0	0	0	+	+	0	+	0	0	+	+	0	+	0	+	3+
3	0	0	0	+	+	+	+	+	0	+	0	+	+	0	+	+	0	+	+	1+

Reaction scale=0 (no reaction) to 4+ (strong reaction)

Table 10: Antibody identification panel using untreated RBC's

Cell	D	C	E	c	e	f	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Gel	Ficin
1	+	+	0	0	+	0	0	+	+	0	+	0	0	+	+	+	+	0	+	3+	1+
2	+	+	0	0	+	0	0	+	0	+	+	+	0	+	0	0	+	+	0	0.5+/ +	0.5+/ 1+
3	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	+	3+	1+
4	+	0	0	+	+	+	0	+	0	0	+	0	0	0	0	+	+	0	+	2+	0.5+/ 1+
5	0	+	0	+	+	+	0	+	0	+	+	+	0	+	0	+	+	0	+	3+	0
6	0	0	+	+	+	+	0	+	+	+	+	+	+	0	+	+	0	+	+	2+	1+
7	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	0	+	0	+	3+	1+
8	0	0	0	+	+	+	0	+	+	0	0	+	0	+	+	+	0	+	0	0	0
9	0	0	0	+	+	+	0	+	+	0	0	+	+	0	+	+	0	0	+	3+	0
10	0	0	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	3+	0
11	+	+	+	0	0	+	+	0	0	+	+	0	+	0	+	+	0	+	+	2+	0- 5+/ 1+

Reaction scale=0 (no reaction) to 4+ (strong reaction)

4. Discussion

One of the most important process in transfusion medicine is management of patients who has red cell single or multiple alloantibodies.

The antibody screen is performed to identify or confirm the presence of antibodies in patient's serum or plasma, as a preoperative and pretransfusion tests. Antibody identification and major crossmatch are provided using indirect antiglobulin test (IAT) to improve safety of red blood administration (Figure 1).

The antibody screen is summarized in Tables 2, 5 and 9. The antibody panel is summarized in Tables 3, 6, 7 and 10. The three examples describe significant single and multiple alloantibodies in clinical practice.

Anti-M documented in first example is a possible warm reacting antibody. For decision making during pregnancy it is of paramount importance obtaining a complete transfusion history and monitoring antibody titer accurately.

Alloantibodies to Rh antigen is observed in the second example. Potentiators such as enzymes or a more sensitive method of antibody identification are necessary to increase weak reactions.

Anti-s recognized in last example is IgG and reactive at 37 °C. Similarly, Anti- Fya is immunogenic and known to cause hemolytic reaction and HDFN. Duffy system includes antigens such as Fya and Fyb. Caucasian individuals express some combination of Fya and/or Fyb, while blacks may express neither antigen. Eventually, it is important to collect information regarding ethnicity, because it can expedite search of red blood units and supply of compatible products.

5. Conclusions

The transfusion staff recommend red blood units for transfusion request based on medical history and immunohematology tests (blood group, antibody screen and identification). In case of unavailability of suitable red blood cell units for transfusion purposes, communication between transfusion specialist and clinician is of paramount importance to plan further laboratory tests, search of red blood units and manage patients properly [22–25].

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