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ANTIBODY SCREENING IN BLOOD DONORS CONTRIBUTES TO BLOOD SAFETY

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Abstract

Screening for irregular red blood cell (RBC) antibodies is a mandatory test for every blood donation in accordance with national blood testing strategy. The aim of this study was to determine the prevalence, specificity and frequency of irregular RBC antibodies detected using column agglutination technology (CAT) based on indirect antiglobulin test (IAT).

A total of 252,641 blood donations collected between 2020 and 2024 were screened using pooled screening cells on immune-hematological analyzer (IH 500/1000). Antibody specificity was determined by using commercially available 11-cell identification panel. Donations were obtained from 205,532 male (81%), 47,109 female (19%), and 47,919 first-time donors (17%).

Irregular RBC antibodies were confirmed in 119 donations corresponding to an overall prevalence of 0.05%. The most frequently detected antibody was anti-M (33%), followed by antibodies of the Rh system (22%) and anti-K (15%) antibodies. Antibodies of the Rh system were anti-D (8%), anti-C (2%), anti-E (9%) and anti-c (3%).

Female donors showed a significantly higher prevalence of irregular RBC antibodies (0.11%) than male donors (0.03%). The most frequent anti-M antibody had similar representation in both genders, 0.015% in male and 0.016% in female donors, as a result of the rejuvenation of our donor pool. Also, in female donors more frequent antibodies were anti-K (0.03%), anti-D (0.02%) and anti-E (0.01%). Positive direct antiglobulin test (DAT) were more frequent among female donors (0.02%).

The low prevalence reflects effective donor selection and sensitive screening methods. Identification of clinically significant antibodies remains essential for ensuring transfusion safety and preventing hemolytic transfusion reactions.

Keywords: antibody screening, irregular RBC antibodies, column agglutination technology (CAT), indirect antiglobulin test (IAT), delayed hemolytic transfusion reactions (DHTR)

Introduction

Blood group antigens are integral components of the red cell membrane and play essential structural, functional, and immunological roles. They contribute to membrane stability, cellular adhesion, transport processes, and immune recognition. These antigens are

crucial for survival and function of erythrocytes and are fundamental to safe blood transfusion practices.

According to the International Society of Blood Transfusion, more than 340 RBC antigens, encoded 45 genes, are classified into 38 blood group systems. Among these, the ABO and Rh systems are of primary clinical importance, followed by Kell, Kidd, Duffy, MNS and Lutheran systems[1].

Other systems, such as Lewis, P, Colton and Dombrock, are generally of lower clinical relevance.

RBC antigens are polymorphic immunogenic determinants capable of inducing immune response and antibody production in antigen-negative recipients following exposure. Such exposure may occur through blood transfusion, pregnancy, or in some cases naturally without an identifiable immunizing event[2].

Irregular RBC antibodies, also known as unexpected antibodies, are non-ABO alloantibodies or autoantibodies detected in serum. Their prevalence in the general population ranges from 0.3% to 2%[3,4]. However, in blood donors it is typically below 0.1%, whereas it may reach 5% to 50% in polytransfused patients[5,6].

These antibodies may cause acute or delayed hemolytic transfusion reaction and can compromise transfusion safety. Therefore, systematic screening of blood donors for irregular antibodies is essential to prevent passive antibody transfer and adverse transfusion outcome.

In recent decades, gel-based microagglutination technique suitable for full automation has been introduced. This method, based on the IAT principle, allows sensitive detection of IgG antibodies and complement components and has significantly improved the reliability of antibody screening.

This testing strategy allows the detection of all clinically significant RBC antibodies with the potential to cause immune hemolysis, particularly regarding passive transfer of antibodies from blood donors to patients.

The selection of screening erythrocytes is crucial for test sensitivity. Screening cells must be group O and express clinically significant antigens in appropriate dosage to ensure detection of relevant antibodies.

Aims

The aims of this retrospective study were to determine the prevalence and specificity of irregular RBC antibodies among blood donors and to analyze their frequency distribution in the period from January 1, 2020 to December 31, 2024 at the Institute of Transfusion Medicine in Skopje.

Material and methods

This retrospective study included 252,641 blood samples collected from voluntary blood donors, between January 1, 2020 and December 31, 2024 at the Institute of Transfusion Medicine in Skopje.

Blood samples (6 ml) were collected in vacuum tubes containing K2EDTA anticoagulant. All samples were subjected to indirect antiglobulin test (IAT) using pooled screening cells and gel microagglutination techniques on fully automated IH-500 and IH-1000 immune-hematological analyzers (Bio-Rad, Switzerland). Commercially available pooled screening erythrocytes from two O group donors were used. The following antigens must be present on at least one RBC included in the screening pool: D, C, E, c, e, M, N, S, s, P, Lea, Leb, K, Fya, Fyb, and Jkb from two blood donors.

Gel cards containing polyspecific anti-human globulin (anti-IgG and anti-C3d) were employed.

First, 50 µl of 0.8% commercially pooled cells were pipetted and brought into contact with 25µl of donor sera. The gel cards were incubated at 37˚C for 15 minutes and centrifugated for 10 minutes at 1000 rpm before interpretation.

The strength of agglutination was graded from 0 (negative) to 4+ (strongly positive). All positive samples were retested using the same pooled and two donor reagent screening cells, DiaCell I+II (Bio-Rad, Switzerland). Persistently positive samples underwent antibody identification using 11-cell identification panels (Bio-Rad, Switzerland and Ortho, Ireland) and when necessary, enzyme-treated cells. Direct antiglobulin test (DAT) was performed on all IAT-positive samples. The specificity of antibodies was interpreted based on antigen profiles provided with the identification panels.

A standardized donor follow-up algorithm was applied.

Procedure when screening for irregular RBC antibodies was positive:

1. The test was performed manually again using the same or another lot of screening cells. All persistently positive samples underwent antibody identification in immunohematology laboratory.
2. The identification results were documented in the institutional information system (IS) e-Delphyin and in a form that is kept in the Classifier/Book for donors with positive screening for irregular RBC antibodies.
3. A temporary donor deferral was generated in the IS and the donor was called for retesting. Depending on the outcome of the retesting, the following is undertaken:

a. Negative screening: the deferral from donation is withdrawn and the donor is notified.

b. Repeatedly positive screening results (specific or non-specific antibodies) in two consecutive tests lead to permanent donor deferral. Donors are notified according to the repeat testing outcome.

All blood units with confirmed irregular RBC antibodies were discarded.

Data and modules for statistical processing were used from blood donor information system.

Statistical analysis

Data were used from informational system e-Delphyn, analyzed using descriptive statistical methods. Categorical variables, including gender, donation frequency, prevalence of irregular RBC antibodies, specificity and frequency, were summarized as absolute numbers and percentage.

Results

During the five-year-period, from 1st of January 2020 to 31st of December 2024, 252.641 donations were collected and screened for irregular RBC antibodies, whereas 119 donations had confirmed irregular RBC antibodies. The prevalence of irregular RBC antibodies was 0.05% among voluntary Macedonian blood donors in this period.

Table 1. Blood donations in the last 5 years and identified irregular RBC antibodies per year

Year	Total number of blood donations/IAT tests	Identified irregular RBC antibodies per year/percentage (%)
2020	42.563	13(0.03)
2021	48.894	32(0.06)
2022	51.474	21(0.04)
2023	53.901	31(0.06)
2024	55.809	22(0.04)
Total	252.641	119(0.05)

The prevalence of irregular RBC antibodies was very similar during this 5-year period. The highest prevalence of irregular RBC antibodies was determined in 2021 during Covid-19 pandemic as well as in 2023, with the same prevalence of 0.06%. The lowest prevalence was determined in 2020, with 0.03% at the beginning of Covid-19 pandemic when the number of blood donations decreased in comparison with other years.

Table 2. Distribution of donors by gender and number of donations in the period from 2020 to 2024

Total number of blood donations (2020-2024)	Donation from male donors (No/%)	Donation from female donors (No/%)	First time donors (No/%)
252,641	205,532 (81%)	47,109 (19%)	47,919 (17%)

Most donations during this period were collected from male donors - 81% (n=205,532), while 19% (n=47,109) were from female donors. Additionally, 17% (n=47,919) were collected from first-time donors. In the group of first-time donors, 89 (0,2%) had a positive screening for irregular RBC antibodies. In observed period of 5 years, 101 blood donors got permanent deferral due to permanent detection of an irregular RBC antibody.

The most frequent antibody from MNS blood group system was anti-M (33%), followed by clinically significant alloantibodies from Rh blood group system with 22% (anti-D with 8%, anti-C with 2%, anti-E with 9% and anti-c with 3%). The most frequent of Kell blood group system antibodies was anti-K accounting for 15% of all identified antibodies. Less frequent were anti-P1 and anti-C^w with 4% each, anti-Le^a with 3%, followed by anti-Le^b, anti-Lu^a and anti-S with 2% each, and anti-Fy^a and anti-Jk^a had the lowest frequency, with 1% each. The frequency of unspecified antibodies was 12%, as it shown in Table 3.

Erythrocytes from all blood samples with identified specific irregular RBC antibodies were antigen-negative for the identified antibody.

Table 3. Specificity of irregular RBC antibodies

Antibody specificity	Identified irregular RBC antibodies No (%)
Anti-D	9(7.6%)
Anti -C	2(1.7%)
Anti -E	11(9.2%)
Anti -c	4(3.4%)
Anti - C^W	5(4.2%)
Anti -K	18(15.1%)
Anti -Fy^a	1(0.8%)
Anti -Jk^a	1(0.8%)
Anti -Le^a	4(3.4%)
Anti -Le^b	2(1.7%)
Anti -S	2(1.7%)
Anti -M	39(32.8%)
Anti -P1	5(4.2%)
Anti -Lu^a	2(1.7%)
Unspecified antibodies	14(11.8%)
Total (n) %	119(100%)

The frequency of irregular RBC antibodies was 0.11% in female donors, which was higher compared to male donors in whom the frequency was 0.03%. The most frequent anti-M antibody was equally represented in both genders. In female donors, the most frequent antibodies were anti-K (0.03%), anti-D (0.02%) and anti-E (0.01%).

Table 4. Prevalence of specific irregular RBC antibodies in blood donors by gender

Antibody specificity	Male donors (n) (n) %	Female donors (n) %	Total prevalence n (%)
Anti-D	1(0.0004)	8(0.02)	9(0.0035)
Anti -C	0	2(0.004)	2(0.00079)
Anti -E	4(0.001)	7(0.01)	11(0.0043)
Anti -c	2(0.0009)	2(0.004)	4(0.0015)
Anti - C^W	4(0,001)	1(0.002)	5(0.0019)
Anti –K	3(0.001)	15(0.03)	18(0.0071)
Anti -Fy^a	0	1(0.002)	1(0.00039)
Anti -Jk^a	0	1(0.002)	1(0.00039)
Anti -Le^a	2(0.0009)	2(0.004)	4(0.0015)
Anti -Le^b	2(0.0009)	0	2(0.00079)
Anti -S	1(0.0004)	1(0.002)	2(0.00079)
Anti -M	31(0.015)	8(0.016)	39(0.015)
Anti -P1	5(0.002)	0	5(0.0019)
Anti -Lu^a	1(0.0004)	1(0.002)	2(0.00079)
Unspecified antibodies	10(0.004)	4(0.01)	14 (0.0055)
Total (n) %	66(0.03%)	53(0.11%)	119(0.047)

Table 5. Blood donors with multiple irregular RBC antibodies by gender

Antibody specificity	Male donors n (%)	Female donors n (%)
Anti-D+C	/	2(0.004)
Anti–c+K	1(0.0004)	/
Anti-C^W+K	1(0.0004)	/

Table 6. Blood donors with autoantibodies (DAT+) by gender

Autoantibodies (DAT+)	Number (n)	Percentage (%)
Male donors	27	0.01
Female donors	11	0.02
Total	38	0.015

Table 7. Distribution of specific RBC antibodies in blood donors per year (2020-2024)

Antibody specificity	Irregular RBC antibodies in blood donors per year (2020-2024)				
	2020	2021	2022	2023	2024
Anti-D	2		1	4	2
Anti -C	1			1	
Anti -E	2	4	2	2	1
Anti -c		1	1	1	1
Anti - C^W	1	1		2	1
Anti –K	4	4	5	3	2
Anti -Fy^a					1
Anti -Jk^a					1
Anti -Le^a	1	2	1		
Anti -Le^b		1		1	
Anti -S			1	1	
Anti -M	2	14	6	10	7
Anti -P1		1	2	1	1
Anti -Lu^a		1			1
Unspecified antibodies		3	2	5	4
Total (n)	13	32	21	31	22

In female donors, there was a higher frequency of multiple identified specific irregular RBC antibodies compared to male donors.

The frequency of autoantibodies in blood donors was 0.015%, of which 0.02% were in female donors and 0,01% were in male donors (Table 6).

In the observed period of 5 years, the most frequent irregular RBC antibody was anti-M, with the highest frequency in 2021 and 2023. The frequency of clinically significant anti-E and anti-K antibodies showed tendency of decreasing in the analyzed period (Table 7, Figures 1 and 2).

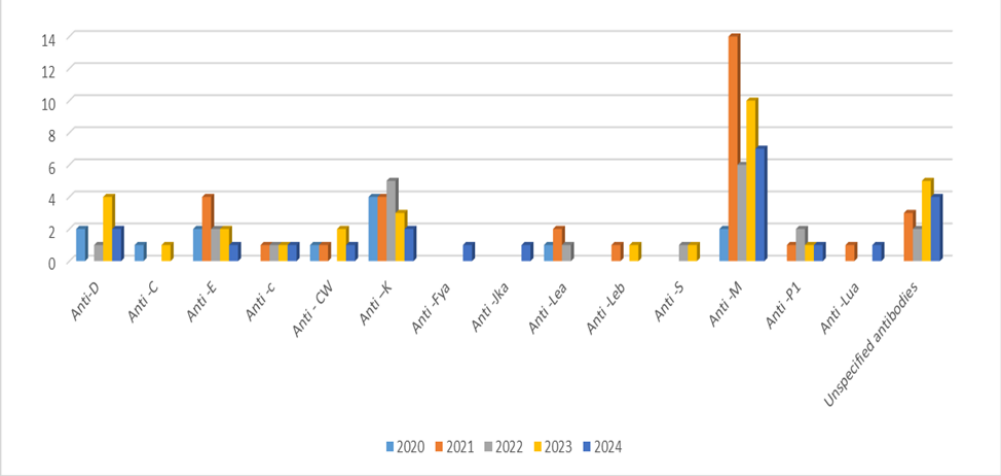

Fig. 1. Distribution of specific RBC antibodies in blood donors per year (2020-2024)

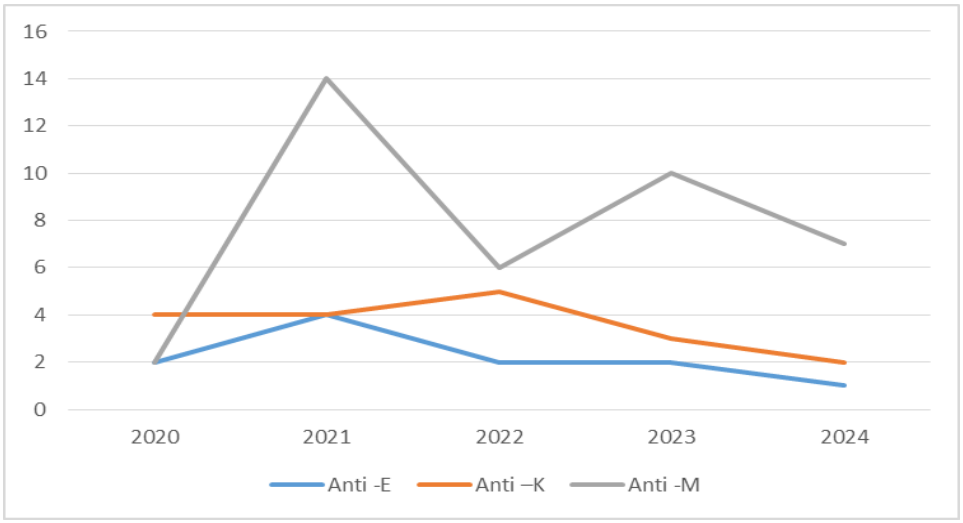

Fig. 2. Frequency trend of the most prevalent RBC antibodies

Discussion

The detection rate of irregular RBC antibodies depends on the test erythrocytes and the screening method. When screening blood donors, erythrocytes can be pooled, i.e., a mixture of up to two donor RBCs of blood group O and Rh phenotype CCDee and ccDEE.

The following antigens must be present on at least one RBC included in the pool: D, C, E, c, e, M, N, S, s, P, Lea, Leb, K, Fya, Fyb, and Jkb. IAT performed by microagglutination technique is currently considered the most sensitive method for the detection of clinically significant RBC antibodies[7,8].

Until the introduction of automation in 2007, screening of blood donors in our country was performed by using an enzyme treated RBC, which is one of the reasons for the higher observed prevalence of irregular RBC antibodies (1%) among blood donors in comparison to the analyzed period in which the frequency was 0.05%.

The prevalence of irregular RBC antibody in our blood donors observed in 2009 and in 2014 was 0.12% and 0.07% respectively, which is also higher in comparison with the analyzed period in this study[9].

The current prevalence of irregular RBC antibodies is 0.05%, with non-significant variations in the investigated period of five years (0.03% in 2020, 0.06% in 2021, 0.04% in 2022, 0.06% in 2023 and 0.04% in 2024). The low prevalence of irregular RBC antibodies in our donor population, which ranges from 0.03% to 0.06%, is a result of the donor selection process and the usage of IAT as a screening technique performed with the microagglutination technique. This contributes to the high sensitivity of the screening and to the reduction of false positive reactions. The prevalence of irregular RBC antibodies is very low in most European countries where blood donation is voluntary and unpaid, with well-established criteria for donor selection, similar to our donor population. In comparison with our data, alloimmunization among blood donors is even rarer (< 0.001%) in other European countries[10].

Certain studies report a significantly higher prevalence of irregular RBC antibodies in blood donors: from 0.17% in blood donors in India to much higher in Brazil with 0.4% occurrence[11,12].

Naturally occurring antibodies (anti-M, anti-Le^a or anti-Le^b) are detected with higher prevalence in donors younger than 30 years. The most frequent antibody was anti-M, representing 33% of all identified antibodies. It was equally represented in both genders, with a frequency of 0.015%.

Anti-M, although naturally occurring in most cases, can be clinically significant, especially if reactive at 37ºC, as detected in our blood donors.

The most frequent clinically significant antibodies were anti-E and anti-K with frequency of 0.0043% and 0.0071%, respectively. These antibodies are alloantibodies which are a result of previous blood transfusion or pregnancy in blood donors. Both antibodies (anti-E and anti-K), showed decreasing tendency in the analyzed period which could be due to the established transfusion practice of donor-recipient matching of RBC units according to the Rh and Kell phenotype, particularly for polytransfused patients, children and women in the reproductive period.

Analysis of irregular RBC antibodies by gender showed a significantly higher prevalence (0.11%) of irregular RBC antibodies in female donors compared to male donors (0.03%), which is probably due to a greater possibility of alloimmunization during pregnancy. In female donors, the clinically significant antibodies - anti-E (0.01%) and anti-K (0.03%) - were also more frequent compared to male donors in whom the frequency of anti-E and anti-K was 0.001% each.

Anti-D antibodies were observed almost exclusively in female donors, which frequency of 0.02%, most likely due to previous pregnancies. The reduced prevalence of anti-D antibodies (0.0035%) in comparison with 0.0037% prevalence in the period 2018-2021, and 0.014% in 2009-2014, supports better prevention of Rh D immunization in pregnant women during the observed period[13].

There was also higher occurrence of two identified specific irregular RBC antibodies (anti-D+C) in female donors in comparison with male donors.

Considering the number of first-time donors, accounting with 17%, and the fact that 47% of detected antibodies in our donor population are clinically significant, caution is warranted when deciding to change the existing strategy for universal screening of irregular RBC antibodies for every donor and every blood donation with a selective screening strategy.

Such a strategy would include screening for RBC antibodies in only first-time donors and those who have had potentially immunizing events (transfusion or pregnancy)[14].

This strategy for selective screening has been proposed in some countries where the antibody screening is performed in first-time donors and after pregnancy, blood transfusion or a significant break between donations (>2 years).

Despite the low frequency of RBC antibodies observed in blood donors, with the prevalence of 0.05%, 37% were clinically significant with the potential of hemolysis of recipient RBC. Antibodies from Rh blood group system were represented with 22% of all identified antibodies, and antibodies from Kell blood group system were represented with 15%.

Anti-Rh alloantibodies are IgG class, formed after sensitization, most often after blood transfusion or pregnancy. Anti-Rh antibodies sometimes activate complement, particularly IgG1 and IgG3 subclasses. As incomplete antibodies, they usually cause delayed hemolytic transfusion reaction with extravascular hemolysis, but sometimes with the activation of complement they can cause acute intravascular hemolysis and can lead to renal failure, shock and even death[15].

The findings of this study are very important, because discarding donations with positive RBC screening helps prevent hemolytic transfusion reactions in recipients of plasma products.

Anti-K1 antibodies in donor plasma can cause a severe hemolytic transfusion reaction after transfusion into patients who pose K antigen on the membrane of their erythrocytes. If these blood components are transfused to pregnant women, they may cause suppression of erythropoiesis in a fetus carrying K antigen inherited from the father[16].

Established anti-Rh and anti-K1 antibodies can be rarely found as antibodies that are naturally occurring without alloimmunization event in blood donors and they are formed without a known external trigger, such as transfusion or pregnancy. They can also lead to transfusion reactions or hemolytic disease of the fetus and newborn (HDFN)[17].

Therefore, the commitment of testing every blood donation is very important to prevent these severe hemolytic reactions.

In the group of first-time donors, 89 (0,2%) had a positive screening for irregular RBC antibodies, which represent high prevalence of irregular RBC antibodies in this group. All blood donors with positive screening were laid on further testing to identify the specificity of the irregular RBC antibodies, by using commercially available 11-cell identification panel and subsequent control testing, during the follow up of these blood donors. Blood donors with constant presence of irregular RBC antibodies were 101 blood donors, they got permanent deferral in this observed period of 5 years. With antibody screening in every blood donation, data suggest acceptable blood loss and blood donor deferral, especially since safe blood was provided to patients.

In our blood donors, anti-M antibodies had the highest prevalence, accounting for 33% of all detected antibodies. They are most commonly naturally occurring, but were reactive at 37˚ C by IAT. Anti-M antibodies most often react optimally at 4˚C with negligible hemolytic potential which is not life threating, but in some cases anti-M antibody could react at 37˚ C and can cause acute or delayed HTR, and rarely HDFN[18].

With undetermined specificity were 12% of all detected antibodies, and were likely nonspecific and naturally occurring. If we compare this result with a previous study involving our blood donors (2009-2014), this represents a higher percentage of antibodies with undetermined specificity, accounting for 45% in that period[9].

Every donation with detected irregular RBC antibodies, specific or unspecific is discarded, which prevents passive transfer of RBC antibodies from a blood donor to a patient. This strategy contributes to safer blood transfusions, especially when fresh frozen plasma, cryoprecipitate and platelets components are to be transfused[4,19-21]. All blood donations with

positive direct antiglobulin test (DAT+) total 38 (0,015%), were also discarded. Autoantibodies may be detected in healthy blood donors, pregnant women and in patients with autoimmune diseases. These autoantibodies may be connected with SARS-CoV-2 infection and immunization. The results from some studies suggest a significant impact of SARS-CoV-2 infection on RBC structural membrane homeostasis. When microorganism-produced toxins damage the RBC membrane, leading to hemolysis, previous crypt-antigens are exposed and they are receptors for certain microbes and antibodies, contributing to the autoimmune component[22-25].

Blood and blood products from donors with a confirmed positive screening for irregular RBC antibodies, regardless of the specificity of the antibodies, are not intended for clinical use, and the donor receives a temporary or permanent deferral for donation based on the repetitive control tests according to existing algorithm. The donor is informed in writing and invited for re-testing in a period of 4 to 6 months. The status of the blood donor is determined depending on the nature of the identified RBC antibody and the results of the control tests.

In case of clinically significant antibodies, the donor is permanently deferred, if the result is confirmed in the control test.

The same dynamics of control testing and donation deferral apply to donors who consistently detect non-specific RBC antibodies, which are more frequent in female donors with 0.01% prevalence. If the control screening is negative, the donor is notified in writing and the blood donor is reinstated in the blood donation process.

Conclusion

Antibody screening in blood donors is essential to detect immunization to RBC antigens, to remove blood units with positive screening from clinical use, which enables prevention of acute or delayed hemolytic transfusion reaction in blood recipients.

The prevalence of irregular RBC antibodies in our blood donors is 0.05%, which is considered to be very low. This is due to the good selection of donors, and above all to the sensitive laboratory methods used for screening, which enable false positive and non-specific reactions to be minimized.

The strategy for screening of irregular RBC antibodies in every blood donor and at every donation provides safer blood transfusion and at the same time maximal prevention of hemolytic transfusion reactions.

The low frequency of irregular RBC antibodies still raises the question of the cost-effectiveness of the existing screening strategy, but we have to take into consideration the logistical and organizational costs to change current practice without impact on blood safety.

Conflict of interest statement. None declared.

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