Guidelines for the Blood Transfusion Services

Chapter 18: Platelet immunology

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Chapter 18:

Platelet immunology

18.1: Reagent manufacture/reference preparations

18.1.1: HPA typing reagents

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

- HPA 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. Information in the ‘Instructions for use’ should further indicate the immunoglobulin class of the antibodies and the presence of any other contaminating antibodies reactive by the recommended methods.

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

18.1.2: Composition of platelet cell panel for HPA 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 HPA antibodies. The panel should consist of platelets typed at a minimum for HPA-1, -2, -3, -5 and -15 by validated HPA typing techniques. Ideally, the panel should contain platelets that are homozygous for HPA-1a, -1b, -2a, -2b, -3a, -3b, -5 a, -5b, -15a and -15b and be from Group O donors.

- HPA typing of a platelet panel donor should be based on two concordant typing results using samples obtained on different occasions.

18.1.3: Selection of normal control sera

Normal control sera should be taken from non-transfused group AB male or ABO compatible blood donors. The sera should be screened and found negative for platelet-reactive-antibodies (e.g. clinically non-

significant autoantibodies or EDTA-dependent antibodies are occasionally detected in apheresis donors). An appropriate number of normal sera should be used so that a statistically relevant normal range in a given assay can be determined.

18.1.4: 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 HPA type of the platelets being used.
In glycoprotein-specific assays a positive control for each glycoprotein used should be included as a minimum.

18.1.5: Reference preparations

- Sensitivity of techniques should be monitored on the basis of the inclusion of a ‘weak positive’ control. For anti-HPA-1a, -3a and -5b, the internal sensitivity control should be calibrated against the WHO International Reference Reagents for anti-HPA-1a (NIBSC code 05/106), anti-HPA-3a (NIBSC code 03/190), anti-HPA-5b (NIBSC code 99/666) ans anti-HPA-15b (NIBSC code 18/220) when diluted as instructed by the manufacturer.
- In-house sensitivity standards, with similar reaction strengths to the above reagents, should be prepared for anti-HPA-1, -3 and -5, and, if possible, for anti-HPA-2 and -15 antibodies.

Table 18.1 Current HPA nomenclature

System	Antigen	Original names	Glycoprotein	CD
HPA-1	HPA-1a	Zw^a, PlA1	GPIIIa	CD61
	HPA-1b	Zw^b, PlA2		
HPA-2	HPA-2a	Ko^b	GPIbalpha	CD42b
	HPA-2b	Ko^a, Sib^a		
HPA-3	HPA-3a	Bak^a, Lek^a	GPIIb	CD41
	HPA-3b	Bak^b		
HPA-4	HPA-4a	Yuk^b, Pen^a	GPIIIa	CD61
	HPA-4b	Yuk^a, Pen^b		
HPA-5	HPA-5a	Br^b, Zav^b	GPIa	CD49b
	HPA-5b	Br^a, Zav^a, Hc^a		

	HPA-6bw	Ca^a, Tu^a	GPIIIa	CD61
	HPA-7bw	Mo^a	GPIIIa	CD61
	HPA-8bw	Sr^a	GPIIIa	CD61
	HPA-9bw	Max^a	GPIIb	CD41
	HPA10bw	La^a	GPIIIa	CD61
	HPA11bw	Gro^a	GPIIIa	CD61
	HPA12bw	Iy^a	GPIbbeta	CD42c
	HPA13bw	Sit^a	GPIa	CD49b
	HPA14bw	Oe^a	GPIIIa	CD61
HPA-15	HPA-15a	Gov^b	CD109	CD109
	HPA-15b	Gov^a		
	HPA-16b	Duv^a	GPIIIa	CD61
	HPA-17b	Va^a	GPIIIa	CD61
	HPA-18b	Cab^a	GPIa	CD49b
	HPA-19b	Sta	GPIIIa	CD61
	HPA-20b	Kno	GPIIb	CD41
	HPA-21b	Nos	GPIIIa	CD61
	HPA-22b	Sey	GPIIb	CD41

	HPA-23b	Hug	GPIIIa	CD61
	HPA-24b	Cab2^{a+}	GPIIb	CD41
	HPA-25b	Swi^{a}	GPIa	CD49b
	HPA-26b	Sec^{a}	GPIIIa	CD61
	HPA-27b	Cab3^{a+}	GPIIb	CD41
	HPA-28b	War	GPIIb	CD41
	HPA-29b	Kha^{b}	GPIIIa	CD61
	HPA-30b	Lab^{a}	GPIIb	CD41
	HPA-31b	Cab4^{b}	GPIX	CD42a
	HPA-32b	Dom^{b}	GPIIIa	CD61
	HPA-33b	Bl^{a}	GPIIIa	CD61
	HPA-34b	Bzh^{a}	GPIIIa	CD61
	HPA-35b	Efs^{a}	GPIIIa	CD61

18.1.6: Quality control schemes

Laboratories should take part in regular external quality control exercises such as the UK NEQAS for Histocompatibility and Immunogenetics schemes for HPA genotyping and HPA antibody detection /specification. Effective mechanisms should be in place to correct poor performance in the quality scheme.

18.1.7: Nomenclature

The current HPA nomenclature must be used for recording platelet-specific alloantigen and alloantibody specificities[1] (see Table 18.1). Any subsequent additions can be found in the Human Platelet Antigen Database (www.versiti.org/products-services/human-platelet-antigen-hpa-database).

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.

18.3: Donor testing

18.3.1: HPA typing

Donors whose products may be used for fetal/neonatal transfusions should be HPA typed twice using samples collected on different occasions. Further HPA typing at subsequent donations is not required after a confirmed type has been established. HPA typing of other donors need only be performed on one occasion and HPA-selected products may be issued on the basis of this ‘unconfirmed’ type.

18.3.2: Investigation of HPA antibodies

HPA antibody specificities should only be assigned when the sample investigated has been tested and a minimum of three positive and three negative reactions obtained. An antibody report can be issued at this stage. A donor with an HPA antibody should receive an HPA antibody card and an information leaflet, where this is available. However, before an HPA antibody card and information leaflet is issued, the donor should be typed and found negative for that antigen.

18.4: Patient testing

18.4.1: HPA typing

Patients should be typed for HPA following the guidelines for donor HPA typing with the following exceptions:

- A provisional type can be issued on the basis of a genotype performed on one occasion. However, it is recommended that a second typing technique be used when quality exercises or routine practice

have revealed technical problems when typing for particular polymorphisms. Typing of subsequent samples will allow a confirmed genotype to be reported.

- HPA typing of fetal amniocytes can be undertaken by molecular techniques using DNA isolated from non-cultured amniocytes and a provisional HPA genotype reported. The HPA genotype should be repeated on DNA extracted from cultured amniocytes and shown to be concordant with the first result.

- HPA typing from cell-free fetal DNA can also be applied, similar to that for blood group typing described in section 15.4.1. Appropriate validation of HPA typing using cell-free fetal DNA against existing techniques using amniocytes should be in place.

18.4.2: Investigation of HPA antibodies

Patients should be investigated for HPA antibodies following the guidelines for donor investigation with the following exceptions:

- Laboratories serving populations with non-Caucasoid patients are advised to include cells in their panels which will aid the detection and identification of additional clinically significant antibodies (e.g. HPA-4, Naka/GPIV). If the acquisition of GPIV negative cells is not possible, an alternative approach is to establish assays capable of identifying GPIV antibodies that are controlled by appropriate positive control sera.

- Laboratories providing diagnostic testing for Neonatal Alloimmune Thrombocytopenia (NAITP) should include HPA typing of the parents and affected baby(ies). This testing will help identify any potential HPA incompatibilities and can be used to direct antibody screening if the father and baby both have a low frequency HPA that is absent in the mother. Laboratories are advised to investigate cases with a clinical diagnosis suggestive of NAITP and with a negative screen for common HPA antibodies , for antibodies against low-frequency or ‘private’ antigens. An effective approach is to use platelets from the child’s father as an additional panel cell (paternal platelets should be HPA typed as a ‘patient sample’). Alternatively, laboratories may refer such cases to a reference laboratory. In the event of a negative antibody screen in a case where NAITP is suspected and there is a potential HPA incompatibility between maternal and baby HPA types, laboratories are advised to repeat the antibody investigation 1 month after delivery.

- Laboratories providing diagnostic testing for platelet refractoriness should follow the algorithm for laboratory investigations of platelet refractoriness in Figure 16.1.

A patient with HPA antibodies should receive an HPA antibody card and, wherever possible, an information leaflet. However, before an HPA antibody card and information leaflet is issued, the patient should be typed and found negative for that antigen.

18.5: References

1. Metcalfe P, Watkins NA, Ouwehand WH, Kaplan C, Newman P, Kekomaki R, De Haas M, Aster R, Shibata Y, Smith J, Kiefel V, Santoso S. (2003). Nomenclature of Human Platelet Antigens. *Vox Sanguinis*, 85, 240–245.