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Practical Solutions for Problems in Blood Grouping and Crossmatching

Kan Grubu ve Çapraz Karşılaştırma Testlerinde Karşılaşılan Sorunlar için Pratik Çözümler

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Abstract

Hematologists often encounter transfusion problems, one of which is crossmatch incompatibility. In many countries, transfusion medicine is not a recognized specialty, there are no reference immunohematology laboratories, and most blood banks can only perform "type and screen" and crossmatch analyses. Therefore, hematologists should have basic knowledge about blood banking procedures and how to use them. This review aims to provide hematologists who do not have access to advanced blood bank laboratories some practical tips for handling problems in pretransfusion testing.

Keywords: Blood grouping, Incompatible crossmatch, Transfusion

Öz

Hematologlar transfüzyon problemleri ile sıklıkla karşılaşmaktadırlar. Bu problemlerden birisi de çapraz karşılaştırmada uyumsuzluktur. Bir çok ülkede transfüzyon tıbbı resmi olarak tanınmış bir bilim dalı değildir, referans immünohematoloji laboratuvarları yoktur ve bir çok kan bankası sadece kan gruplama ve antikor tarama ve çapraz karşılaştırma testlerini yapabilmektedir. Bu nedenle hematologların kan bankacılığı teknikleri ile ilgili temel bilgi sahibi olmaları gerekmektedir. Bu derlemenin amacı, ileri kan bankası laboratuvarlarına erişimi olmayan hematologlar için kan uygunluk testlerinde karşılaşılan problemlerin çözümü için pratik ipuçları sağlamaktır.

Anahtar Sözcükler: Kan grubu, Uyumsuz çapraz karşılaştırma, Transfüzyon

Introduction

Red blood cell (RBC) compatibility testing is a crucial step for erythrocyte concentrate (EC) transfusion. Blood grouping, antibody screening, antibody identification, direct antiglobulin test (DAT), and crossmatching are different aspects of RBC compatibility testing. This review aims to provide information for practicing hematologists on how to use these tests to solve problems in blood grouping and crossmatching.

Tests Used in RBC Compatibility Testing

Most blood bank laboratories use column agglutination technology, commonly referred to as gel testing or card testing. Figure 1 shows the reaction strengths in gel testing.

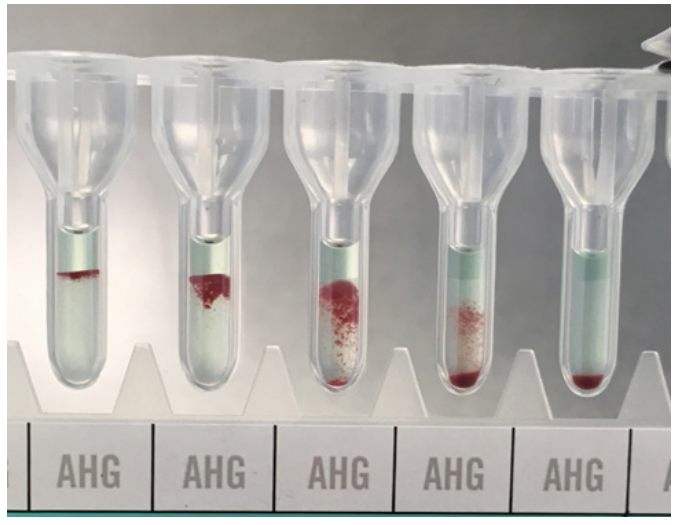

Figure 1. Reaction strengths in gel testing. 4+, 3+, 2+, 1+, and negative from left to right.

Blood Grouping

There are more than 40 blood group systems and over 300 RBC antigens [1]. The ability of a substance to induce antibody production is called immunogenicity. ABO system antigens and

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the D antigen from the Rh system are the most immunogenic antigens. Anti-A and anti-B are naturally occurring antibodies and are usually of the immunoglobulin (Ig) M type. Blood grouping involves two steps, forward grouping (reacting anti-A, anti-B, and anti-D antibodies with the person's RBCs) and reverse grouping (reacting commercially available A- and B-type RBCs with the person's plasma). Forward grouping reactions in particular must be very strong (+4) and the results of forward and reverse grouping should be complementary, as seen in Table 1. Subtypes of A and B antigens can cause weaker or mixed field reactions in forward grouping and sometimes anti-A1 can be identified in reverse grouping. The D antigen has numerous variations that affect serological reactions. If the D antigen is normal in structure but has fewer antigenic sites, it is called weak D (formerly Du). If it has a qualitative structural defect, it is called partial D. The most common partial D variant in white people is DVI. There are some special panels for the differentiation of weak D from partial D, but it is impossible to make this differentiation by routine serological testing. Therefore, we refer to these different types as D variants. People with partial D may produce anti-D when transfused with D-positive EC, while people with weak D will not [2]. The other immunogenic RBC antigens can be identified serologically using specific antibodies. Table 2 shows the most important antigens and antibodies in transfusion medicine.

Direct Antiglobulin Test (DAT)

A positive DAT shows that the RBCs are coated with antibodies. In DATs, the antigen-antibody reaction occurs in vivo. Adding anti-human globulin (AHG) to RBCs enables the reaction to be visualized in vitro. AHG is an antibody against human antibodies and can be polyspecific or monoclonal against IgG or complement C3.

Antibody Screening

Antibody screening detects antibodies against antigens other than A and B and is performed by indirect antiglobulin test (IAT) technique. In this test, the person's plasma is mixed with at least two (preferably four) commercially available O-type RBCs, which should be selected to screen for antibodies against most immunogenic antigens. Figure 2 shows an antibody screening result. Positivity indicates that the patient's plasma contains antibodies against RBC antigens that are reactive at body temperature. A positive screening test should be followed by antibody identification.

Antibody Identification

This test is technically the same as antibody screening but is performed with more types of RBCs and aims to identify the antibody(ies) detected in the screening test. The O-type RBCs used in antibody identification tests are collectively referred to as a "panel." A panel should consist of at least 11 types of RBCs. An example of an antibody identification panel is shown in Figures 3 and 4. Interpreting the identification panel requires some experience and is rather time-consuming.

Crossmatching

CM looks for unexpected antibodies in the recipient's plasma against the RBCs in the EC. It is done by IAT technique and is

Table 1. Forward and reverse blood typing.

Blood type	Forward typing		Reverse typing	
	Anti-A	Anti-B	A cells	B cells
A	+	-	-	+
B	-	+	+	-
AB	+	+	-	-
O	-	-	+	+

Table 2. Common blood groups [1,3].

System name	Symbol	Antigens					Clinical significance of antibodies against antigens
ABO	ABO	A	B	A, B	A1		Severe AHTR
Rh	RH	D	C	c	E	e	Severe AHTR and DHTR
Kell	KEL	K	k	Kp^a	Kp^b		Severe AHTR and DHTR
Kidd	JK	Jk^a	Jk^b				AHTR, DHTR
Duffy	FY	Fy^a	Fy^b				AHTR, DHTR (most common cause of DHTR)
MNS	MNS	M	N	S	s		M and N rarely cause HTR if active at 37 °C S and s may cause AHTR and DHTR
Lewis	LE	Le^a	Le^b				Clinically insignificant
Lutheran	LU	Lu^a	Lu^b				Mild DTHR

AHTR: Acute hemolytic transfusion reaction; DHTR: delayed hemolytic transfusion reaction.

thus actually an antibody screening test. A negative result is called CM-compatible.

Practical Solutions for Problems in Blood Grouping and Crossmatching

When there is a problem in EC CM, the physician should ask the following questions:

a. Is there a problem with the recipient's ABO and D (Rh) blood grouping?

b. Has the patient received any transfusions or been pregnant before (including miscarriages and abortions)? If so, was that within the last 3 months?

c. Does the patient currently have hemolysis?

d. What are the patient's results from DAT and antibody screening (and antibody identification, if available)?

e. What is the "pattern" of positive reactions in antibody screening (and antibody identification, if available)?

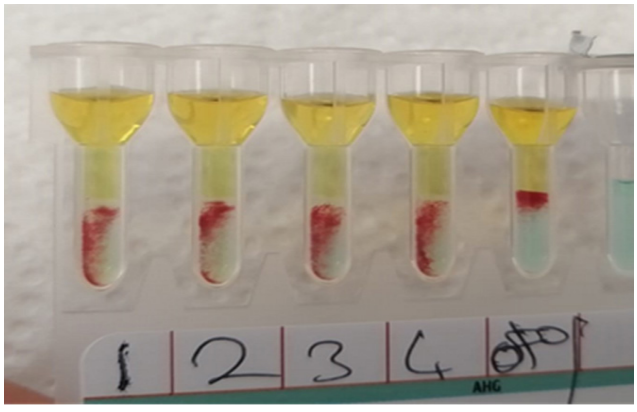

Figure 2. Antibody screening result showing equal positivity with all cells and positive auto-control.

Problems in Blood Grouping

These problems are usually solved in blood bank laboratories. If the patient has subtype A, it will be safe to transfuse him or her with a CM-compatible O group EC, because A subtype recipients can produce anti-A1.

D variant recipients should be considered D-negative when planning transfusions because if they carry a partial D variant, they may produce anti-D if transfused with D-positive EC. This method may result in the unnecessary transfusion of Rh-negative EC to weak D patients but it is a safer approach unless genetic testing can be done. Double populations or mixed field reactions can be seen in patients who have undergone hematopoietic stem cell transplantation (HSCT). Figure 5 shows one example. The blood bank should be informed about these patients and there should be algorithms for EC transfusions during the engraftment period of ABO-incompatible HSCT cases. Table 3 shows the algorithm that the Turkish Society of Hematology published in 2020 [3]. Ultimately, if the ABO type cannot be determined, CM-compatible O blood group transfusions almost always solve the problem.

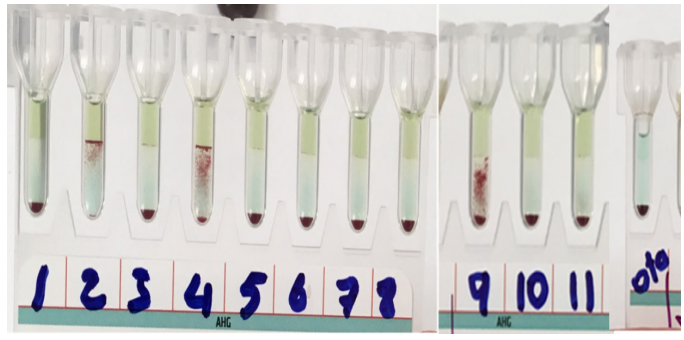

Figure 3. Antibody identification result with positive and negative cells.

Table 3. Erythrocyte concentrate transfusion algorithm for ABO-mismatch hematopoietic stem cell transplantation.

	Recipient blood type	Donor blood type	Erythrocyte concentrate type*
Major mismatch transplantation	O	A	O
	O	B	O
	O	AB	O
	A	AB	A, O
	B	AB	B, O
Minor mismatch transplantation	A	O	O
	B	O	O
	AB	O	O
	AB	A	A, O
	AB	B	B, O
Bidirectional mismatch transplantation	A	B	O
	B	A	O

*Listed in order of preference.

Antibody Problems: Incompatible Crossmatch

Incompatible CM means that the recipient has an antibody against the RBCs in the EC. The first step is to determine whether it is an autoantibody (against the patient's own RBC antigens), alloantibody (against non-self RBC antigens, i.e., foreign RBC antigens from transfusion or pregnancy), or both. Here are some clinical situations in which the questions above should be asked to plan the safest transfusion for the patient.

If the patient has no history of transfusion or pregnancy and has a positive DAT result, the antibodies coating the RBCs should be autoantibodies because there was no exposure to foreign antigens. If the patient has hemolysis, the diagnosis is autoimmune hemolytic anemia (AIHA). In this case, if the patient needs to be transfused, there is no need to waste time trying to find a CM-compatible EC because that will be impossible. Autoantibodies will react with every RBC from every EC. Physicians should take responsibility and transfuse an EC with close follow-up. Such patients will hemolyze these transfused RBCs at the same speed they hemolyze their own RBCs. Autoantibodies are almost always against public antigens. Public antigens are high-frequency antigens, which means they can be found in nearly all RBCs. Therefore, autoantibodies will react and give the same reaction strength with all RBCs. A typical antibody screening result for a patient with autoantibodies can be seen in Figure 6. Ideally, antibody identification should be performed. All identification panel cells would give the same reaction, including the auto-control (Figure 6). If there is a reaction with all RBCs in the panel, it is called a pan-reactive panel.

If the patient has been transfused or pregnant but not within the last 3 months and the DAT is positive, then once again, the antibodies coating the RBCs should be autoantibodies. However, since the patient was exposed to foreign antigens previously, there is a possibility that the patient's plasma contains alloantibodies. If there are both allo- and autoantibodies, antibody screening and antibody identification will be pan-reactive but we will see a wavy pattern (Figure 7). If the autoantibody is strong and causes

Antibody Panel																			
Panel Cell	D	C	c	E	e	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	M	N	S	s
1	+	+	–	–	+	+	+	0	+	0	+	0	+	+	+	+	+	+	+
2	+	+	–	–	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+
3	+	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	+	+	+	0	+
6	0	0	+	+	+	+	+	0	+	0	+	0	+	+	0	+	0	+	+
7	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+
8	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+
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
Patient's cells																			

Figure 4. Interpretation sheet of identification panel.

Figure 5. Mixed field result in ABO typing.

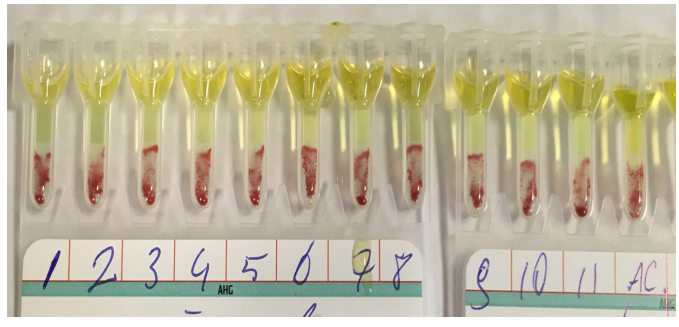

Figure 6. Antibody identification panel with all equal positivities and positive auto-control.

a 4+ reaction, it may mask the alloantibodies. To eliminate the autoantibodies, auto-adsorption can be performed. Adsorption is a procedure in which antibodies are adsorbed onto the RBCs. The patient's own RBCs can be used for this, but since self-RBCs are already coated with antibodies, there will be few antigenic sites left and the procedure must be repeated several times. If the positive reaction seen in the DAT is +3 or +4, it will be very difficult to adsorb the autoantibodies. In such a case, one alternative method is to phenotype the patient and find RBCs that match the patient's negative antigens to use for adsorption, and another method is to subject the patient's RBCs to gentle heat elution (see below) and use these "naked" RBCs for adsorption [4]. Antibody identification is done after every adsorption procedure. If there is an autoantibody, positivity will decrease with every adsorption and eventually the panel will be negative. CM can be done with this auto-adsorbed plasma and should be compatible. If there is an alloantibody accompanying the autoantibody, alloantibody(ies) will be left alone after adsorbing and the appearance will be like that in Figure 3. After that, antibody identification can be done. This process is clearly time-consuming; if a patient with AIHA needs to be transfused, delaying the transfusion can be life-threatening. If the antibody screening panel (and identification panel, if done) is equally pan-reactive and if there is no time to do the tests above, I suggest transfusion of CM-incompatible EC with close monitoring of vital signs. Besides ABO and D antigen matching, Ee, Cc, and K antigen matching is suggested. There is no need to select O RhD-negative blood if there is no doubt in the patient's blood grouping.

If there is a wavy pan-reactive pattern in the antibody screening (and identification panel) (Figure 7) of an AIHA patient and there is no time or opportunity to perform adsorption, then I recommend transfusing the patient with an EC that gives the same reaction strength on DAT or auto-control.

If the DAT is positive and the patient was transfused or pregnant within the last 3 months, foreign RBCs may be present in the patient's circulation. In this case, we cannot easily attribute DAT positivity to autoantibodies; we have to perform elution. Elution is a process in which the antibodies coating the RBCs are dissociated into a liquid called the eluate. After elution, antibody identification is performed on the eluate to identify the antibodies on the RBCs. In clinical practice, if you do not have the time or opportunity to perform elution, and if the patient has received a transfusion due to AIHA within the last 3 months, the DAT was positive before transfusion, and antibody screening suggests an autoantibody, you can proceed with transfusing the patient.

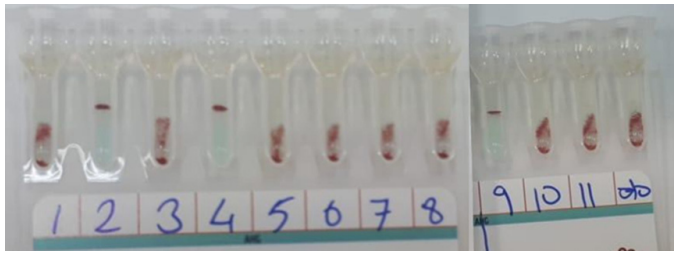

Figure 7. Pan-reactive panel with different positivity strengths.

In the case of delayed hemolytic reaction (DHR), the DAT is positive, antibody screening results are wavy and positive like in Figure 7, and the patient has a history of transfusion, usually within the last 15 days. The classical clinical picture will be a patient transfused for some reason other than immune hemolysis and the first transfusion is CM compatible, and then after 7 to 15 days, hemolysis starts, the DAT becomes positive, and antibody screening is positive. The DAT positivity is caused by RBCs from the previously transfused EC. In this case, elution will reveal an alloantibody. Antibodies like Fy^a, which may be cleared from the plasma in as little as 3 months, are usually responsible for these reactions. To avoid DHR, if a patient is known to have had an alloantibody in the past, always transfuse with the corresponding antigen-negative EC.

If the DAT is negative and antibody screening is positive, it must be due to an alloantibody. The patient will report a history of transfusion and/or pregnancy. Antibody identification should be performed in order to define the antibody(ies) and an EC that is antigen-negative for the corresponding antibody(ies) should be selected for CM. If there is no time or opportunity to identify the antibody, then the only option is to perform CM tests with different units and try to find one that is compatible. If there are negative cell(s) in the antibody screen panel (Figure 3), then one can guess it will not take long to find a CM-compatible EC. If the screening panel is pan-reactive, it would be wise to contact an experienced blood bank laboratory without wasting time trying to find compatible units. Not all alloantibodies have the same hemolytic risk (Table 2). Rh, Kell, Kidd, and Duffy system antibodies and anti-S or anti-s antibodies in the MNS system can cause clinically significant hemolytic transfusion reactions [5]. In the event of an alloantibody, if the antibody is one of the risky ones or cannot be identified, do not agree to transfuse a CM-incompatible EC unless the patient's life is in danger. Unlike autoantibodies, hemolysis with alloantibodies is often unpredictable. One or two positive CM-incompatible units may cause a serious hemolytic transfusion reaction. On the other hand, if transfusion is absolutely necessary, than it should be done. If a CM-compatible EC cannot be found for a patient with alloantibody(ies), one option is to perform extensive phenotyping of the recipient. This phenotyping should include the most immunogenic antigens: Ee, Cc, K, Fy^a, Fy^b, Jk^a, Jk^b, and Ss. The recipient and donor RBCs should match. For example, if the patient is E-negative, the EC must be E-negative. If the patient has been transfused recently, there may be double populations, indicating antigen mismatch in previous

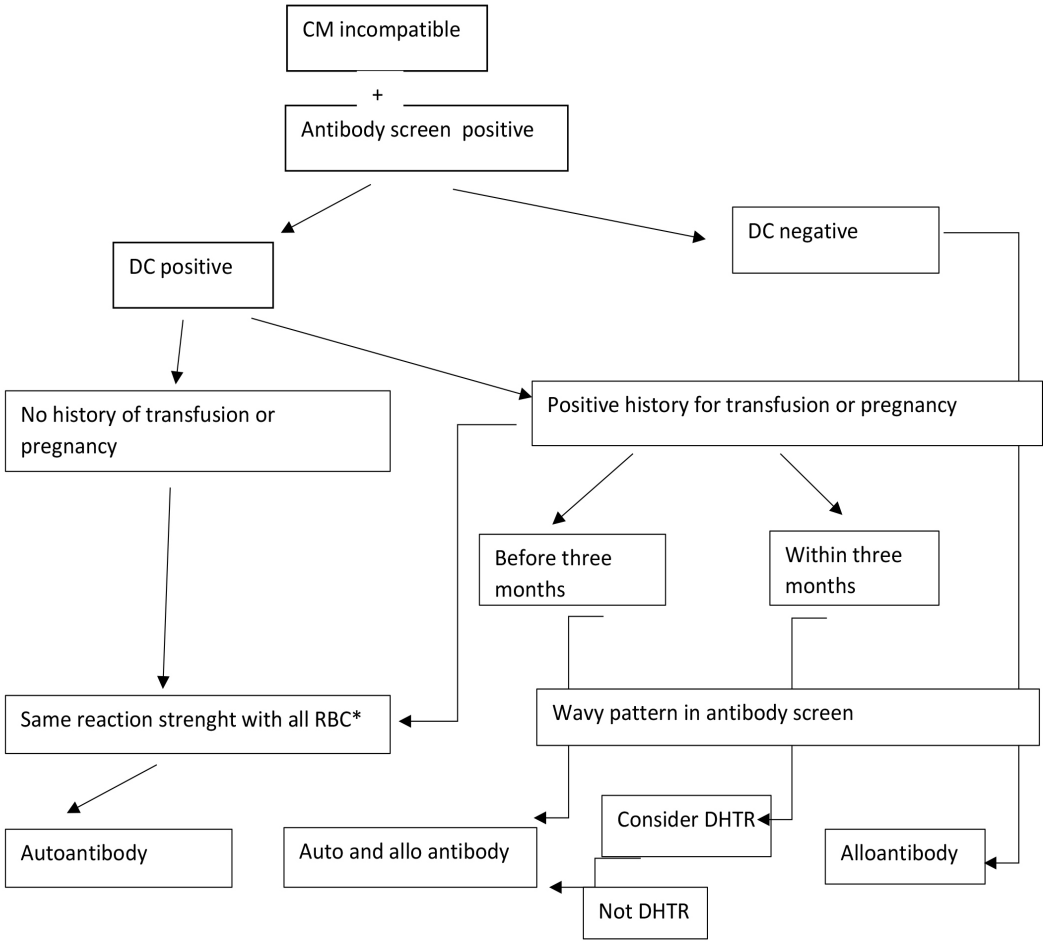

Figure 8: How to differentiate the type of antibody causing CM incompatibility.

*Strong autoantibodies can mask alloantibodies. The same reaction strength does not rule out alloantibodies. Please refer to the text for more information. DC: Direct Coombs test; DHTR: Delayed hemolytic transfusion reaction.

transfusions, which makes phenotyping complicated. Another option is to transfuse the patient with the least incompatible EC and hope for the best. This is definitely not recommended but can be done if transfusion is urgently needed. In this case, the EC should be transfused slowly with close monitoring of the patient. After transfusion of 10-15 mL of EC, testing for intravascular hemolysis with an interim blood sample is strongly advised. However, an uneventful transfusion in this case should not be completely reassuring because it does not guarantee normal RBC survival in vivo.

A simple scheme that summarizes how to differentiate auto- and alloantibodies can be seen in Figure 8.

Conclusion

This review covers the most frequently encountered compatibility testing problems. However, the reader should keep in mind that there are important exceptions in all clinical scenarios. Open communication and collaboration between clinicians and blood bank personnel can solve many problems.

Ethics

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References

1. International Society of Blood Transfusion. Red Cell Immunogenetics and Blood Group Terminology. Amsterdam, ISBT, no date. Available online at http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology.
2. Huang CH, Liu P, Cheng JG. Molecular biology and genetics of the Rh blood group system. Semin Hematol 2000;37:150-165
3. Turkish Society of Hematology. Grup uyumsuz allojenik hematopoietik kök hücre naklinde transfüzyon önerileri. Ankara, THD, no date. Available online at https://www.thd.org.tr/thdData/Books/2113/grup-uyumsuz-allojenik-hematopoietik-kok-hucre-naklinde-transfuzyon-onerileri.pdf.
4. Walker SP. Identification of antibodies to red cell antigens. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM (eds). AABB Technical Manual. 18th Edition. Bethesda, American Association of Blood Banks, 2014.
5. Storry JR. Other blood group systems and antigens. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM (eds). AABB Technical Manual. 18th Edition. Bethesda, American Association of Blood Banks, 2014.