Guidelines for the Blood Transfusion Services

18.2: Methods

http://www.transfusionguidelines.org/red-book/chapter-18-platelet-immunology/18-2-methods

18.2: Methods

18.2.1: HPA typing methods

- HPA types should be determined using antibody-based and/or DNA/PCR-based techniques that have been validated in the laboratory.

- Polyclonal human anti-HPA antisera used in serological techniques should be well characterised. When used in techniques with ‘intact’ platelets the antisera should be ABO compatible with the platelets to be typed. Alternatively, anti-A and anti-B antibodies may be removed by absorption or neutralisation. This is not a requirement when using human antisera in glycoprotein capture assays, but reactivity against ABO incompatible platelets should be assessed. Sera shown to contain anti-A /B activity in these assays should be subject to the same requirements as those used in ‘intact’ platelet assays.

18.2.2: HPA antibody detection methods

- There are several techniques for the detection of HPA-reactive antibodies. These techniques can be divided into non-specific (where intact platelets are used, e.g. platelet immunofluorescence test, solid phase adherence assay) and specific assays (where glycoprotein capture, or purified glycoproteins or recombinant antigens are used, e.g. monoclonal antibody-specific immobilisation of platelet antigen assay). Laboratories should use tests with adequate sensitivity for the detection and specification of HPA-reactive antibodies.

- The combination of chosen technique(s) and the composition of the cell panel cells (if applicable) must ensure:
    - the detection of clinically significant HPA-reactive alloantibodies in the HPA-1, HPA-2, HPA-3, HPA-5 and HPA-15 systems
    - the identification of HPA-reactive antibodies and their specificity in samples containing a mixture of HPA and HLA-reactive antibodies
    - the identification of the specificities in samples containing mixtures of alloantibodies against several HPA antigens (i.e. avoiding the masking of certain HPA specificities by the composition of the panel).

- Assays for the detection of platelet antibodies, which utilise:
    1. glycoproteins isolated from human cells or soluble recombinant antigens attached to a solid phase, or
    2. recombinant cell lines expressing HPA

  should be used in parallel with established human platelet based tests, either 'in house' or at a reference laboratory.

- An antibody specificity determined on the basis of reactivity with a single recombinant antigen or single isolated membrane glycoprotein should be viewed as indicative rather than definitive. Further work should be undertaken to confirm the antibody specificity using other sources of the implicated antigen. If the ‘indicative’ antibody specificity is confirmed by other techniques the original result can be used as supporting evidence to satisfy the requirements in 18.3.2. The existing advice that, wherever possible, a patient or donor with suspected HPA specific alloantibodies should either be genotyped to determine if they are negative for the allele encoding the implicated antigen or be phenotyped to ensure the absence of the antigen (18.3.2) should be applied.

- Where HPA-reactive antibodies are detected, but the specificity cannot be determined, the samples should be referred to a reference laboratory for antibody specificity investigations. However, all reasonable efforts should be made to screen against the widest possible range of HPA antigens.

18.2.3: Validation of laboratory kits

- Kits for HPA typing should be validated for specificity on a batch basis with samples that possess homozygosity and heterozygosity for the relevant HPA polymorphism that is included in the test kit.

- Kits for the detection of HPA-reactive antibodies should be validated for sensitivity and specificity on a batch basis using a panel of clinically representative HPA antisera. It is recommended that for monitoring of the sensitivity of HPA antibody detection the panel of antisera should contain ‘weak’ reactive HPA antibodies (not obtained by dilution of strongly reactive HPA typing sera). A panel of sera shown to be inert for HPA and HLA antibodies should also be used.