Guidelines for the Blood Transfusion Services

17.1: Reagent manufacture/reference preparations/cell panels

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17.1: Reagent manufacture/reference preparations/cell panels

17.1.1: HNA typing reagents

There are several human neutrophil antigen (HNA) genotyping and phenotyping techniques. The latter are generally based on the use of polyclonal HNA alloantibodies obtained from immunised donors or patients or monoclonal antibodies. HNA typing techniques that do not require polyclonal antibodies derived from donors or patients are the techniques of choice.

HNA typing reagents prepared from human source material should comply with the guidelines in section 11.1.4.10.

An ‘Instructions for use’ sheet (package insert) should be prepared and supplied with antibody typing reagents, see section 11.1.4.12. Information in the instructions for use sheet should further indicate the immunoglobulin class of the antibodies and the presence of any other contaminating antibodies reactive by the recommended methods.

HNA typing reagents used in genomic DNA and polymerase chain reaction (PCR)-based techniques should comply with the guidelines in Chapter 14.

17.1.2: Composition of granulocyte cell panel for HNA antibody detection

It is recommended that laboratories make all reasonable efforts to include cells in their panel that will aid the detection and identification of clinically significant HNA antibodies. The panel should consist of granulocytes typed for HNA-1a, 1b, 1c, 2, 3a, 3b, 4a, 4bw, 5a and 5bw by validated HNA typing techniques. A minimum panel should include granulocytes that are homozygous for HNA-1a and HNA-1b and preferably be from Group O donors. The panel can be expanded to include granulocytes homozygous for other HNA as indicated by the results of laboratory testing.

HNA typing of a granulocyte panel donor should ideally be based on two concordant typing results performed on samples obtained on different occasions. Wherever possible, both phenotyping and genotyping should be performed for the above antigens.

17.1.3: The preparation of granulocytes/lymphocytes

Granulocytes and lymphocytes for use in serological investigations should be prepared with regard to the following criteria:

- Granulocytes/lymphocytes should be prepared from donors/patients within 24 hours of venesection. Precautions must be taken to minimise activation of granulocytes during isolation.
- Granulocyte/lymphocyte preparations should be essentially free from red cells that would otherwise interfere with the technique or its reading.

- The viability of isolated granulocytes should be sufficient as to not interfere in the technique used.

17.1.4: Selection of normal control sera

Normal control sera should be taken from untransfused male blood donors. The sera should be screened and found negative for granulocyte-reactive antibodies (e.g. clinically non-significant autoantibodies are occasionally detected in apheresis donors). An appropriate number of normal sera should be used, so that in any given assay a statistically relevant normal range can be determined.

17.1.5: Selection of positive control sera

At least one positive control should be included in each assay. The selection and number of positive control sera will depend on the technique and the HNA type of the granulocytes being used. In glycoprotein-specific assays, a positive control for each glycoprotein used should be included as a minimum. If different capture monoclonal antibodies are used, the positive control selected should be reactive with the monoclonal antibody selected.

17.1.6: Reference preparations

Sensitivity of techniques should be monitored on the basis of the inclusion of a ‘weak positive’ control. For anti-HNA-1a, the internal sensitivity control should be calibrated against the WHO International Reference Reagents for anti-HNA-1a (NIBSC code 09/284) when diluted as instructed by the manufacturer.

In-house sensitivity standards, with similar reaction strengths to the above reagent, should be prepared for other HNA antibodies.

17.1.7: Quality control schemes

Laboratories should take part in regular external quality control exercises such as the International Granulocyte Immunology Workshops for HNA antibody detection and for HNA genotyping. Effective mechanisms should be in place to correct poor performance in the quality scheme.