ORIGINAL REPORT

A simple approach to screen rare donors in Brazil

C.P. Arnoni, F.R.M. Latini, J.G. Muniz, R.D.M. Person, T.A.P. Vendrame, D. Gazito, and L. Castilho

Providing blood units for patients with an antibody to a high-prevalence antigen or with multiple common antibodies is a constant challenge to the blood banks. Finding a compatible donor requires extensive screening, which incurs a large amount of investment. In this article, we share our experience of organizing a rare donor inventory with limited resources, we include the strategy used for finding rare donors, and we share the difficulties found during the implementation of the approach and the results obtained. ***Immunohematology* 2015;31:20–23.**

Key Words: rare donor, high prevalence antigen, phenotyping, genotyping

Rare donor identification began to get attention in the 1960s when the American Red Cross and American Association of Blood Banks began compiling a rare donor database.[1] Both institutions realized that ensuring blood supply for patients with antibodies to high prevalence antigens or with multiple common antibodies was a big challenge.[1,2] Thereafter, rare donor programs were created in some countries and, in 1998, a national American program was formed (the American Rare Donor Program)[1] to manage and supply rare blood, which currently provides approximately 1800 units per year in the United States.[1,3] Concomitantly, in 1965, the International Society of Blood Transfusion (ISBT) established the International Panel of Rare Blood Donors gathered by the International Blood Group Reference Laboratory in Bristol, U.K. Consequently, since 1984, when the ISBT Working Party on Rare Donors was formed, the provision of an effective exchange program has been discussed worldwide.[4,5] In 2004, the ISBT Working Party on Rare Donors reported that the International Panel of Rare Blood Donors held 4000 rare donors registered from 24 countries.[5,6] Other national rare donor programs were also established—for example, the French program conducted by the French National Reference Laboratory for Blood Groups[7] and the Israeli program headquartered by the Magen David Adom National Blood Services.[8] Unfortunately, however, there are still some countries without a national program.[2]

Currently, we are starting discussions in Brazil to establish a national panel of rare donors. Implementation steps include the screening of large numbers of donors, the education of blood center personnel, the development of a computer system, recruitment of donors, and maintenance of the rare units.[2] Since 2011, Colsan, a blood bank in São Paulo, has implemented a process to screen for rare blood types, trying to set a reliable, useful, and efficient strategy to find rare blood donors. Taking into account the limitations we have related to financial support and the use of different methods, we developed an approach to converge resources and strengthen the efforts that aim to find the best combination of tests to successfully screen large numbers of donors, reduce costs, and increase the chances of encountering rare phenotypes and genotypes.

Different serology methods, as well as DNA typing, are being used to screen rare donors.[2] As serology has some limitations attributable to the scarcity of commercial and potent antisera to type rare antigens, molecular protocols for large-scale genotyping have emerged as a tool to overcome these restrictions.[9,10]

Because the definition of what can be considered as rare blood differs between countries, the first step is to establish which antigens should be screened. It is very difficult to obtain a negative example of a high prevalence antigen when it only occurs in less than 1 in 1000 individuals.[11] Then, finding donors whose red cells are negative for multiple common antigens can also be a challenge. Therefore, a donor whose red cells lack multiple common antigens is also considered a rare donor.

In a multi-ethnic population such as we find in Brazil, the prevalence of the different blood group antigens varies significantly, and it is necessary to have a range of rare blood types available. According to the prevalence of rare blood groups in the Brazilian population, we established an approach to search for S–s–, k–, Rh_{null}, r′r′, r″r″, Di(b–), Vel–, Wr(b–), Co(a–), Yt(a–), Js(b–), Kp(b–), Jo(a–), and Hy– donors. Additionally, we searched for R_2R_2 donors with a combination of negative clinical antigens. This approach includes serologic and molecular screening in repeated donors, in D– donors, and in donors with RhCE variants. We herein share our approach, the difficulties we experienced during implementation, and the results obtained after approximately 3 years of the program.

Materials and Methods

Rare Donor Screening Strategy

Screening for Rare Phenotypes in Repeat Donors

Repeat donors, including group O donors with at least two prior donations, are selected for screening by serology as well as by DNA typing.

1. Serologic Screening: We first perform a cross-match between the donor's red blood cells (RBCs) and plasmas containing antibodies, such as anti-K, anti-Di^a, anti-s, and anti-E by gel test in an automated instrument (Wadianna, Grifols, Barcelona, Spain). To optimize the searching and to reduce costs, we mix plasmas with anti-K and anti-Di^a and perform the tests on the pool. If the test is positive, K and Di^a are individually typed. All the positive results are confirmed with commercial sera.

   The aim of this serologic strategy is to find donors with k–, S–s–, and Di(b–) phenotypes and R_2R_2 donors with an interesting combination of antigen-negative results. Figure 1 shows the four scenarios obtained with this strategy and the further steps performed with serologic and molecular protocols.
2. Molecular Screening: Taking into account the lack of commercial antisera to type the majority of high-prevalence antigens and the limitations of conventional polymerase chain reaction (PCR), we developed a SNaPshot protocol to identify rare donors, as previously described by Latini et al.[12]

Briefly, SNaPshot is a mini-sequencing assay that permits analysis of several single nucleotide polymorphisms (SNPs) from numerous donors in a short period of time. The protocol we developed identifies alleles of the Diego, Colton, Cartwright, KEL, Dombrock, and VEL blood group systems. All samples previously typed by serology, such as Di(a+) and/or K+, R_1r, R_1R_1, R_2R_2, or R_0r are selected for this molecular analysis.

Screening of Rare Phenotypes in D-Negative Donors

All D– donors are typed for C, E, and K by hemagglutination in microplates using an automated instrument (Neo, Immucor, Birkenfeld, Germany). This approach identifies rare Rh phenotypes such as r′r′, r″r″, and Rh_{null} and also the k– phenotype in the samples typed as K+.

Molecular Screening for RhCE Variants

Molecular screening for RhCE variants is performed in repeat donors of group O African descendants and in donors with altered expression of C, c, E, and e. We also perform molecular searching for CE variants in donors with altered expression of D because RhD variants have been shown to be associated with RhCE variants. In these cases, we select samples with weak D expression for immediate spin with two monoclonal anti-D reagents (IgM RUM-1 and blend D175+D415) on an automated instrument (Neo, Immucor).

Because the most important RhCE variants are those silencing the high prevalence antigens, hr^S and hr^B, our strategy includes screening the following alleles: *RHCE*ceAR, RHCE*ceEK, RHCE*ceAG, RHCE*ceMO, RHCE*ceBI, RHCE*ceSM, RHCE*ceCF,* and *RHCE*ce^S (RHCE*ce733G)* that cause the hr^S– and/or hr^B– phenotypes. To identify these variants, we first use a PCR–restriction fragment–length polymorphism (PCR-RFLP) for detection of the SNPs 712A>G, 254C>G, 667G>T and 733C>G. Depending on the results obtained, we then use a strategy to identify the variant, as shown in Figure 2.

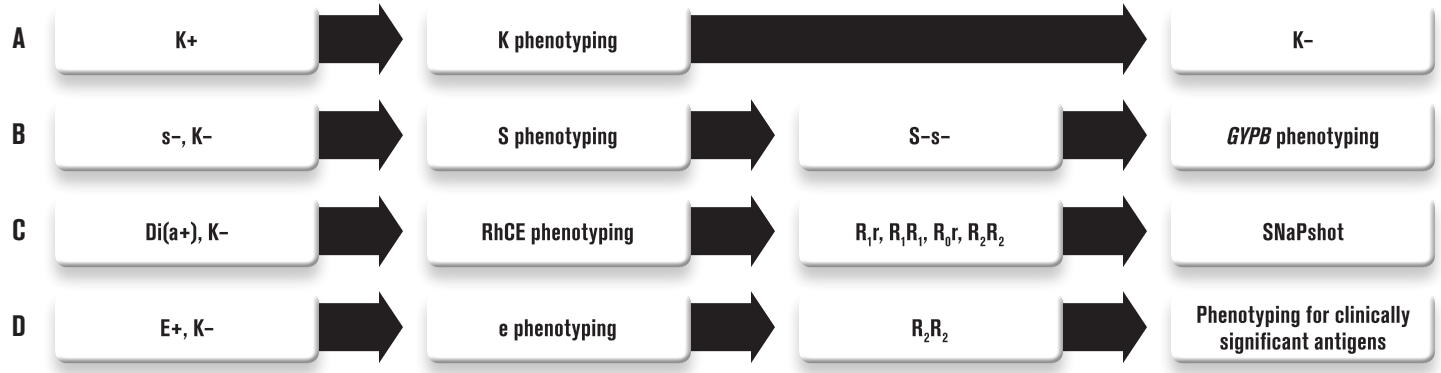

Fig. 1. This figure shows the four scenarios obtained by serologic screening. (A) All K+ RBCs are typed for k to identify k– phenotypes. (B) s– and K– RBCs are typed for S and all S–s– samples are genotyped for *GYPB* to identify U– and U+^{var} phenotypes. (C) All Di(a+) and K– samples are typed for RhCE. R_1r, R_1R_1, R_0r, and R_2R_2 RBCs are molecular typed by SNaPshot. (D) All E+ K– RBCs are typed for e, and all R_2R_2 samples are typed for other clinically relevant antigens.

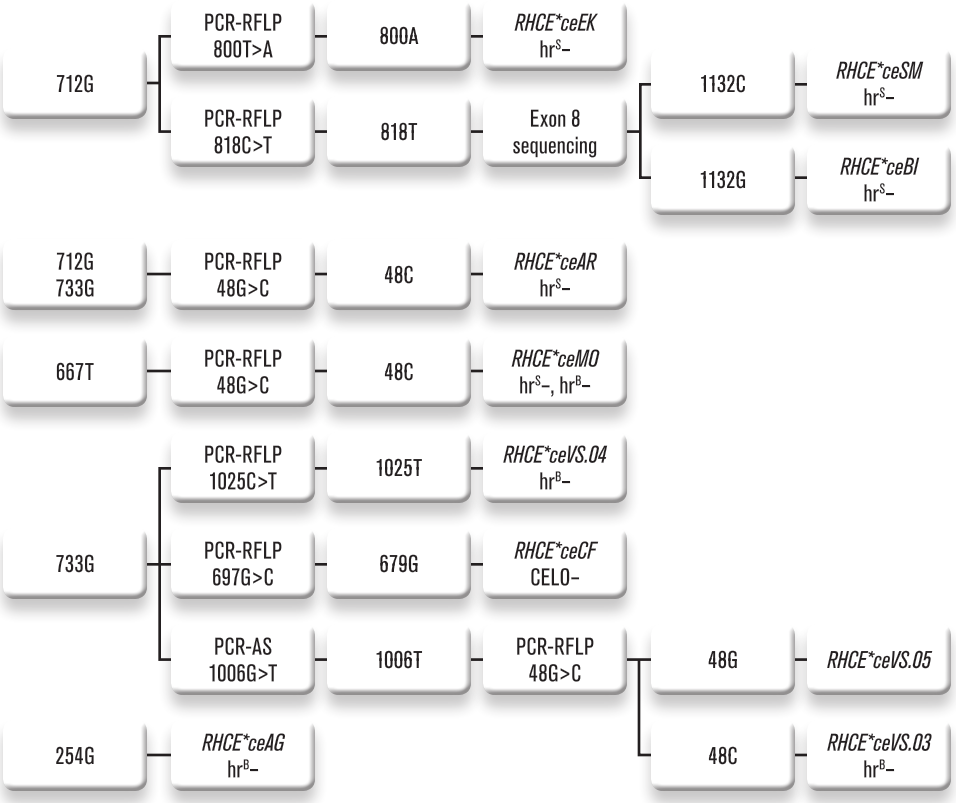

Fig. 2. Flowchart used to identify hr^S– and hr^B– donors. The investigation starts with the use of polymerase chain reaction–restriction fragment–length polymorphism to detect the single nucleotide polymorphisms 712A>G, 254C>G, 667G>T, and 733C>G. According to the results, we perform the analyses indicated in the flowchart to identify the *RHCE* variants.

We also perform molecular tests to identify RhD variants in all samples with RhCE variants, based on previously published protocols.[13]

Results

Identified rare donors are included in our rare donor registry. Table 1 presents the results obtained in our 3 years of experience of building our rare donor inventory. To all donors included in this inventory, we send a folder with a simple explanation of their rare blood type and instructions regarding their next donation. We also ask that they keep their contact information updated.

Additionally, we also phenotype and genotype all the clinical relevant antigens and freeze RBC aliquots in liquid nitrogen. Phenotype information on rare donors is submitted to the database housed in our blood center. The database allows the identification of the donors and facilitates searching for a requested phenotype. The steps of the process are schematically described in Figure 2.

The inventory of donors with RhCE variants in the homozygous state or in the heterozygous state with Ce or cE haplotypes allows for RH molecular matching to patients with sickle cell disease, preventing Rh alloimmunization and delayed hemolytic transfusion reactions. Six of our 11 donors with RhCE variants were related to RhD variants; all hr^S– linked to weak D type 4.2.2 and all hr^B– linked to weak D type 4.0.

Discussion

The strategy presented herein has allowed us to search for rare donors and to create a rare donor inventory at our institution. After the complete implementation of the molecular laboratory, the investment is approximately $2.00 per sample for serologic screening and $13.00 per sample for SNaPshot screening for *RHCE* variants. The search for D– donors is part of our laboratory routine; the additional k typing is the equivalent of $0.70 per sample. The development of the SNaPshot method has helped increase throughput and reduce costs. Therefore, this strategy to find rare donors, based on a focused search, is cost-effective

Table 1. Rare donor inventory

Screening	Total number of donors screened	Phenotypes and predicted phenotypes from genotypes	*n*
Serologic screening	4500	U+^{var}*	6
		U–*	1
		k–	5
Molecular screening	1600	Yt(a-b+)	4
		Js(a+b–)	1
		Co(a–b+)	1
		Kp(a+b–)	1
		Di(a+b–)	2
RhD negative	2000	r″r″	2
		r′r′	2
		k–	2
RhCE variants	590	hr^S–	7
		hr^B–	3
		hr^S–, hr^B–	1
		TOTAL	**38**

*U– and U+^{var} were determined by polymerase chain reaction–allele specific and restriction fragment–length polymorphism.[14]

as compared with other strategies using microarrays and may help other blood centers to build their rare donor inventory.

The benefit of this investment is documented when an alloimmunized patient with a rare phenotype requires a rare unit of blood and is successfully transfused with a compatible unit. In a period of 3 years, we received 18 requests for rare units, and 8 of these were fulfilled using our rare donor inventory. Applying this approach, we intend to meet the needs of the patients with a rare phenotype in our institution and help other blood centers. Nevertheless, we still have some limitations to ensure that blood is made available to specific patients. Although we observed a great collaboration of the donors when recruited, some of these may be unable to donate when required. Freezing blood would be a good option, but this procedure is currently a challenge in Brazil, since the blood bag and the glycerol solution used for freezing are not licensed by our regulatory agency.

Limitations of the Approach

Our strategy for searching for rare donors does not include screening for D– –, Ge:-2, and K_o phenotypes, which have already been found in the Brazilian population, but our future screening activities will focus on the identification of such donors. Another unfavorable point of this strategy is the long duration of the whole process. In general, when we find a rare donor, the RBC unit has already been transfused and we need to wait until the next donation to freeze RBC aliquots and to perform complete phenotyping.

Conclusion

It is widely accepted that a rare donor program is important to ensure a safe transfusion. In our experience, we realized that the implementation of a rare donor program requires efforts from both technical and administrative areas. On the other hand, we show herein that a simple and focused strategy can help fulfill the requests for units of rare blood for patients with antibodies against high-prevalence antigens or patients with multiple antibodies. Exchanges of experience and collaboration between different centers, through a national program, will allow us to ensure that blood will be made available to patients with rare blood phenotypes.

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Carine Prisco Arnoni, PhD (corresponding author), Adjunct Director; Flavia R.M. Latini, PhD, Director; Janaína Guilhem Muniz, Biologist; Rosangela Duarte de Medeiros Person, Biologist; Tatiane Aparecida de Paula Vendrame, Biologist; Diana Gazito, Biologist, Technical and Scientific Department, Colsan-Associação Beneficente de Coleta de Sangue, Avenida Jandira 1260, Indianópolis 04080-006, São Paulo, SP, Brazil; and Lilian Castilho, PhD, Professor and Researcher, Hemocentro-Unicamp, Rua Carlos Chagas 480, Barão Geraldo 13083-878, Campinas, SP, Brazil.