

## Guidelines for the Blood Transfusion Services

### 15.4: Methods available for molecular blood grouping

<http://www.transfusionguidelines.org/red-book/chapter-15-molecular-typing-for-red-cell-antigens/15-4-methods-available-for-molecular-blood-grouping>

### 15.4: Methods available for molecular blood grouping

#### 15.4.1: Fetal typing

The usual technology employed for fetal blood group typing, in which the mother lacks the antigen to be tested, is real-time quantitative PCR (RQ-PCR) on cell-free DNA isolated from the maternal plasma. For D, probes and primers are designed to detect two to four regions of *RHD*. There are numerous variants of D that could give rise to a false answer. Any test for D must reveal the D negative genes *RHD* and *RHD-CE-D*<sup>S</sup>, which are common in people of African origin. Testing for at least RHD exons 5 and 7, with the test for the former being designed to give a negative result with *RHD*, is the minimum required.

Tests for fetal C, c, E and K involve RQ-PCR with allele-specific primers.

A test for the housekeeping gene CCR5 is also included to confirm that DNA is present and that there is not an excess of maternal DNA.

The following DNA controls for fetal *RHD* are used: *RHD* positive; *RHD* negative and *RHD* pseudogene positive. The control DNA can be cell-free fetal DNA from maternal plasma, or cell-derived DNA appropriately diluted to simulate fetal DNA. In addition, an International Reference Reagent is available to purchase from the National Institute for Biological Standards and Control (see Annex 1 and [www.nibsc.org](http://www.nibsc.org)), to use as a standard for minimum acceptable potency for the detection of *RHD/SRY* in cell-free plasma DNA<sup>3</sup>.

Positive and negative DNA controls are used for C, c, E and K.

#### 15.4.2: Typing from DNA obtained from peripheral blood

There are a variety of platforms for detecting single nucleotide polymorphisms for the purpose of predicting blood group phenotypes of donors and patients from genomic DNA isolated from blood.<sup>4,5</sup> These include low-throughput methods involving allele-specific primers and gel electrophoresis, a very comprehensive DNA microarray platform that identifies many D variants, and higher throughput platforms such as allelic discrimination technology and platforms involving the application of fluorescent beads coated with oligonucleotide probes. Those platforms that offer the possibility of high-throughput testing do not include testing for ABO or D.

The usual tests for blood group polymorphisms that would be required for testing donors and patients would be D, C, c, E, e, M, N, S, s, K, k, Fy<sup>a</sup>, Fy<sup>b</sup>, Fy-null, Jk<sup>a</sup>, Jk<sup>b</sup>, Do<sup>a</sup> and Do<sup>b</sup>. Often some others are also included. See Table 15.1.

Homozygous positive, homozygous negative and heterozygous controls are used when available. In addition, International Reference Reagents which can be purchased from the National Institute for Biological Standards and Control (see Annex 1 and [www.nibsc.org](http://www.nibsc.org)) could be useful in the standardisation of blood group genotyping.

There are certain precautions that are required for all molecular testing and they are described in Chapter 14. In addition, there are certain tests in molecular blood grouping that must be carried out to ensure a reasonable level of accuracy. The hazards of ABO grouping are described above. There are numerous variants of D that could give rise to a false answer. Any test for D must reveal the D negative genes *RHD* and *RHD-CE-D<sup>S</sup>*, which are common in people of African origin. Testing for at least *RHD* exons 5 and 7, with the test for the former being designed to give a negative result with *RHD*, is the minimum required. C typing should not depend on the *RHCE* nucleotide 48 polymorphism; testing for the *RHCE* intron 2 insert is more reliable. Duffy typing must include a test for the *GATA* mutation to detect the common silent allele.