ORIGINAL ARTICLE

UDC: 615.381
DOI: https://doi.org/10.2298/VSP231105022R

Prevalence and identification of irregular erythrocyte antibodies in voluntary blood donors in the territory of Southeast Serbia

Prevalencija i identifikacija iregularnih eritrocitnih antitela kod dobrovoljnih davalaca krvi na teritoriji Jugoistočne Srbije

Milica Randjelović*, Ana Veličković*, Jelena Vojinović†‡, Zoran Stanojković*

***Blood Transfusion Institute of Niš, Niš, Serbia; †University Clinical Center of Niš, Pediatric Internal Diseases Clinic, Niš, Serbia; ‡University of Niš, Faculty of Medicine, Niš, Serbia**

Abstract

Background/Aim. The purpose of tests for transmittable diseases, as well as determining ABO group, Rh typing, and the presence of clinically significant erythrocyte antibodies, is to enable the appropriate selection of blood/blood components for transfusion, to prevent or minimize adverse effects of transfusion, and to identify donors whose units of blood/components are not suitable for transfusion. The aim of the study was to evaluate the presence of irregular erythrocyte antibodies as well as to determine the specificity and origin of these antibodies in voluntary donors. **Methods.** This prospective observational study, conducted from January until July 2023, included samples of voluntary blood donors (BDs) for antibody screening in the population of voluntary BDs in the territory of Southeast Serbia. A total of 23,082 samples from healthy BD were examined through this period. **Results.** The overall prevalence of irregular anti-erythrocyte antibodies was 0.1125%, with anti-K, anti-D, and anti-E being the most frequently identified alloantibodies. Out of a total of 26 donors with positive screening results, 11 (42.32%) had alloantibodies, and 15 (57.68%) had antibodies of undetermined specificity. A positive antibody test was observed more frequently in male donors, and the age range of 51 years and above accounted for the majority of positive cases. **Conclusion.** Immunohematological testing, including antibody screening in voluntary BDs, is important for safe blood transfusion and is of great clinical importance. Likewise, it reduces the risk of complications due to incompatible transfusions.

Key words:
abo blood-group system; antibodies; blood donors; serbia.

Apstrakt

Uvod/Cilj. Svrha testiranja na transmisivne bolesti, kao i određivanje ABO grupa, Rh tipizacije i prisustva klinički značajnih eritrocitnih antitela je da se omogući odgovarajući izbor krvi/komponenti krvi za transfuziju, spreče ili svedu na minimum neželjeni efekti transfuzije i da se identifikuju davaoci čije jedinice krvi/komponente nisu pogodne za transfuziju. Cilj rada bio je da se utvrdi prisustvo iregularnih eritrocitnih antitela, kao i da se odredi specifičnost i poreklo ovih antitela kod dobrovoljnih davaoca. **Metode.** Prospektivnom opservacionom studijom, sprovedenom od januara do jula 2023. godine, obuhvaćeni su uzorci dobrovoljnih davalaca krvi za „skrining“ na antitela u populaciji dobrovoljnih davalaca krvi na teritoriji Jugoistočne Srbije. U ovom periodu, pregledano je ukupno 23 082 uzoraka zdravih davalaca krvi. **Rezultati.** Ukupna prevalencija iregularnih anti-eritrocitnih antitela iznosila je 0,1125%, pri čemu su anti-K, anti-D i anti-E aloantitela bila najčešće identifikovana. Od ukupno 26 davaoca sa pozitivnim „skriningom“, njih 11 (42,32%) je imalo aloantitela, a 15 (57,68%) je imalo antitela neutvrđene specifičnosti. Pozitivan test na antitela češće je utvrđen kod donora muškog pola, a većina pozitivnih slučajeva bila je u životnom dobu od 51 godine i starijih. **Zaključak.** Imunohematološko testiranje, uključujući „skrining“ antitela kod dobrovoljnih davalaca krvi, važno je za bezbednu transfuziju krvi i ima veliki klinički značaj. Takođe, ovo testiranje smanjuje rizik od komplikacija usled nekompatibilnih transfuzija.

Ključne reči:
krvne grupe, abo sistem; antitela; krv, davaoci; srbija.

Correspondence to: Milica Ranđelović, Blood Transfusion Institute of Niš, Bulevar dr Zorana Đinđića 48, 18 000 Niš, Serbia. E-mail: milicarandjelovic990@gmail.com

Introduction

Blood groups are antigens located on the erythrocyte membrane, which represent the hereditary characteristics of each person and can be proven by specific antibodies. They reflect fine structural differences in the composition of the erythrocyte membrane. The Working Party on Red cell Immunogenetics and Blood Group Terminology of the International Society for Blood Transfusion (ISBT) has, so far, registered 360 antigens. These proven blood group antigens are divided into 45 blood group systems, of which the most important in blood transfusion are the ABO and Rh blood group systems [1].

The erythrocyte antigens vary significantly in their composition and can be proteins (Rh, M, and N), glycolipids (ABH, Lewis, Ii, and P), or glycoproteins. All this affects the different immunogenicity of blood group substances [2].

Anti-A and anti-B anti-erythrocyte antibodies are naturally occurring antibodies present in human serum that are routinely tested and found depending on the blood type. Any other unexpected antibodies are called irregular red blood cell (erythrocyte) antibodies. Unexpected erythrocyte antibodies may be either IgG class or IgM class antibodies and may cause agglutination, hemolysis, or sensitization. Most of the clinically significant antibodies are the IgG class reacting at a temperature of 37 °C. The IgM class antibodies react optimally at a temperature of 22 °C or lower. Some antibodies require special techniques to be detected. The *in vitro* procedure for the detection of unexpected erythrocyte antibodies involves screening procedures that investigate unknown serum/plasma (donors' or patients') with known test cells (reagents) at different temperature conditions, in different environments, and with different incubation times [3]. There are two types of irregular antibodies to erythrocytes: alloantibodies and autoantibodies. Alloantibodies are circulating, naturally occurring, immune antibodies that appear as a result of contact with foreign erythrocyte antigens. For anti-erythrocyte alloantibodies, exposure occurs during pregnancy, blood transfusion, or transplantation. Autoantibodies react against one's own tissue. They can cause a post-transfusion reaction, and sometimes, it is difficult to determine their specificity. They react in a wide temperature range. The transfusion management of such patients is quite complex [4].

In terms of their clinical importance, the anti-erythrocyte antibodies are divided into the following: clinically significant antibodies − Rh (C, E, c, e), Kell (K, k, Ku), Duffy (Fy^a, Fy^b, Fy3), Kidd (Jk^a, Jk^b, Jk3), Diego (Di^a, Di^b, Wr^b), MNS (S, s); antibodies with variable clinical significance − MNS (U, Vw, Mur), Vel, Ge, Yt^a, Hy; antibodies usually without clinical significance − Lutheran (Lu^a, Lu^b), Lewis (Le^a, Le^b), MNS (M, N), A1, P1, Cw; antibodies of unconfirmed significance – Chido/Rodgers (Ch^a, Rg^a), JMH, Bg, Cs^a, Xg^a [5].

Erythrocyte antibodies that exist in healthy blood donors (BDs) can be responsible for serious hemolytic post-transfusion reactions in blood recipients. Fairly uncommon are hemolytic reactions caused by irregular erythrocyte antibodies (IEAs) in the blood of donor [6]. Nevertheless, in neonatal and pediatric recipients, as well as in cases that require massive transfusion, they can be responsible for a grave post-transfusion reaction [7].

The scope of mandatory immunohematological testing of BDs in our country is defined by legislation and is carried out during each blood donation. Sample testing of all voluntary donors of blood/blood components is performed at each donation of blood in the same degree with the minimum requirements according to the Guide and Standard Operating Procedures that describe each step in all work procedures [8, 9].

Each collected unit of blood/blood component is subject to at least immunohematological tests and immunoserological tests. The authorized blood transfusion institution performs automated immunohematological and immunoserological testing of BD samples [9].

Through immunohematological testing of BD samples, the authorized blood transfusion institution determines the following: ABO blood group, presence or absence of RhD antigens, Rh phenotype (C, c, E, e) in those being RhD antigen-negative, and presence of IEAs [10].

In the case of BDs who donate blood/blood component for the first time, it is mandatory to perform a double determination of the ABO blood group system (forward and reverse ABO blood typing with determination of antigens and corresponding antibodies and confirmatory ABO blood typing with determination of antigens) and the presence of the RhD antigen, whereby the determination must be automated. It is mandatory to use the monoclonal test reagents of different clones and/or polyclonal test reagents of different series [4].

To determine the RhD antigen, it is necessary to use an anti-D test serum that detects the D^{VI+} variant. The presence of a weak variant of the RhD antigen is determined by the indirect antiglobulin testing method. The Rh phenotype of RhD-negative BD is determined during the first and second donation.

Anti-A1 lectin is used to determine subgroups of the ABO system [9].

In the case of multiple donors of blood/blood components, the determination of the ABO blood groups system and the presence of the RhD antigen is performed using a single automated technique [9].

The presence of IEAs in BD samples is tested by an indirect antiglobulin test using a single test reagent of a mixture of test-erythrocytes (two donors) that must be phenotyped at least for Rh (D, C, c, E, e) and Kell (Kk) system antigens [9].

Units of blood/blood components with consistent results in immunohematological testing can be validated, labeled, and used for therapeutic purposes. For units of blood/blood components that had a positive screening for IEAs during immunohematological testing, additional tests are carried out [9].

Additional immunohematological tests include all other tests that should fully define the status of the blood/blood component units. Among additional immunohematological tests, particular focus is placed on the determination of antibody specificity and extended phenotyping to different blood group systems [4].

If the IEAs screening result is positive, the release of blood units is prevented, and the BD sample is additionally tested according to the algorithm for additional testing of BD samples in case of a positive IEAs screening result. If the result of screening for IEAs (with pooled erythrocytes) is positive, additional tests are performed, such as screening (with test reagents consisting of phenotyped erythrocytes), the direct antiglobulin test (DAT), and antibody identification. If the DAT result is positive, the erythrocytes are discarded, and the donor is informed and excluded from further blood donation. When antibodies are not identified, the whole blood, platelets, and plasma are blocked, and erythrocytes can be used. All data must be entered into the database and available when testing the next blood donation. If the screening result is positive, and non-specific antibodies are detected, the whole blood, platelets, and plasma are blocked, and erythrocytes can be used. If specific antibodies are detected in a positive screening, the unit of blood is discarded, and the donor is informed and permanently excluded from further blood donation [9].

The aim of this study was to investigate the incidence of IEAs, as well as determine the specificity and origin of IEAs, in voluntary BDs in the territory of Southeast Serbia.

Methods

The research was conducted according to the valid permission of the Ethics Committee of the Blood Transfusion Institute of Niš, Serbia (No. 1557, from May 15, 2023). All voluntary BDs included in the study signed the approval. All BDs were screened after completing the BD questionnaire form. This form included the basic profiles of the donors (name, age, gender, address, etc.), medication intake, medical history, tests or treatment, jaundice, high-risk behavior, and any other clinically relevant illness. The BD questionnaire form does not contain questions about previous pregnancies and previous blood transfusions; these data were not subsequently collected. Antibody screening of voluntary BDs was performed as a routine immunohematological procedure. Samples from donors of blood/blood components were tested for erythrocytes antibody screening from January until July 2023. For the examination period, samples from 23,082 healthy BDs, 19,358 (83.87%) male and 3,724 (16.13%) female persons, were tested for erythrocyte antibodies.

Venous blood samples of 5 mL from each BD were collected in a container with an anticoagulant. Ethylenediaminetetraacetic acid was used as an anticoagulant. For the processing of samples from all voluntary BDs, fully automated systems were used for immunohematological blood group testing and antibody screening using the microtiter plate method (NEO IRIS, Immucor Inc., Norcross, GA, USA) and the micromethod gel test (Erytra, Grifols, Barcelona, Spain). Positive screening samples were further tested to identify the specificity of the present IEAs. Identisera Diana Extend and Identisera Diana Extend P panels (12–15 cells) were used for the identification of IEAs by the micromethod gel test (Erytra, Grifols, Barcelona, Spain) and for the identification of antibodies on microtiter plates (NEO IRIS, Immucor Inc., Norcross, GA, USA), Capture-R Ready-ID panels were used. The manufacturer's instructions were strictly followed [10, 11].

Results

During the study period, 23,082 IEA screenings were performed. Of the total number of tested samples, 19,358 (83.87%) were male, and 3,704 (16.13%) were female. Positive IEA screening was recorded in 26 (0.1125%) BDs, more precisely, in 15 (57.68%) males and 11 (42.32%) females. From a total of 26 cases, 11 donors (5 men and 6 women, i.e., 42.32%) had specific anti-erythrocyte alloantibodies, and 15 (10 men and 5 women, i.e., 57.68%) had a positive screening for erythrocyte antibodies of undetermined specificity (Table 1).

The average age of donors with positive IEA screening ± standard deviation (SD) was 48.81 ± 9.46 years. The highest number (n = 7; 26.91%) of donors who had positive IEA screening were in the age group 51–55 years (Table 2).

Table 1

Profile of the donors tested for irregular erythrocyte antibody (IEA) screening

Group	Male	Female	Total
Total donors	19,358 (83.87)	3,724 (16.13)	23,082
IEA positive	15 (57.68)	11 (42.32)	26
Positive IEA screening with inconclusive results	10 (76.91)	5 (19.23)	15

All values are expressed as numbers or numbers (percentages).

Table 2

Distribution of blood donors with positive irregular erythrocyte antibody screening results according to age

Age group (years)	Number of positive donors
18–25	–
26–30	1
31–35	2
36–40	3
41–45	3
46–50	3
51–55	7
56–60	4
61–65	3
Total	26

Table 3

The characteristics of blood donors with positive irregular erythrocyte antibody screening

Blood donors	n (%)
Gender	
male	15 (57.68)
female	11 (42.32)
ABO blood group	
A	7 (26.92)
B	7 (26.92)
AB	5 (19.23)
O	7 (26.92)
Rh blood group	
D-positive	21 (80.77)
D-negative	5 (19.23)

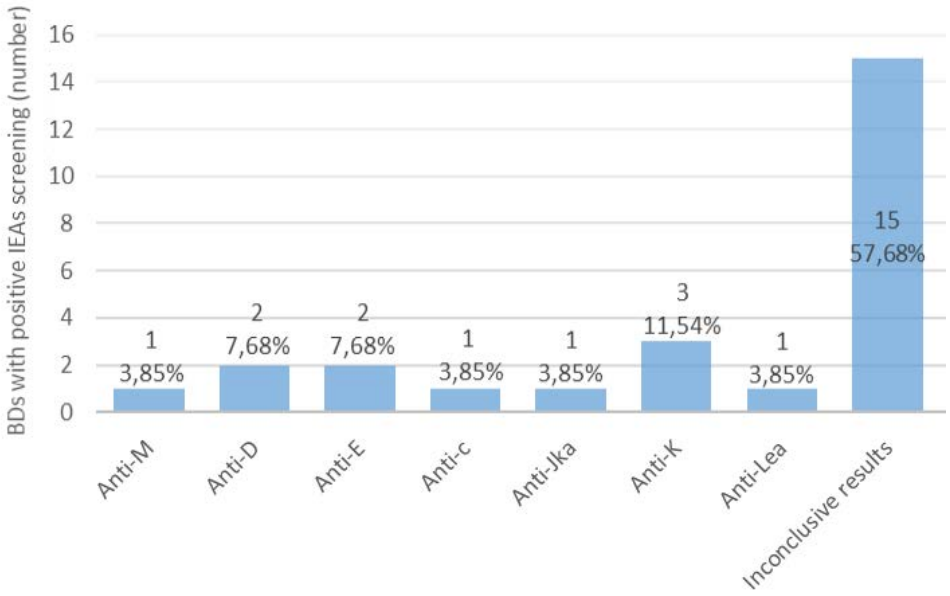

Fig. 1 – Distribution of irregular erythrocyte antibodies (IEAs). BDs – blood donors.

From the total number of positive IEA screening tests, the A, O, and B blood groups had 7 (26.92%) cases each, while 5 (19.23%) cases were with the AB blood group. Regarding the Rh blood group system, the majority of cases, 21 (80.77%), were D-positive, while 5 (19.23%) were D-negative (Table 3).

The most frequently identified IEA antibody was anti-K with 3 (11.54%) cases, followed by anti-D and anti-E, with 2 (7.68%) cases each, and anti-M, anti-c, anti-Jk^a, and anti-Le^a, with one (3.85%) detected case each (Figure 1).

Discussion

Notably, there is diversity in the occurrence and specificity of IEAs found through screening. This depends on participants, blood group, genotype, screening method, and used platforms [12].

Various studies involving healthy BDs have shown an alloimmunization frequency in the range from 0.05 to 4.0% [13–18]. According to this research, the comprehensive prevalence of anti-erythrocyte antibodies was 0.1125%.

In similar studies conducted by others, the prevalence of anti-erythrocyte antibodies was 0.05% [19]. A study conducted in the Delhi population reported a prevalence of 0.09% among the whole BD population [20].

Just the opposite, Giblett [21] revealed that the prevalence of anti-erythrocyte antibodies in BDs was 0.32%. Some studies announced that the percentage of BDs varies between 0.32% and 2.4% [21, 22]. A study in Minnesota from 2001 reported a prevalence of 0.89% [23].

In our consideration, the highest frequency of irregular anti-erythrocyte alloantibodies was identified in BDs in the age group 51–55. Our data present that the incidence of alloantibodies increased with age, and the age range of 51 years and older accounted for the majority of positive cases. This may be caused by the greater availability of alloantigens in the elderly people. The most important reasons responsible for anti-erythrocyte alloantibodies in healthy adult donors are previous pregnancies and transfusions [19]. During pregnancy, females are exposed to alloantigen more frequently and subsequently have a higher possibility of being alloantibody positive.

The most frequent anti-erythrocyte antibodies identified were from the Kell system, namely the anti-K antibody, with a frequency of 11.54%. This antibody is the primary cause of hemolytic disease of the newborn (HDN) and hemolytic transfusion reaction elsewhere [24–26].

The most common anti-erythrocyte alloantibodies in Western Europe and the United States are alloantibodies against Rh and Kell antigens [27]. In Olmsted County, the most frequent alloantibody was anti-E, and the second most frequent alloantibody was anti-K. Clarification for this distinction lies in divergent improvement methods. In examinations that use techniques that improve the recognition of Rh-specific antibodies, anti-E is identified more frequently [23].

Furthermore, in the survey by Reyhaneh et al. [28], the most abundant anti-erythrocyte antibodies were anti-Kell (23.53%), anti-E (20.59%), and anti-c (17.56%), which is a similar result to the prevalence of antibodies against antigen K (11.54%) and E (7.68%) in our study.

The anti-erythrocyte antibodies of the Kell system are mainly IgG class, most often IgG1. They are considered clinically significant because they react at a temperature of 37 °C and can induce complement activation, leading to acute or delayed hemolytic reactions, while in pregnancy, they can lead to HDN [29]. There are many considerations in the literature as to why the anti-K antibody leads to very severe forms of hemolytic disease of the fetus and the newborn in some cases and not in others, which is why determining the anti-K antibody titer is not a significant predictor in assessing the severity of the clinical picture in this disorder, as well as because of the way the antibodies destroy the erythrocytes of the fetus. Some published data indicate that anti-K antibodies produced by immunization in pregnancy cause severe forms of hemolytic disease more often than anti-K produced by stimulation after blood transfusion, but these claims have not yet been confirmed definitively. The determination of the anti-K antibody titer is not a significant prognostic factor for assessing the severity of the clinical picture of hemolytic disease, mainly due to insufficient experience of the staff in the technical performance. It has been established that if an individual determines the anti-K antibody titer daily, their accuracy improves immeasurably. In any case, the anti-K antibody appears to have a tendency to lead to much more severe forms of hemolytic disease at lower titers than most other alloantibodies [4].

The most abundant anti-erythrocyte antibodies, after anti-K, in this study were against antigens D (7.68%) and E (7.68%). Ameen et al. [22] also listed anti-D as the most common antibody. In this study, both donors with anti-D antibodies were multiparous women who may have developed anti-D from previous pregnancies or blood transfusions. Similar results were obtained by Nathani et al. [30], where the most common anti-erythrocyte antibody was anti-D (19.17%).

Antigen D is extremely immunogenic. The anti-D anti-erythrocyte antibody is mainly of the IgG class, although some tested sera also contain an IgM component. Despite the successful use of RhD immunoprophylaxis, anti-D is still the most common cause of hemolytic disease of the fetus/neonate [31, 32].

Antigens E and c are highly immunogenic, and anti-E and anti-c anti-erythrocyte antibodies are often demonstrated after transfusions of blood components containing erythrocytes. Anti-E antibody is the most commonly demonstrated anti-erythrocyte antibody in examined hospitalized patients, but anti-c is the second most frequent cause of hemolytic disease of the fetus and the newborn in Great Britain [31]. It should be noted that anti-c antibody is involved in much more complex and severe forms of hemolytic transfusion reactions than anti-E. Anti-E antibody usually does not cause the HDN, and if it does, it is mild in nature [4]. In the study conducted by Al-Joudi et al. [33], anti-E was the most frequent antibody, and this outcome is also comparable to the research by Thakral et al. [34] and Zaman et al. [35].

The least represented irregular anti-erythrocyte antibodies in this research are anti-c, anti-M, anti-Le^a, and anti-Le^b, with one case each and a frequency of 3.85%, respectively.

The anti-M anti-erythrocyte antibody is usually a clinically benign IgM class antibody, although some examples containing an IgG component may react *in vitro* at 37 °C in an indirect antiglobulin test. If there is an anti-M antibody that does not react at 37 °C, the compatible blood in the cross-reaction test does not need to be tested for the presence of the antigen M [32].

All anti-Kidd anti-erythrocyte antibodies, especially the anti-Jk^a antibody, are known to be labile both *in vivo* and *in vitro*. Anti-Kidd antibodies are usually IgG class and induce complement activation more frequently than most other IgG class antibodies. Antibodies of this blood group system often show dosage effects and can cause a severe, and often fatal, delayed transfusion reaction. In contrast, anti-Kidd antibodies, except for one or two isolated cases, cause mild forms of hemolytic disease in the fetus and the newborn [36, 37].

Anti-Le^a anti-erythrocyte antibody is usually an IgM class and has very rarely been reported to cause a hemolytic transfusion reaction, while anti-Le^b antibody, which is usually IgM, is clinically insignificant. None of the antibodies cause clinically significant hemolytic disease in the newborn [38].

Regarding the prevalence of unexpected erythrocyte antibodies in the ABO blood groups of the BDs, it has been established that A, B, and O blood groups had equal representation (26.92%), while Rh-positive BDs had higher frequency (80.77%) of unexpected antibodies, which is correlated with the total representation of these blood groups in our population [39].

Our study has determined that IEAs were unidentified in 15 (57.68%) positive cases, which is a higher prevalence compared to similar studies [18].

Conclusion

We established that the total prevalence of unexpected alloantibodies against erythrocytes antigens was 0.1125%, with anti-K, anti-D, and anti-E being the most frequently identified alloantibodies in BDs in our Institute, followed by antibodies to other Rh antigens. A positive antibody test was observed more frequently in male donors and donors aged 51

years or above. It was identified that donors of A, B, and O blood groups had an equal number of positive antibody screenings. The reason may be because of the higher frequency of men as BDs, as well as exposure during past transfusions.

The study showed that immunohematological testing of voluntary BDs is necessary in order to improve the quality and safety of blood transfusion in recipients and that blood screening of voluntary donors for the presence of anti-erythrocyte alloantibodies is of great clinical importance.

R E F E R E N C E S

1. *International Society of Blood Transfusion.* Red cell Immunogenetics and Blood Group Terminology [Internet]. Amsterdam, the Netherlands: ISBT; 2023 [cited on 2024 Feb 22]. Available from: https://www.isbtweb.org/isbt-working-parties/rcibgt.html
2. *Stites DP, Terr AI.* Basic and clinical immunology. 7th ed. Norwalk, CT: Appleton and Lange; 1991. 817 p.
3. *Mollison PL, Engelfriet CP, Contreras M.* Blood Transfusion in Clinical Medicine. 9th ed. London: Blackwell Scientific; 1993. 1056 p.
4. *Kovač M, Balint B, Bogdanović G.* Basic and clinical transfusionology. Beograd: Medicinski fakultet Univerziteta u Beogradu, Akademska misao; 2020. 540 p. (Serbian)
5. *Shaz BH.* Antibody identification. In: *Shaz BH, Hillyer CD, Zimring JC, Abshire TC,* editors. Transfusion medicine and hemostasis: clinical and laboratory aspects. Amsterdam: Elsevier; 2009. p. 102–10.
6. *Mohn JF, Lambert RM, Bowman HS, Brason FW.* Experimental transfusion of donor plasma containing blood-group antibodies into incompatible normal human recipients. I. Absence of destruction of red-Kell and anti-M. Br J Haematol 1961; 7: 112–29.
7. *Makroo RN, Rajput S, Agarwal S, Chowdhry M, Prakash B, Karna P.* Prevalence of irregular red cell antibody in healthy blood donors attending a tertiary care hospital in North India. Asian J Transfus Sci 2018; 12(1): 17–20.
8. *Council of Europe.* Guide to the preparation, use and quality assurance of blood components. Recommendation No. R(95)15. 21th ed [Internet]. France: Council of Europe; 2023 [cited on 2024 Feb 23]. Available from: https://www.edqm.eu/en/blood-guide
9. *Legal information system of the Republic of Serbia.* Rulebook on the quality in the field of transfusion medicine. Official Gazette of the Republic Serbia, No 6/2019 [Internet]. Serbia: Official Gazette of the Republic Serbia; 2019 [cited on 2024 Feb 23]. Available from: https://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/ministarstva/pravilnik/2019/6/9 (Serbian)
10. *Legal information system of the Republic of Serbia.* Law on transfusion medicine, Official Gazette of the Republic Serbia, No 40/2017 [Internet]. Serbia: Official Gazette of the Republic Serbia; 2017 [cited on 2024 Feb 23]. Available from: https://www.pravno-informacionisitem.rs/SlGlasnikPortal/eli/rep/sgrs/skupstina/zakon/2017/40/2. (Serbian)
11. *Immucor.* NEO Iris, Operator Manual. Norcross. GA: Immucor; 2017. Available from: https://www.scribd.com/document/437855305/NEO-Iris-OperatorManual-JUNE2017
12. *Diagnostic Grifols.* Erytra, Instructions for Use. Barcelona, Spain: Grifols; 2016.
13. *Brecher ME.* Technical manual. American Association of Blood Banks. 14th ed. Bethesda: Maryland; 2002. p. 315–97.
14. *Kaur D, Bains L, Kandwal M, Parmar I.* Erythrocyte alloimmunization and autoimmunization among blood donors and recipients visiting a tertiary care hospital. J Clin Diagn Res 2017; 11(3): EC12–5.
15. *Promwong C, Siammai S, Hassarin S, Buakaew J, Yeela T, Soisangwan P,* et al. Frequencies and specificities of red cell alloantibodies in the Southern Thai population. Asian J Transfus Sci 2013; 7(1): 16–20.
16. *Karafin MS, Tan S, Tormey CA, Spencer BR, Hauser RG, Norris PJ,* et al. Prevalence and risk factors for RBC alloantibodies in blood donors in the Recipient Epidemiology and Donor Evaluation Study-III (REDS-III). Transfusion 2019; 59(1): 217–25.
17. *Keokhamphoui C, Urwijitaroon Y, Kongphaly D, Thammavong T.* Red cell alloantibodies in Lao blood donors. Southeast Asian J Trop Med Public Health 2014; 45(1): 194–7.
18. *Faramawy MSM, Hashem AEA, Mostafa GMH.* Red blood cell alloantibodies in healthy Egyptian blood donors. Egypt J Hosp Med 2018; 72(7): 4913–8.
19. *Pahuja S, Kushwaha S, Sethi N, Pujani M, Jain M.* Screening of blood donors for erythrocyte alloantibodies. Hematology 2012; 17(5): 302–5.
20. *Garg N, Sharma T, Singh B.* Prevalence of irregular red blood cell antibodies among healthy blood donors in Delhi population. Transfus Apher Sci 2014; 50(3): 415–7.
21. *Giblett ER.* Blood group alloantibodies: an assessment of some laboratory practices. Transfusion 1977; 17(4): 299–308.
22. *Ameen R, Al-Eyaadi O, Al-Shemmari S, Chowdhury R, Al-Bashir A.* Frequency of red blood cell alloantibody in Kuwaiti population. Med Princ Pract 2005; 14(5): 230–4.
23. *Winters JL, Pineda AA, Gorden LD, Bryant SC, Melton LJ 3rd, Vamvakas EC,* et al. RBC alloantibody specificity and antigen potency in Olmsted County, Minnesota. Transfusion 2001; 41(11): 1413–20.
24. *Lee S, Zambas ED, Marsh WL, Redman CM.* The human Kell blood group gene maps to chromosome 7q33 and its expression is restricted to erythroid cells. Blood 1993; 81(10): 2804–9.
25. *Marsh WL, Oyen R, Alicea E, Linter M, Horton S.* Autoimmune hemolytic anemia and the Kell blood groups. Am J Hematol 1979; 7(2): 155–62.
26. *Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IA.* Inhibition of erythroid progenitor cells by anti-Kell antibodies in fetal alloimmune anemia. N Engl J Med 1998; 338(12): 798–803.
27. *Natukunda B, Schonewille H, van de Watering L, Brand A.* Prevalence and specificities of red blood cell alloantibodies in transfused Ugandans with different diseases. Vox Sang 2010; 98(2): 167–71.
28. *Reyhaneh K, Ahmad G, Gharib K, Vida V, Raheleh K, Mehdi TN.* Frequency & specificity of RBC alloantibodies in patients due for surgery in Iran. Indian J Med Res 2013; 138(2): 252–6.
29. *Jovanović Srzentić S, Veljković DK.* Immunobiological and clinical significance of blood groups. Beograd: Intra.Net Communication; 2009. (Serbian)
30. *Nathani KJ, Patel J, Modi P, Pandya A, Wadhwani S, Jarag M.* Prevalence of red cell alloantibodies & autoantibodies in patient & donor attending a tertiary care hospital in South Gujarat. Int J Contemp Med Res 2021; 8(3): C1–5.
31. *Daniels G.* Human Blood Groups. 3rd ed. Oxford: Wiley Blackwell; 2013 p. 560.
32. *Fung MK, Grossman BJ, Hillyer CD, Westhoff CM.* ABO, H and Lewis blood groups and structurally related antigens, Rh system and other blood group system. In: *Fung MK, Grossman BJ, Hillyer CD, Westhoff CM*, editors. Technical manual. 18th ed. Bethesda, Maryland: American Association of Blood Banks; 2014. p. 304–51.

33. *Al-Joudi F, Ali AB, Ramli MB, Ahmed S, Ismail M.* Prevalence and specificities of red cell alloantibodies among blood recipients in the Malaysian state of Kelantan. Asian J Transfus Sci 2011; 5(1): 42–5.
34. *Thakral B, Saluja K, Sharma RR, Marwaha N.* Red cell alloimmunization in a transfused patient population: A study from a tertiary care hospital in north India. Hematology 2008; 13(5): 313–8.
35. *Zaman S, Chaurasia R, Chatterjee K, Thapliyal RM.* Prevalence and specificity of RBC alloantibodies in Indian patients attending a tertiary care hospital. Adv Hematol 2014; 2014: 749218.
36. *Daniels G.* Human Blood Groups. 1st ed. Oxford, UK: Blackwell Scientific; 1995.
37. *Matson GA, Swanson J, Tobin JD.* Severe hemolytic disease of the newborn caused by anti-Jka. Vox Sang 1959; 4(2): 144–7.
38. *Combs MR.* Lewis blood group system review. Immunohematology 2009; 25(3): 112–8.
39. *Vojvodić S.* Distribution of the ABO, Rh, MNSs, Kell and Duffy blood group antigens in the population of Vojvodina. Med Pregl 2003; 56(3–4): 173–7. (Serbian)

Received on November 5, 2023
Revised on November 30, 2023
Revised on February 15, 2024
Accepted on February 20, 2024
Online First April 2024