

## Guidelines for the Blood Transfusion Services

### 17.4: HNA antibody detection methods

<http://transfusionguidelines.org/red-book/chapter-17-granulocyte-immunology/17-4-hna-antibody-detection-methods>

### 17.4: HNA antibody detection methods

There are several techniques for the detection of HNA-reactive antibodies. These techniques

can be divided into non-specific (where intact granulocytes are used, e.g. granulocyte immunofluorescence test, granulocyte agglutination test) and specific assays (where glycoprotein capture, or purified glycoproteins or recombinant antigens are used, e.g. monoclonal antibody immobilisation of granulocyte antigen test). Laboratories should use tests with adequate sensitivity for the detection and identification of HNA-reactive antibodies. It is recommended that more than one technique is used to detect HNA-specific antibodies.

The combination of chosen technique(s) and the composition of the cell panel cells (if applicable) must ensure:

- The detection of clinically significant HNA-reactive alloantibodies to the antigens of the HNA-1, HNA-2, HNA-3, HNA-4 and HNA-5 systems.
- The detection and identification of HNA-reactive antibodies in samples containing a mixture of both HNA and HLA-reactive antibodies, including antibodies to HNA-3 system antigens, which are expressed on both granulocytes and lymphocytes.
- The identification of the individual HNA specificities in samples containing mixtures of alloantibodies against several HNA antigens (e.g. masking of certain HNA specificities by composition of the panel).
- Techniques should be available to detect cytotoxic and non-cytotoxic anti-lymphocyte antibodies and thereby aid the distinction between granulocyte-specific, lymphocyte-reactive and HLA Class I and Class II antibodies.
- Assays for the detection of granulocyte antibodies, which utilise glycoproteins isolated from human cells, soluble recombinant antigens attached to a solid phase or recombinant cell lines expressing HNA should be used in parallel with established human granulocyte-based tests, either 'in house' or at a reference laboratory, while further data on the performance of these tests is gathered. 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 17.5.2. The existing advice that, wherever possible, a patient or donor with suspected HNA 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 (17.5.2) should be applied.

Where granulocyte-specific antibodies are detected, which appear to have allo-specificity, but the specificity cannot be determined, the samples should be referred to a reference laboratory for further antibody specificity investigations. However, laboratories should make all reasonable efforts to screen against the widest possible range of HNA antigens.

#### 17.4.1: Validation of laboratory kits

- Kits for the detection of HNA-reactive antibodies should be validated for sensitivity and specificity on a batch basis using a panel of clinically representative HNA antisera. It is recommended that the sensitivity of HNA antibody detection should be monitored using a panel of antisera containing 'weak' reactive HNA antibodies (not obtained by dilution of strongly reactive HNA typing sera). A panel of sera shown to be inert for HNA and HLA antibodies should also be used.
- Kits for HNA typing should be validated for specificity on a batch basis using nine donors (three donors homozygous for each HNA allele together with three heterozygotes).