How do we identify RHD variants using a practical molecular approach?

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Serologic resolution of Rh discrepancies due to partial D or weak D phenotypes is a frequent problem encountered during routine typing that can be solved by *RHD* genotyping because it provides better characterization of these variants. The objective of the current study was to develop algorithms for identification of D variants in multiethnic populations based on a logic sequence of molecular tests using a large number of atypical RhD specimens. Thus, a total of 360 blood samples with atypical D antigen expression were analyzed. A previously published multiplex polymerase chain reaction (PCR) procedure was performed and depending on multiplex PCR analysis, the associated *RHCE* allele, and D variant frequency in our population, an algorithm was developed composed of six flow charts using specific PCR–restriction fragment length polymorphism and/or specific exon sequencing. This strategy allowed the identification of 22 different variants with few assays and a much reduced cost. This study describes a simple and practical algorithm that we use to determine *RHD* genotypes in samples with unknown *RHD*. This strategy is relatively easy to implement and the algorithm can be adapted to populations with various ethnic backgrounds after an initial assessment of the type and frequency of D variants. Essentially, we demonstrate that sequencing of all *RHD* exons is not necessary for the identification of the majority of known D variants.

The majority of weak D phenotypes result from single-nucleotide polymorphisms (SNPs) in *RHD* encoding amino acid changes within either the membrane-spanning domains or the cytoplasmic loops of the protein. These changes can interfere with the integration of the RhD protein in the membrane leading to a reduced number of D antigen sites on red blood cells (RBCs).[1] Partial D, in contrast to weak D, is characterized by amino acid changes outside of the membrane that can alter or create new epitopes.[2] Therefore, individuals with partial D can make anti-D when stimulated by transfusion or pregnancy. In fact, many partial D are typed as D+ by direct agglutination and these individuals will be identified only after anti-D formation.[3] *RHD* genotyping is useful for precise characterization of partial D and weak D types in donors and recipients and is a clinically important approach to prevent alloimmunization of recipients with partial D, usually typed as D+, when exposed to D+ RBCs or to some of the weak D RBCs typed as D– by serologic methods. However, *RHD* genotyping is not easy, because it is influenced by the size of the gene, by the presence of rearrangements with *RHCE*,[3] and by the high number of SNPs that are recognized in variants found among different ethnic groups.[4]

A multitude of different primers and probes is available for molecular genotyping of *RHD* making difficult the

ABBREVIATION: SNP(s) = single-nucleotide polymorphism(s).

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