GUIDELINE FOR RED CELL GENOTYPING IN THE RED CELL REFERENCE LABORATORY

REASON FOR ISSUE: Rewording of Rh D Characterisation bullet in section 6.

1. PURPOSE

This guideline describes indications for red cell genotyping in the following situations:

- Resolving ABO discrepancies.
- Management of Haemolytic Disease of the Foetus and Newborn(HDFN).
- Transfusion Support in Patients with Warm Autoantibodies.
- Sickle Cell Disease and Thalassaemia
- Rh D Characterisation

2. SCOPE

Haemagglutination is a simple technique and when done correctly, has a specificity and sensitivity that is appropriate for the clinical management of the vast majority of patients. However, in some aspects, haemagglutination has limitations. For example, it gives only an indirect measure of the potential complications in an at-risk pregnancy, it cannot be relied upon to type some recently transfused patients, and it requires the availability of specific reliable antisera. This guideline is primarily for use by the Red Cell Reference Laboratory when red cell genotyping is considered to be appropriate. In all situations the guidance of a Transfusion Medicine Specialist (TMS) must be sought prior to a decision to testing

3. KEY RESPONSIBILITIES

- Red Cell Reference Laboratory personnel will consult the TMS on the appropriateness of red cell genotyping in any of the described clinical situations.
- The TMS will make the decision on whether red cell genotyping is appropriate.

4. RELATED DOCUMENTS

136G001 Managing Patients with Warm Autoantibodies (Current and Historic) and Use of PAM Blood.

5. DEFINITIONS

- **Prophylactic antigen matched (PAM) blood:** red cell components matched with the patient's phenotype. The fundamental reason for provision of PAM blood is to prevent alloantibody formation and the subsequent negative consequences of haemolytic transfusion reactions. The predicted phenotype could be used to precisely match blood types, thereby reducing the need to perform extensive serologic testing. In particular the need for regular absorption studies can be virtually eliminated in many regularly transfused patients.
- **Extended phenotype/genotype:** includes C, E, c, e, K, Jka, Jkb, Fya, Fyb, S, s
- **Haemolytic Disease of the Foetus and Newborn (HDFN):** Condition resulting from sensitisation of the mother to foetal red cell antigens and causing increased red cell destruction the consequence of which is foetal anaemia and neonatal anaemia and jaundice

6. INDICATIONS FOR RED CELL GENOTYPING

- **Resolution of ABO typing discrepancies:** Occasionally inherited or acquired variant ABO phenotypes are encountered in blood donors and patients. Genotyping can aid in the differentiation between subgroup alleles and acquired weakened agglutination and allows proper ABO identification of both donors and patients. Specific clinical applications may include resolving ABO typing discrepancies for patients awaiting transplant, or determination of the original type of a bone marrow transplant recipient, or confirmation of an ABO subgroup in a kidney donor.

- **Prenatal Setting:** Antigen prediction by genotyping has particular value in this setting to identify a foetus who is not at risk of HDFN, that is, antigen negative. In these situations aggressive monitoring of the mother is not necessary. Certain criteria need to be met before obtaining foetal DNA for genotyping: the mother's serum contains an IgG antibody of potential clinical significance and the father is, or may be, heterozygous for the gene encoding the antigen of interest, or when paternity is in doubt.

  Foetal D status is also useful in determining whether Rh(D) immunoglobulin prophylaxis is required.

- **Patients with warm autoantibodies and transfusion dependent:** The provision of prophylactic antigen-matched blood where feasible in patients with warm autoantibodies requiring regular transfusion is advocated good practice. The practice allows the provision of safe blood for transfusion and significantly reduces, and at times eliminates, the need for repeated absorption studies. In a majority of patients extended red cell phenotyping is achieved using serological means. However in some previously multitransfused patients this is not possible. In this situation red cell genotyping can be used to allow a PAM protocol to be used. Since genotyping complements serological phenotyping, careful consideration must be given to the cost of genotyping.

  - ✓ Patients with a confirmed diagnosis of warm autoimmune haemolytic anaemia who require frequent transfusions and are essentially transfusion dependent genotyping may be very beneficial. This will eliminate the need for regular absorption studies.
  - ✓ Some patients would have already formed one or more alloantibodies from previous transfusion(s). These patients could be good responders with the ability to form more alloantibodies. Genotyping may be considered appropriate in this situation.

  The demonstration of the presence of warm autoantibodies alone should not trigger the requirement for extended red cell phenotyping/genotyping. The patient's clinical records must be scrutinised by a TMS/MO and the transfusion records must be carefully checked to confirm expected future transfusion requirements.

- **Sickle Cell Disease (SCD) and Thalasaemia: High alloimmunisation rates are postulated** particularly in SCD when an antigenic disparity is present within a population e.g. African Americans and whites. This is presently not a problem in New Zealand but consideration may need to be given to genotyping where clinically appropriate. Patients with Thalassaemia major who are expected to be long term transfusion dependent can be considered for a PAM protocol. This may require genotyping where serological phenotyping is not possible for all clinically significant antigens. Where patients have formed one or more clinically significant alloantibodies, it is recommended that a PAM protocol is applied since these patients could be good responders. Genotyping may be necessary in this situation.

- **RhD Characterisation:** Our present protocols for RhD characterisation are based on serology where two different reagents and occasionally the Partial RhD Typing Kit are used. The major limitation of serology is that we cannot confidently characterise weak D types 1, 2 and 3 in patients, thus calling them Rh positive. Presently, based on serology, these patients are characterised as being RhD negative for transfusion and RhD immunoglobulin administration purposes. Genotyping is now recommended to characterise RhD type 1,2 and 3 which account for the majority of D variants.

7. LIMITATIONS OF RED CELL GENOTYPING

There are rare situations where the genotype determination will not correlate with the antigenic expression on the RBC. In some centres appropriate assays are available to detect a change that silences a gene. If such a situation does arise then a TMS can be consulted on whether it is appropriate to refer samples abroad for such testing.