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The Impact of Extended Typing On Red Blood Cell Alloimmunization in Transfused Patients

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Abstract

BACKGROUND: Red blood cell (RBC) alloimmunization is still an actual problem in our transfusion practice. In 2011, in addition to the regular ABO/D blood group typing, phenotyping for Rh (C, c, E, e) and Kell antigens was introduced for blood donors and patients undergoing blood transfusion. Our aim was to evaluate the impact of the extended RBC typing and donor/recipient matching on the incidence of RBC alloimmunization.

METHODS: A retrospective comparative study was conducted by reviewing RBC request records for about 36,000 patients transfused with RBC in the period from 2013 to 2015 in comparison to the similar study conducted on 47,000 transfused patients in the period from 2005 to 2008. Pre-transfusion serologic testing data were retrieved for analysis. Blood samples with positive antibody screening and positive cross-match were further subjected to antibody identification. All the tests were performed using column agglutination technique (CAT) with ID-cards and reagents from DiaMed in both studies.

RESULTS: Irregular RBC alloantibodies were detected in 116 (0.32%) out of 36,000 transfused patients. Multiple transfusions (15.8 units/patient) were given to 450 patients from which 79 (17.5%) had RBC allontibodies. The incidence of RBC alloimmunisation in the rest of the 35,550 transfused patients from which 37 had RBC alloantibodies was 0.10%. A total of 117 alloantibodies were identified in 96 out of the 116 patients with irregular RBC antibodies. Their specificity was as fallows: anti-E (25.6%), -C (6.0%), -c (8.5%), -e (0.85%), -C^w (5.1%), -K (12.8%), -Fy^a (10.2%), -Fy^b (2.5%), -Jk^a (7.7%), -Jk^b (2.5%), -M (9.4%), -S (1.7%), -s (0.85%), -Lu^a (1.7%), -Le^b (3.4%) and anti-Le^b (0.85%). Multiple antibodies were identified in 22 of the transfused patients out of which 15 (68.2%) received multiple transfusions. Anti-E was the most common antibody found in more of the 50% of the multiple antibody cases.

CONCLUSIONS: The overall incidence of RBC alloimmunization in transfused patients decreased from 0.51% which was the estimated incidence for the period before the introduction of the extended RBC typing (2005-2008) to 0.32% (2013-2015). This is due to the decreased incidence of RBC alloimmunization in the multiply transfused patients from 33.9% to 17.5% respectively. The current frequency of anti-E (25.6%) and -K (12.8%) antibodies in transfused patients are significantly lower than their previous estimated frequencies of 30.4% and 24.0% respectively, as well as the overall frequency of RBC antibodies to Rh+Kell antigens which decreased from 72.4% to 53.8%. Extended donor-recipient matching for C, c, E, e and Kell antigens has proved a beneficial effect on the incidence of RBC alloimmunization in multiply transfused patients.

Introduction

Red blood cell alloimmunization results from the genetic red blood cell antigen disparity between donor and recipient or from mother and fetus. The surface of the red blood cell (RBC) is coated with antigens (sugars and proteins) that are integrally linked to membrane proteins or lipids. The clinical relevance of these antigens for blood component transfusion and tissue/organ transplantation lies in the ability of these surface molecules to initiate an immune response [1].

The way the immune system reacts depends on several factors. The immune response to carbohydrate antigens is usually thymus independent. Multivalent antigens directly stimulate B cells to synthesise antibodies without the aid of helper T cells resulting in the majority of cases in the production of IgM antibodies. Individuals lacking a particular carbohydrate blood group antigen on their red cells can have ‘naturally occurring' IgM antibodies, which are most probably stimulated by cross-reacting antigens present in the environment, such as on gut bacteria. The most important carbohydrate antigens for blood transfusion practice are the A- and B-antigens. Normal individuals who lack either the A or

B antigen make IgM B or A antibodies, respectively. Since IgM antibodies are complement-binding, these antibodies can cause immediate and severe intravascular hemolysis after transfusion of incompatible red cells, which can lead to serious or fatal complications [2].

The discovery of the AB0 blood group system, by the Austrian pathologist Karl Landsteiner [3] and the introduction of the AB0 blood grouping test for selected donors by Ottenberg [4] in 1911 greatly reduced the fatalities associated with blood transfusion in the early days of transfusion therapy. Despite the ABO matching, hemolytic transfusion reactions were reported in 1921 by Unger [5], and additional tests were recommended to exclude the possibility of a recipient's serum agglutinating the donor's red cells due to the presence of non-ABO antibodies, now known as irregular alloantibodies. These alloantibodies are directed against the numerous other blood group antigens which reside on membrane proteins and comprise polymorphic determinants dependent primarily on amino acid sequence. These protein antigens can stimulate a thymus-dependent immune response, and the resulting IgG antibodies can cause extravascular clearance of antigen-positive cells. These IgG antibodies may also cross the placenta, resulting in hemolytic disease of the newborn.

Currently, 35 blood group systems and 342 antigens are recognised by the Working Party committee on terminology for red cell surface antigens of the International Society of Blood Transfusion (ISBT). The most important irregular red blood cell alloantibodies in daily transfusion practice, in terms of clinical significance and frequency of occurrence, are directed towards the RH (anti-D, -C, -E, -c and -e), KEL (anti-K), FY (anti-Fya and -Fyb), JK (anti-Jka and -Jkb) and the MNS (anti-M, -S and -s) blood group systems [6]. Of these, the D-antigen is the most immunogenic, resulting in more than 80% of immunocompetent D negative persons becoming allo immunised after a transfusion of D-positive erythrocytes [7]. This has resulted in the prophylactic matching of red cell transfusions for the D-status. Such routine is not sufficient to prevent other RBC antibodies, for which we rely on serologic screening before transfusion.

Retrospective studies in the general population reported antibody frequencies after transfusion of less than 1 to 3 percent. However, in multi-transfused patients alloimmunization occurs from 10-38% and up to 60-70% of patients [8-11].

Whether the recipient's immune system will produce RBC antibodies depends on genetic and acquired patient-related factors, antigen frequency in the population, dose and number of expositions (transfusions) and the immunogenicity of the RBC antigen as it is shown in Table 1 [12].

Table 1: Frequent clinically significant RBC antibodies

Antigen	System	Antibody frequency	Antigen frequency (Whites)	Antigen frequency (Blacks)	Potential*
E	Rh	16-40%	30%	2%	4%
Kell (Kl)	Kell	5-40%	9%	3%	9%
D	Rh	8-33%	85%	92%	70%
C	Rh	4-15%	80%	99%	4%
Jk(a)	Kidd	2-13%	77%	91%	0.14%
Fy(a)	Duffy	4-12%	63%	10%	0.46%
c	Rh	2-10%	70%	32%	0.22%
e	Rh	2-3%	98%	98%	1%
Jk(b)	Kidd	2%	72%	43%	0.06%
S	MNSs	1-2%	55%	31%	0.08%
s	MNSs	<1%	89%	97%	0.06%

* Percentage of antigen-negative recipients who become allo immunized if transfused with antigen-positive units.

Clinical manifestations of RBC antibodies are acute intravascular hemolytic transfusion reactions (rarely a consequence of alloimmunization and almost always caused by ABO antibodies), delayed hemolytic transfusion reactions (DHTRs) (hemolysis caused by RBC alloantibodies at least 24 hours posttransfusion) and hemolytic disease in newborns (mother's alloimmunization against fetal antigens, most often resulting from previous pregnancies).

Nowadays, after 70 years of transfusion practice, RBC alloimmunization is still an actual problem in our pre-transfusion testing. It is a cause for a delay in transfusion treatment of the alloimmunized patients because antigen-negative RBC must be provided which can be difficult in a patient with rare and multiple alloantibodies.

In 2011, in addition to the regular ABO/D blood group typing, routine phenotyping for Rh (C, c, E, e) and Kell antigens was introduced in blood donors and patients undergoing a blood transfusion. Our aim was to evaluate the impact of the extended RBC typing and donor/recipient matching on the incidence of RBC alloimmunization.

Methods

A retrospective comparative study was conducted by reviewing RBC request records for about 36,000 patients transfused with RBC in the period from 2013 to 2015 in comparison to the similar study conducted on 47,000 transfused patients in the period from 2005 to 2008. Pre-transfusion serologic testing data were retrieved for analysis.

Blood samples with positive antibody screening or/and positive cross-match were further subjected to antibody identification. All the tests were performed using column agglutination technique (CAT) with ID-cards and reagents from DiaMed in both studies.

We compared the sex, number of transfusions and specificity of the revealed alloantibodies as well

as the overall incidence of alloimmunization in the two groups of transfused patients in the above mentioned periods, i.e., the period before and after the introduction of routine extended Rh (C, c, E, e) and Kell antigen typing.

Results

In the period from 2005 to 2008 irregular red cell antibodies were detected in 53 (0.11%) out of 46,459 transfused patients and in 188 (33.9%) out of 554 multiply transfused patients. The overall incidence rate of immunisation due to RBC transfusion in the total of 47,013 transfused patients was 0.51%, and the total number of patients with positive antibody screening was 241 (Table 2).

In the period from 2013 to 2015 irregular red cell antibodies were detected in 37 (0.10%) out of 35,550 transfused patients and in 79 (17.5%) out of 450 multiply transfused patients. The overall incidence rate of immunisation due to RBC transfusion in the total of 36,000 transfused patients was 0.32%, and the total number of patients with positive antibody screening was 116 (Table 2).

The incidence of alloimmunization in the multiply transfused patients decreased from 33.9% to 17.5% which lead to the decrease of the overall incidence of RBC alloimmunization in transfused patients from 0.51% to 0.32% respectively. However, irregular RBC antibodies were significantly more frequent in the multiply transfused patients in comparison to transfused patients in both periods ($p < 0.05$).

Table 2: Immunisation to red blood cell antigens in transfused patients

Transfused patients No		Immunization patients No (%)		Transfusions Average No	
(2005-08)	(2013-15)	(2005-08)	(2013-15)	(2005-08)	(2013-15)
554	450	188 (33.9)	79 (17.5)	16.7	15.8
46459	35550	53 (0.11)	37 (0.10)	1.74	1.72
47013	36000	241 (0.51)	116 (0.32)		

According to the sex, there was no difference between the number of male and female transfused patients in the two observed periods, i.e. the period before (2005-2008) and the period after the introduction of extended RBC typing (2013-2015). Significant difference in the occurrence of immunization between male 60 (32.4%) and female 128 (67.7%) multiply transfused patients was observed in the period from 2005 to 2008, as it was in the period from 2013 to 2015 being 31 (39.3%) male and 48 (60.7%) female patients ($p<0.05$).

There was no significant difference between the two groups of multiply transfused patients according to the number of transfusions (Table 2).

Also, there was no significant difference in the rate of alloimmunization between the two groups of multiply transfused patients according to the diagnosis as shown in Table 3.

Table 3: Rate of RBC alloimmunization according to the diagnosis

Diagnosis	Patients No		Alloimmunization No (%)	
	(2005-2008)	(2013-2015)	(2005-2008)	(2013-2015)
Leucosis in adults	217	125	107 (56.9)	43 (54.4)
Leucosis in children	78	82	16 (8.5)	7 (8.8)
Chronic renal failure	109	116	29 (15.4)	16 (20.2)
Internal patients	83	94	23 (12.2)	9 (12.6)
Surgical patients	67	33	13 (6.9)	4 (5.0)
Total	554	450	188 (33.9)	79 (17.5%)

In 126 (52.3%) out of 241 transfused patients with irregular antibodies, 138 clinically significant antibodies were identified in the period from 2005-to 2008 in comparison to 117 alloantibodies which were identified in 96 (82%) out of the 116 transfused patients in the period from 2013 to 2015. Specificity of the identified antibodies is shown in Table 4.

Table 4: Antibody specificity in transfused patients

Antibody	Period 2005-2008 No (%)	2013-2015 No (%)
E	42 (30.4)	30 (25.6)
C	15 (10.8)	7 (6.0)
c	8 (5.8)	10 (8.5)
e	2 (1.4)	1 (0.85)
C^w	4 (2.9)	6 (5.1)
K	33 (24.0)	15 (12.8)
Fy^a	6 (4.3)	12 (10.2)
Fy^b	1 (0.7)	3 (2.5)
Jk^a	8 (5.8)	9 (7.7)
Jk^b	2 (1.4)	3 (2.5)
M	5 (3.6)	11 (9.4)
S	/	2 (1.75)
s	1 (0.72)	1 (0.85)
Lu^a	2 (1.4)	2 (1.7)
Le^b	3 (2.2)	4 (3.4)
Le^a	4 (2.9)	1 (0.85)
Total	138	117

Multiple antibodies were identified in 9 (7.1%) out of 126 patients with specific alloantibodies from which 7 (77.7 %) were multiply transfused in the period from 2005 to 2008. In comparison to that, 22 (18.9%) out of 96 patients had multiple antibodies from which 15 (68.2%) received multiple transfusions. The current occurrence of multiple alloantibodies (68.2%) was lower in a patient with multiple transfusions in comparison to the period before extended RBC typing was introduced. Anti-E was the most common antibody found in more of the 50% of the multiple antibody cases.

The risk of alloimmunization to anti-E and anti-K is 297.47 times greater (RR=68.21 $r<0.05$) in multiply transfused patients in comparison to transfused patients. The frequency of RBC antibodies to Rh+Kell antigens decreased from 100 (72.4%) to 63 (53.8%) identified alloantibodies which are mostly due to the decreased frequency of E and K antibodies.

The current frequency of E which is 30 (25.6%) antibodies and for K which is 15 (12.8%) antibodies in transfused patients is significantly lower

(χ^2 = 12.7, p < 0.01) than their previous (2005-2008) estimated frequencies of 42 (30.4%) antibodies and 33 (24.0%) antibodies respectively.

Discussion

Despite the relatively high frequency of RBC alloimmunization, clinical manifestations of hemolytic transfusion reactions are rare (approximately 0.05% of patients transfused) due to the regular ABO/D matching and serologic patient/donor cross-matching [12]. Acute hemolytic transfusion reactions due to ABO mismatched RBC transfusions almost always occur as a clerical error (misidentification of the patient/blood sample or incorrect blood component transfused) [13].

Delayed hemolytic transfusion reactions (DHTRs) can occur if the alloantibody titer drops below detectable levels (which is nowadays rarely the case with the column agglutination technique used for the compatibility testing), or in very rare cases of primary immunisation after an initial transfusion. Based on recent UK haemovigilance data from Serious Hazards of Transfusion (SHOT), a total of 1681 cases were reported in 2014. Of these cases, there were 151 (8.9%) cases with RBC alloantibodies and 46 (2.7%) cases with HTRs from which 18 acute and 28 delayed hemolytic transfusion reactions [14]. HTRs and related mortality have been reported to occur at approximately 1 in 76,000, and 1 in 1.8 million units transfused, respectively [15, 16]. The risk of death from a DHTR is approximately 1 fatality per 3.85 million units (1 per 1.15 million units in patients who have received transfusions) [12].

Nowadays, we see RBC alloimmunization more as a problem of the laboratory to provide compatible blood for transfusion because this process is time-consuming and often causes a delay in transfusion treatment. The most frequent alloantibodies identified in transfused patients and different groups of chronically transfused patients (sickle cell disease, myelodysplastic syndrome or chronic myelomonocytic leukaemia as well as in nonhematologic patients) are within the Rh and Kell system [10, 11, 17, 18]. These findings suggest that it is vice to perform extended typing for chronically transfused patients at least for Rh (C, c, E, e) and Kell antigens before the initial RBC transfusion. For this purpose, it is also rational to have already typed RBC in the blood supply.

Sickle cell disease patients are at particular risk of alloimmunization, which can be reduced by red cell phenotyping before the first transfusion followed by routine matching for at least the Rh and Kell groups [19]. This has been challenged by a recent study that showed no difference in alloimmunization rates between centres in the United States that provided closer antigen matching compared to those who did not [20].

However, most investigators agree that the extended RBC typing (beyond the routine ABO/D typing) has a beneficial effect on the incidence of alloimmunization in thalassemia major patients [18, 21]. It has been shown that nonhematooncologic alloimmunized patients are high antibody responders, with a more than 20 times increased risk to form antibodies compared to first-time alloimmunization risk. If extended matching for C, c, E, K, Fy(a), and Jk(a) antigens in the future is considered, this group should be taken into account [22]. According to the data on the estimated frequency of clinically significant red blood cell antigens, there is no significant difference between the multiply transfused patients and the blood donors in the absence of race differences [11]. Individuals from ethnic minority groups have an increased risk of alloimmunization from transfusion because notable differences exist in the frequency of blood cell antigens between races. Efforts to increase the blood supply from minority donors are essential to reduce the frequency of alloimmunization in these groups [12].

However, total prevention of RBC alloimmunization and HTRs is unrealistic, because current immunohematological methods of testing are not sensitive enough to detect all RBC antigen and antibodies. In the early 2000s the EU BloodGen group was established with the aim of improving patient safety and blood transfusion compatibility through validation and standardisation of molecular genotyping approaches and to prove its superiority over currently applied serological testing [23]. BLOODchip is an integrated circuit that determines the main allelic variants of blood groups and platelet antigens by analysing 128 genetic polymorphisms. It is used to prevent potential transfusion reactions in those patients for whom conventional serologic tests are insufficient. It is still too early to determine whether proposals to minimise RBC alloimmunization through large-scale molecular RBC typing for all patients would be more efficient since the genotyping is no longer regarded as being more costly than serology [24]. Speaking of RBC alloimmunization, blood banks and immunohematology laboratories can not influence the demographic structure of the patients, neither the number of transfusions. What they can do is to choose and to optimise the method and the extent of RBC antigen typing and donor/recipient matching are providing more timely the most compatible blood for transfusion.

In conclusion, there was no significant difference according to the sex, diagnosis and number of transfusions, except for the incidence of alloimmunization between the group of transfused patients in the period before and the patients

transfused after the introduction of extended RBC typing. Although, the incidence of alloimmunization did not change (0.11% vs. 0.10%) over the time in the patients who were not multiply transfused it was not the case for the multiply transfused patients. The incidence of alloimmunization in the multiply transfused patients significantly decreased from 33.9% to 17.5% which lead to the decrease of the overall incidence of RBC alloimmunization in transfused patients from 0.51% to 0.32% respectively.

The current frequency of anti-E (25.6%) and -K (12.8%) antibodies in transfused patients are significantly lower than their previous estimated frequencies of 30.4% and 24.0% respectively, as well as the overall frequency of RBC antibodies to Rh+Kell antigens which decreased from 72.4% to 53.8%. We believe that the extended donor-recipient matching for C, c, E, e and Kell antigens has a beneficial effect on the incidence of RBC alloimmunization especially for multi transfused patients. Currently, approximately 30% of the available RBC units are typed for CEce/K within the corresponding ABO/D blood group. Our goal is to reach the number of 50% of Rh, and Kell typed RBC in our blood supply which would be sufficient to meet the needs for antigen negative RBC for most of the alloimmunized patients at any time. At this point and with the currently employed serologic methods there is no rational for routine donor/patient typing beyond ABO, Rh, and Kell systems in our transfusion practice.

References

1. Daniels G, Poole J, de Silva M, Callagan T, MacLennan S, Smith N. The clinical significance of blood group antibodies. Transfusion Med. 2002; 12: 287-295. https://doi.org/10.1046/j.1365-3148.2002.00399.x

2. Flegel WA. Pathogenesis and mechanisms of antibody-mediated hemolysis. Transfusion. 2015; 55(2):47-58. https://doi.org/10.1111/trf.13147 PMid:26174897 PMCid:PMC4503931

3. Landsteiner K. Ueber Agglutinationserscheinungen normalen menschlichen Blutes. Wien Klin Wochenschr. 1901;14:1132-1134.

4. Ottenberg R. Studies on isoagglutination. Transfusion and the question of intravascular agglutination. J Exp Med. 1911; 13:425-438. https://doi.org/10.1084/jem.13.4.425

5. Unger LJ. Precautions necessary in the selection of a donor for blood transfusion. JAMA. 1921; 76:9-11. https://doi.org/10.1001/jama.1921.02630010013003

6. Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. Transfus Med Rev. 2007;21:58–71. https://doi.org/10.1016/j.tmrv.2006.08.003 PMid:17174221

7. Urbaniak SJ, Robertson AE. A successful program of immunizing Rh-negative volunteers for anti-D production using frozen/thawed blood. Transfusion. 1981;21:64-69. https://doi.org/10.1046/j.1537-2995.1981.21181127486.x

8. Singerb ST, Wu V, Mignacca R. et al. Alloimmunisation and erythrocyte autoimmunisation in transfusion-dependent thalassemia patients of predominant Asian descent. Blood. 2000; 96(10):3369-3373.

9. Daniels G, Bromilow I. Essential Gide to Blood Groups. Blackwell publishing, 1st Ed., 2007: 1-5. https://doi.org/10.1002/9780470750926.ch1 PMid:17150014

10. Hoeltge GA, Domen RE, Rybicki LA, Schaffer PA. Multiple red cell transfusions and alloimmunization. Experiences with 6996 antibodies detected in a total of 159,262 patients from 1985-1993. Arch Pathol Lab Med. 1995;119:42-45. PMid:7802552

11. Makarovska-Bojadzieva T, Blagoevska M, Kolevski P, Kostovska S. Optimal blood gouping and antibody screenining for safe transfusion. Contributions Sec Biol Med Sci MASA. 2009; 30 (1): 119-128.

12. Oren E. Alloimmunization from transfusion emedicine medscape com/article/134958.

13. Bolton-Maggs P, Poles D, Watt A, Cohen H, Thomas D. Serious Hazards of Transfusion (SHOT) haemovigilance and progress is improving transfusion safety. Br J Haematol. 2013; 163(3): 303–314. https://doi.org/10.1111/bjh.12547 PMid:24032719 PMCid:PMC3935404

14. SHOT Annual Report 2014. Available from: http://www.shotuk.org/wp-content/uploads/ SHOT-2014-Annual-Reportv11-Web-Edition.pdf [Accessed 4 August 2015].

15. Strobel E. Hemolytic transfusion reactions. Transfus Med Hemother. 2008; 35:346-353. https://doi.org/10.1159/000154811 PMid:21512623 PMCid:PMC3076326

16. Mazzei CA, Popovsky MA, Kopko PM. Noninfectious complications of blood transfusion. In: Roback JD, Combs MR, Grossman BJ, et al., editors. Technical Manual. 16th Ed. Bethesda, MD: American Association of Blood Banks, 2008: 715–749.

17. Sanz C, Nomdedeu M, Belkaid M, Martinez I, Nomdedeu B, Pereira A. Red blood cell alloimmunization in transfused patients with myelodysplastic syndrome or chronic myelomonocytic leukemia. Transfusion. 2013; 53(4):710-5. https://doi.org/10.1111/j.1537-2995.2012.03819.x PMid:22845746

18. Azarkeivan A, Ansari S, Ahmadi MH, Hajibeigy B, Maghsudlu M, Nasizadeh S, Shaigan M, Toolabi A, Salahmand M. Blood transfusion and alloimmunization in patients with thalassemia: multicenter study. Pediatr Hematol Oncol. 2011; 28(6):479-85. https://doi.org/10.3109/08880018.2011.568595 PMid:21854216

19. Vichinsky EP, Luban NL, Wright E, Olivieri N, Driscoll C, Pegelow CH, Adams RJ. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. Transfusion. 2001; 41(9):1086-92. https://doi.org/10.1046/j.1537-2995.2001.41091086.x PMid:11552063

20. Miller ST, Kim HY, Weiner DL, Wager CG, Gallagher D, Styles LA, Dampier CD, Roseff SD. Red blood cell alloimmunization in sickle cell disease: prevalence in 2010. Transfusion. 2013; 53(4):704-9. https://doi.org/10.1111/j.1537-2995.2012.03796.x PMid:22804353 PMCid:PMC5096391

21. Cheng CK, Lee CK, Lin CK. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. Transfusion. 2012; 52(10):2220-4. https://doi.org/10.1111/j.1537-2995.2012.03570.x PMid:22339270

22. Schonewille H, van de Watering LM, Brand A. Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? Transfusion. 2006; 46(4):630-635. https://doi.org/10.1111/j.1537-2995.2006.00764.x PMid:16584440

23. Avent ND, Martinez A, Flegel WA, et al. The Bloodgen Project of the European Union, 2003-2009. Transfus Med Hemother. 2009; 36 (3): 162-167. https://doi.org/10.1159/000218192 PMid:21113258 PMCid:PMC2980524

24. Flegel WA, Gottschal JL, Denomme GA. Implementing mass-scale red cell genotyping at a blood center. Transfusion. 2015;55(11):2610-5. https://doi.org/10.1111/trf.13168 PMid:26094790 PMCid:PMC4644091