13.3: Pre-transfusion testing

13.3.1: Resolution of anomalous grouping

ABO grouping is the most important pre-transfusion serological test performed. Fully automated ABO and D grouping procedures have significantly improved the accuracy and security of results, and should be used wherever possible.

When anomalous ABO groups are encountered laboratory protocols should support investigation of the following findings.

Missing agglutinins in reverse grouping:

- obtain the patient’s history, and review for information which may explain missing agglutinin (e.g. age, immunodeficiency)
- repeat the reverse group, increasing the sensitivity of the test, consider the use of tube techniques, lower incubation temperature, increased plasma:cell ratio and enzyme-treated red cells.

Unexpected additional reactions in the reverse group:

- investigate the presence of allo- or autoantibodies active at temperatures below 37°C
- consider repeating the reverse group at 37°C
- consider repeating the reverse group using cells negative for any identified alloantibody.

Unexpected reactions in the forward or D grouping, including positive diluent control:

- check for immunoglobulin coating of the patient’s cells by performing a direct antiglobulin test (DAT)
- consider repeating tests using unpotentiated reagents in tube techniques
- consider techniques to remove or reduce immunoglobulin coating (e.g. warm wash to remove IgM) and repeat tests with appropriate controls.

Unexpectedly weak or mixed field reactions in forward or D group:

- obtain the patient’s history, and review for information which may explain results (e.g. recent non-ABO identical transfusion, haemopoietic cell transplant)
- consider additional investigations which may include adsorption/elution, and flow cytometry
• panels of monoclonal anti-D reagents are commercially available for the investigation of partial and weak D phenotypes.

Genotyping is useful in resolving grouping problems, particularly weak and partial D types (see section 15.2). Genotyping alone must not be used to determine the ABO group for use in selection of blood for transfusion. Where the patient ABO group cannot confidently be assigned by serology, group O (high-titre negative) blood must be selected.

13.3.2: Antibody identification

In all cases of the investigation of alloantibodies laboratories should focus on:

• secure identification of alloantibodies detected

• exclusion of additional specificities to those identified

• selection of blood for transfusion (Daniels et al. 2002).7

When antibodies which cannot be identified have been detected, laboratories should consider referral to the International Blood Group Reference Laboratory (IBGRL).

When patients with rare phenotypes are encountered, laboratories should, when practicable, exchange material with other RCI departments via the UK Rare Red Cell Exchange to ensure continued supply of valuable materials.

Antibody identification techniques and protocols are described in BCSH guidelines and should be adhered to. More complex problems encountered by RCI laboratories and not covered by BCSH are considered below.

13.3.2.1: Complex antibody mixtures

When investigating complex antibody mixtures RCI laboratories should consider:

• extended phenotyping of the patient, e.g. C, c, D, E, e, K, M, N, S, s, P, LeA, LeB, FYa, FYb, JKa, Jkb

• if this is impossible due to previous transfusion or heavy IgG sensitisation, genotyping offers an alternative source of information

• extending the range of techniques and incubation temperature to identify component antibodies

• using cells matching the patient’s phenotype/genotype to confirm the presence of multiple antibodies rather than an antibody to a high-frequency antigen

• careful use of alloadsorption techniques to confirm the specificity of elements of the mixture.

13.3.2.2: Antibodies known as high-titre low-avidity (HTLA)

Antibodies traditionally known as HTLA include anti-Ch, –Rg, –Kn, McC, –Yk, –Cs and –Si. Typically HTLA antibodies present as reacting with most panel cells by indirect autoglobulin test (IAT) with variable strength, with or without similar patterns using enzyme-treated cells. Experienced operators can recognise characteristic agglutination by microscopic examination of tube IAT, which have been described as ‘loose’, ‘stringy’ or ‘gritty’. In investigating samples suspected to contain HTLA antibodies RCI laboratories should consider:
• Neutralising anti-Ch or -Rg specificities by incubating the patient’s plasma with pooled group AB donor plasma before IAT is undertaken. Reactivity of these antibodies is usually abolished. A dilution control in which the patient’s plasma is incubated with phosphate-buffered saline should be prepared and tested in parallel with the neutralised plasma.

• The use of a panel of cells lacking HTLA antigens.

13.3.2.3: Antibodies to high-frequency antigens (HFA)

Typically antibodies to HFA present with positive reactions of similar strength against all routine screen and identification panel cells. The most commonly encountered specificities include anti-k, –Lu\(^b\), –Kp\(^b\), –Vel, –Co\(^a\), –Yt\(^a\), –Fy3, –U and –In\(^b\). In investigating samples suspected to contain antibodies to HFA, RCI laboratories should consider:

• the ethnicity of the patient

• extended phenotyping as in section 13.3.2.1

• typing the patient’s red cells with antibodies to HFA. Where possible, CE-marked reagents must be used, otherwise results must be considered in context of the reliability of the reagent in use, supported by adequate controls.

13.3.2.4: Antibodies to low-frequency antigens (LFA)

Typically antibodies to LFA present with negative antibody screen and are detected in crossmatch. The most commonly encountered specificities include anti-Kp\(^a\), –Wr\(^a\) and –Co\(^b\). In investigating samples suspected to contain antibodies to LFA, RCI laboratories should consider:

• testing the patient’s plasma with a panel of red cells expressing LFA

• phenotyping the incompatible unit(s) for LFA.

13.3.3: Autoantibodies

Autoantibodies are frequently encountered in pre-transfusion testing, and may be the cause of autoimmune red cell destruction, or may be clinically benign. In either case autoantibodies may interfere with pre-transfusion testing, either due to coating of patient’s cells with immunoglobulin, or as pan-reactive antibody in patient’s plasma. In providing safe transfusion in the presence of autoantibodies, RCI laboratories may adopt the following strategies.

13.3.3.1: ABO and Rh grouping in the presence of autoantibodies

Most modern test systems support routine, accurate grouping of the majority of patients whose cells are coated with immunoglobulin and who give a positive DAT. Cases which are problematic may present with reaction patterns that cannot be assigned to an ABO group, weak additional reactions and positive reagent controls. Such cases should be investigated as in section 13.3.1.

Laboratories should make a clear documented assessment, based on the recommendations of reagent and test system suppliers, how to manage cases with anomalous ABO and D groups. This is particularly important when potentiated reagents are included in test systems.

13.3.3.2: Alloantibody detection and identification in the presence of autoantibodies

In dealing with cross-reacting autoantibodies, which complicate the detection and identification of underlying alloantibodies, RCI laboratories should consider:
• The characteristics of available, validated IAT in testing patient plasma-containing pan-reacting autoantibodies. Some workers consider tube IAT to be less prone to interference by autoantibodies than column technologies.

• The use of the patient’s own cells to adsorb autoantibody from the plasma, permitting detection and identification of alloantibodies.

• The use of cells from two or more selected donors to adsorb autoantibody. Typically these cells are enzyme treated to optimise removal of autoantibody.

### 13.3.4: Management of patients with autoantibodies

Consideration should be given to close matching of recipient and donor red cell types. This is to safeguard against the presence of alloantibodies undetected by tests on modified plasma, and to prevent further alloimmunisation. In patients who cannot be grouped by conventional serology, due to sensitisation of red cells or previous transfusion, genotyping offers a solution.

In patients with autoantibodies requiring regular transfusion, close matching of transfused red cells with the patient’s own phenotype, to manage risk of transfusion reactions, may be used as a basis by scientists and clinicians to assess, and potentially reduce, the required frequency of testing. Such assessments should be fully documented and subject to planned review.