Guidelines for the Blood Transfusion Services

Chapter 15: Molecular typing for red cell antigens

http://transfusionguidelines.org/red-book/chapter-15-molecular-typing-for-red-cell-antigens

Chapter 15:

Molecular typing for red cell antigens

15.1: Introduction

Genes for all of the blood group systems have been isolated and the molecular bases for most of the clinically important blood group antigens are known. So it is now possible to predict, with a high level of accuracy, most blood group phenotypes from genomic DNA.

This technology is generally applied when:

- we need to know a blood group phenotype, but do not have a suitable red cell sample
- molecular testing will provide more or better information than serological testing
- molecular testing is more efficient or more cost-effective than serological testing.

15.2: Clinical applications of blood group molecular typing

Various clinical applications of blood group molecular typing are listed below:

- **Fetal typing:**[1] Typing of fetuses, usually for D, but also K, C, c or E, of alloimmunised women, to assess whether the fetus is at risk of haemolytic disease of the fetus and newborn (HDFN). The DNA source is cell-free fetal DNA in the mother's plasma. This technology is now also applied to high-throughput non-invasive prenatal testing (NIPT) for fetal *RHD* genotype of D negative pregnant women, to determine their requirement for antenatal anti-D prophylaxis.[2]
- **Transfused patients:** Typing of multiply transfused patients, where serological testing cannot be used because of the presence of transfused red cells.
- **Immunoglobulin-coated red cells:** Typing of red cells giving a positive direct antiglobulin test (DAT), usually in patients with autoimmune haemolytic anaemia, to help in the identification of underlying alloantibodies.
- **Determining Rh variants:** Molecular methods are used for identifying Rh variants, especially the weak and partial variants of D, to assist in the provision of the most suitable blood for transfusion.
- **Confirmation of D negative:** Detection of *RHD* in an apparently D negative donor could signal very weak D expression, which could immunise a D negative patient.
- ***RHD* zygosity:** Quantitative PCR can reveal whether a D positive person is homozygous or hemizygous for *RHD*. This cannot be done by serological methods. Testing fathers of fetuses at risk of HDFN provides limited information on the D type of the fetus.

- **Testing when suitable reagents are not available:** Molecular methods can replace serological methods when suitable serological reagents are unreliable or not available, e.g. Dombrock typing of donors.
- **Supporting the serological reference laboratory:** Molecular methods are valuable for supporting the serological reference laboratory in sorting out difficult problems.

15.2.1: Testing donors for multiple blood groups

It is probable that molecular methods will replace serology in the near future for testing donors for multiple blood groups. The new high-throughput molecular technology will be more accurate than serological methods and will probably be more cost-effective. Molecular tests could also be applied to screening for donors with rare blood group phenotypes such as S s U, Lu(b), k, Js(b), Yt(a), Co(a) and Vel.

For prediction of blood group phenotypes from DNA of donors, results should either be confirmed by serological testing or by testing twice by molecular methods. This does not apply to ABO and RhD, which are always determined by serological testing.

15.3: ABO typing by molecular genetics

Whereas ABO typing by serological means is straightforward and extremely accurate, the genetics of ABO is complex, rendering ABO molecular typing by available methods unreliable. This is particularly so in people of African origin, where hybrid ABO alleles are present. As it is never acceptable to obtain a false ABO typing, prediction of ABO phenotype by molecular methods is not currently recommended.

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*^{S}, 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), to use as a standard for minimum acceptable potency for the detection of *RHD/SRY* in cell-free plasma DNA [3].

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.[4,5] 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^a, Fy^b, Fy-null, Jk^a, Jk^b, Do^a and Do^b. 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 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^S, 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.

15.5: External quality assurance

It is important that any laboratory performing this testing for clinical purposes participates in some sort of external quality assurance scheme. There is a National External Quality Assurance Scheme (NEQAS) available for molecular blood grouping. The International Society of Blood Transfusion (ISBT) organises workshops every 2 years in which DNA samples from ‘patients’ and plasma from D negative pregnant women are distributed. In addition, it may be possible to set up sampleexchange schemes with other laboratories carrying out similar work.

Table 15.1 Some blood group polymorphisms and associated gene sequence changes

System	Gene	Antigens	Molecular test
MNS	*GYPA*	M/N	59 C/T, 71 G/A
Rh	*RHD*	D	Presence/absence
	RHCE	C	Intron 2 insertion

		c	307 C
		E/e	676 G/C
Lutheran	*LU* or *BCAM*	Lu^a/Lu^b	230 A/G
Kell	*KEL*	K/k	578 T/C
		Kp^a/Kp^b	841 T/C
		Js^a/Js^b	1790 C/T
Duffy	*FY* or *ACKR1*	Fy^a/Fy^b	125 G/A
		Fy-null	67t>c
Kidd	*JK* or *SLC14A1*	Jk^a/Jk^b	838 G/A
Diego	*DI* or *SLC4A1*	Di^a/Di^b	2561 T/C
Yt	*YT* or *ACHE*	Yt^a/Yt^b	1057 C/A
Dombrock	*DO* or *ART4*	Do^a/Do^b	793 A/G
Colton	*CO* or *AQP1*	Co^a/Co^b	134 C/T

15.6: References

1. Daniels G, Finning K, Martin P, Massey E (2009). Non-invasive prenatal diagnosis of fetal blood group phenotypes: current practice and future prospects. *Prenatal Diagnosis*, 29, 101–107.
2. National Institute for Health and Clinical Excellence (2016) High-throughput non-invasive prenatal testing for fetal *RHD* genotype. *Diagnostics Guidance DG25*. London: NICE.
3. Metcalfe P, Rigsby P, Tait E, Urbaniak S (2012). An international reference reagent for the detection of *RHD* and *SRY* DNA in plasma. *Vox Sanguinis*, 102, 243-249.
4. Anstee DJ (2009). Red cell genotyping and the future of pretransfusion testing. *Blood*, 114, 248–256.
5. Veldhuisen B, van der Schoot CE, de Haas M (2009). Blood group genotyping: from patient to high-throughput donor screening. *Vox Sanguinis*, 97, 198–206.