Letter to the Editor

Precautions when using commercial kits to determine the molecular basis of blood groups

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In the past few years, CE-marked kits for blood group genotyping have become available. These can facilitate the work in laboratories with high throughput, potentially acting as a screening method to identify common genotypes (and in the case of RHD, even rare variants, given that more than 30 different partial D can be identified) and selecting the others for more complex investigations in reference laboratories. Although the costs are high, the use of commercially available kits may also be a reasonable option for routine laboratories with limited human or technical resources, in order to perform sequence-specific primer (SSP) - polymerase chain reaction (PCR) methods (using pre aliquoted and dried reaction mixes including internal amplification controls).

Two companies, INNO-TRAIN (Diagnostik GmbH, Kronberg/Taunus, Germany) and BAGene (Biologische Analysensystem GmbH, Lich, Germany), have CE-marked reagents. The BAGene kits are also licensed for in vitro diagnostics. Nevertheless, there are risks of misinterpretation due to incorrect execution of the test or interpretation of the results, or even because of problems in the design of the tests. This situation can be important in rare cases, when sometimes the molecular basis is not well understood or when there are insufficient rare samples for test validation. With regards to these aspects, the RHD alleles are different from other blood groups, for which identification is generally based on single nucleotide polymorphisms (SNPs).

One of these situations is the RHD category VI type 4. This novel variant, described1 as a RHD-Ce(2/3-5)-D hybrid allele, is frequent in Spain and Portugal, and potentially important in transfusion medicine: as recipients, individuals of this phenotype are at risk of anti-D immunisation; as donors, they could be mistyped as normal D positive by serological methods and cause immunisation by inducing anti- BARC.

Our laboratory received eleven samples, eight from Lisbon (in southern Portugal) and three from Oporto (in the north of the country), which were referred with the suspicion of being DVI based on serological studies.

DNA was extracted from whole blood EDTA-samples, using the MagNA Pure LC (Roche Molecular Biochemicals, Mannheim, Germany) with the Total Nucleic Acid Isolation Kit. Real-time PCR with a SYBRGreen approach was performed in the Light Cycler (Roche)2, using primers specific for RHD exons1.

The pattern obtained was compatible in seven cases (all Rh ccEe) with DVI type 1 (negative reactions on exons 4 and 5), and in four (all Rh Ccee) with DVI type 4 (negative reactions on exons 3, 4 and 5).

We analysed these samples with the Partial D-TYPE (BAGene) and CDE-SSP (INNO-TRAIN) kits, carrying out the tests according to the manufacturers’ instructions.

The results using the InnoTrain kit were consistent with the genotype determined by real-time PCR. However, the DVI type 4 samples could not be discriminated from the DHMii phenotype by the BAGene kit since both the DVI type 4 and DHMii phenotype samples give an identical
pattern in this test system. The rare DHMii variant has a distinct molecular basis, different serological pattern with monoclonal anti-D reagents and does not have the BARC antigen. While the kit clearly identified the samples as RHD variants, the results illustrate that care should be exercised when using commercial kits to determine the molecular basis of blood groups, especially those with rare variants.

References