

A new strategy to identify rare blood donors: single polymerase chain reaction multiplex SNaPshot reaction for detection of 16 blood group alleles

Flavia Roche Moreira Latini¹, Diana Gazito¹, Carine Prisco Arnoni¹, Janaína Guilhem Muniz¹, Rosângela de Medeiros Person¹, Fabricio Oliveira Carvalho¹, Wilson Baleotti Jr.², Lilian Castilho³, José Augusto Barreto¹

¹Colsan - Blood Collection Centre, São Paulo; ²FAMEMA - Faculty of Medicine of Marília, Marília, SP; ³INCTs-Unicamp Blood Centre, Campinas, SP, Brazil

Background. As an alternative to phenotyping, large-scale DNA-based assays, which are feasible for high-throughput donor red blood cell typing, were developed for determination of blood group polymorphisms. However, high-throughput genotyping platforms based on these technologies are still expensive and the inclusion of single nucleotide polymorphisms and analysis of the alleles depend on the manufacturer's determination. To overcome this limitation and in order to develop an assay to enable the screening of rare donors, we developed a SNaPshot assay for analysis of nine single nucleotide polymorphisms related to antigens that are difficult to assess using conventional serology.

Materials and methods. The single polymerase chain reaction multiplex SNaPshot reaction was optimized to identify nine single nucleotide polymorphisms determining 16 alleles: *KEL*3/KEL*4*, *KEL*6/KEL*7*, *DI*1/DI*2*, *DI*3/DI*4*, *YT*1/YT*2*, *CO*1/CO*2*, *DO*1/DO*2*, *DO*4*, *DO*5*. We designed a single multiplex PCR with primers encompassing the blood group single nucleotide polymorphisms and performed an internal reaction with probe primers able to discriminate the alleles after fragment analysis. The SNaPshot assay was validated with 140 known alleles previously determined by PCR restriction fragment length polymorphism.

Results. We were able to simultaneously detect nine single nucleotide polymorphisms defining 16 blood group alleles on an assay based on a multiplex PCR combined with a single base extension using genomic DNA.

Discussion. This study demonstrates a robust genotyping strategy for conducting rare donor screening which can be applied in blood centers and could be an important tool for identifying antigen-negative donors and, therefore, for providing rare blood.

Keywords: rare blood donors, SNaPshot, screening, genotyping.

Introduction

The blood group antigens of patients and donors can be easily typed using high sensitivity haemagglutination when this is done correctly. However, as haemagglutination has certain limitations, genotyping assays are offering a good alternative for problems encountered by serology¹.

Detection of rare blood donors is more complex, but can be achieved in a couple of ways, including antigen identification when a patient with a rare blood type needs a transfusion, and through a selective search by testing donors in blood banks. The detection should comprise serological and/or molecular techniques². However, when a good commercial antibody, a potent antiserum in sufficient volume or a reagent at a reasonable price is not available, DNA-based approaches are being used as an alternative for screening donors¹. The molecular bases of the majority of the blood group antigens are already known and differences

between antithetical antigens within the same blood group system are associated with single nucleotide polymorphisms (SNP)³. Several molecular methods have been developed for the prediction of red blood cell (RBC) phenotypes³. Depending on the purpose of the blood centre, RBC genotyping may include polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (PCR-RFLP), allele-specific PCR (AS-PCR) or real-time-based allele-specific extension assays^{3,4}. Although reliable, these are low or medium-throughput approaches. Commercial microarrays are an excellent option for performing high-throughput genotyping of a large panel of blood group antigens in numerous donors^{3,4}. Although microarray platforms will bring high-throughput extended blood group typing into blood donor laboratories, the analysis of the alleles depends on the manufacturers' determinations and high costs could be a barrier, particularly in emerging countries.