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SECTION 5.0 NZBMDR TISSUE TYPING STANDARDS

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SECTION 5.0 NZBMDR TISSUE TYPING STANDARDS

Accreditation

All Tissue Typing for the NZBMDR is carried out at the NZBS Tissue Typing laboratory in Auckland. This laboratory holds accreditation with the American Society for Histocompatibility and Immunogenetics (ASHI) for functions and tests performed by serology or molecular typing methods. (refer: Section 2 Form ATT).

Minimal Matching Requirement

This Standard outlines minimal matching requirements for Tissue Typing Laboratories providing donors from the NZBMDR for matched unrelated bone marrow transplantation and distinguishes these requirements from additional ones deemed important by individual transplant units.

Four levels of matching are considered:

1. HLA-A, B, DR typing at registration.
2. HLA-DR requests – Class II typing by generic DNA typing
3. HR requests (High Resolution Typing by DNA) and repeat Class I typing.
4. CT Requests (Confirmatory testing) – Provision of a blood sample for confirmatory and additional tests performed by the Laboratory serving the Transplant Centre to determine the degree of donor/recipient compatibility.

Level 1

HLA-A, B, DR TYPING – Class I and II Tissue Typing of Newly Recruited Donors by DNA Typing

Molecular tests will be used for registration with the level of discrimination shown in Table 6 (molecular) which must be obtained.

Class II typing of young donors with an emphasis on male donors under the age of 41 years is performed by molecular techniques and must cover DR1-DR16. Subtyping of DR6 and DR2 must be performed (see Table 7).

Level 2

HLA-DR REQUESTS – Class II Typing by Generic DNA Typing

Class II typing on recall should be performed by generic DNA typing. DR1-DR16 should be tested. DR6 and DR2 subtyping must be included in generic typing but these tests may be reported in two stages (Table 7). On the basis of these results the potential donor can either be excluded or further tests performed.

Level 3

HR REQUESTS (High Resolution Typing by DNA)

High resolution typing of DRB1 and DQB1. Table 8 (DRB1) and Table 9 (DQB1) lists the level of resolution which must be included in the testing algorithm. For the DRB1 locus all ambiguities are to be resolved (exon 3 excepted apart from distinguishing DRB1*14:01 and DRB1*14:54) so that either one (homozygote) or two (heterozygote) alleles are reported.

CT Requests (Confirmatory Testing) – Provision of a Blood Sample for confirmatory typing and additional tests performed by the laboratory serving the Transplant Centre to determine the degree of Donor/Recipient compatibility, namely antibody screening and identification.

Level 4

CONFIRMATORY TYPING

i) DNA Testing

HLA-A, B, C, DQB1 and DRB1 high resolution DNA typing should be performed for both patient and donor by the recipient laboratory. The degree of resolution required is shown in Tables 10 & 11 .

DPB1 have been shown to influence outcome in bone marrow transplantation but is not generally used in the matching algorithm. DRB3, DRB4 and DRB5 have not been shown to influence outcome at this stage but should be typed to assist in deciding between several donors who are otherwise matched.

ii) Donor/recipient compatibility testing – HLA Antibody Screening and Identification by Luminex

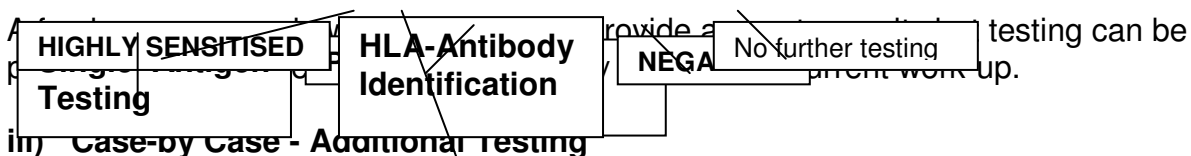
Recent studies have associated donor specific HLA antibodies with graft failure and rejection in both unrelated cord transplantation (Takanashi et al 2008) and conventional HSC transplantation (Bray et al 2007).

The advantages associated with this assay are as follows:

- a) Detection of **both** HLA-Class I and HLA-Class II antibodies
- b) Detection of **both** complement and non-complement fixing antibodies
- c) Detection of low titre antibodies
- d) Detection of **only** HLA-antibodies
- e) Does not require donor lymphocytes

The National Tissue Typing Laboratory has employed this assay for 3 years as part of the routine testing of patients requiring a solid organ transplant.

Testing Strategy:



In those cases where antibodies are determined against HLA loci not routinely typed for, additional HLA typing of the donor and/or patient may be required. For example, HLA-DP in the case of HSC with a MUD or HLA-Cw and/or HLA-DQ in cord transplants or mismatched related cases.

iv) Class I Serology HLA Typing

HLA Class I serology typing to check for allele expression will complement the current molecular techniques used for high resolution typing and will aid the exclusion of rare alleles.

Summary

In summary therefore, in order to provide an NZBMDR matched donor the following must be performed: -

- i) Intermediate or High resolution by DNA-based HLA-A, B, C, DQB1 and DRB1 typing at final matching level.
- ii) NZBMDR allows a 1 antigen mismatch at HLA A, B, DRB1* between patient and donor.

Standards for DRB1 DNA Assignments

Information on new alleles can be obtained from the IMGT web site or from the WHO nomenclature report which is published in Human Immunology and Tissue Antigens. Both sources are updated regularly. The list of alleles shown in the attached tables will be updated on a 12 monthly basis.

New sequences should be incorporated into generic and allele specific typing strategies and all ambiguous allele combinations from this point onwards are to be documented. The DRB1 high resolution strategy should as far as possible provide unambiguous typing for the alleles listed in Table 8. All ambiguities however (exon 3 excluded) should be resolved at CT stage.

Where more than one donor meets the minimum matching criteria DRB3, 4, 5 should be used to select the best matched available donor.

This Standard has attempted to summarise what is required in terms of testing to define a matched donor. At the point this is achieved the NZBMDR has fulfilled its function, that is, to provide a matched unrelated bone marrow donor for use in clinical transplantation. Other tests which supplement the base level of testing should be seen at this stage as additional local requirements to be used where applicable.

TABLE 6

Minimal Requirements for Molecular Class I Typing at Registration

Recommended Requirements HLA A (DNA based typing)		
A*01:XX	02:XX	03:XX
11:XX	23:XX	24:XX
25:XX	26:XX	29:XX
30:XX	31:XX	32:XX
33:XX	34:XX	36:XX
43:XX	66:XX	68:XX
69:XX	74:XX	80:XX

HLA-B

Recommended Requirements HLA B (DNA based typing)		
B*07:XX	08:XX	13:XX
14:XX	18:XX	27:XX
35:XX	37:XX	38:XX
39:XX	40:05	41:XX
42:XX	44:XX	45:XX
46:XX	47:XX	48:XX
49:XX	50:XX	51:XX
52:XX	53:XX	54:XX
55:XX	56:XX	57:XX
58:XX	59:XX	40:XX
67:XX	15:XX	78:XX
81:XX	73:XX	82:XX
	83:XX	

* note: it is recognised that different molecular methods will give different allele strings, but each laboratory should endeavour to split the B15 alleles into the serological splits (B62, 75, 76, 70, 71, 72, 63, 77). The same principle applies for B40, (B60, B61). Examples of strings for these antigens are shown.

B*14:01 and B*14:02 must be part of different strings except when a homozygote B14.

HLA-C

Recommended Requirements HLA C (DNA based typing)			
C*	01:XX	02:XX	03:XX
	04:XX	05:XX	06:XX
	07:XX	08:XX	12:XX
	14:XX	15:XX	16:XX
	17:XX	18:XX	

Table 7

Generic DRB1 Requirements at Registration

DRB1*		
01	15	16
03	04	11
12	13	14
07	08	09
10		

DR6 may be reported if the result includes rare allele combinations of DR13 and DR14.

Table 8

DRB1 Alleles for High Resolution Stage Typing

DRB1
01:01 – 01:20, 15:01 – 15:31, 16:01 – 16:13N
03:01 – 03:40, 04:01 – 04:78, 11:01 – 11:70
12:01 – 12:18, 13:01 – 13:87, 14:01 – 14:83
07:01, 07:03 – 07:16, 08:01 – 08:36
09:01-09:08, 10:01- 10:03

No requirement to distinguish between DRB1*12:01,12:06 AND 12:10.

No requirement to distinguish silent substitutions

Table 9

DQB1 Allele Requirements for High Resolution Stage Typing

DQB1
02:01/02, 02:03-05, 04:01-04:03, 05:01 – 05:05
06:01-06:34
03:01/09, 03:02-03:23

Alleles that differ by silent substitutions are not required to be resolved.

Exon 3 differences do not require resolution.

Table 10

CT Matching Level Requirements for HLA-A

Serological Requirements	Molecular Typing Requirements
A1 A2 A3	A*01:01-33 02:01-99 02:101-151 03:01-46
A11 A23 A24	11:01-40 23:01-19 24:02-97
A25 A26 A29	25:01-08 26:01-37 29:01-19
A30 A31 A32	30:01-28 31:01-24 32:01-18
A33 A34 A36	33:01-23 34:01-08 36:01-04
A43 A66 A68	43:01 66:01-08 68:01-46
A69 A74 A80	69:01 74:01-13 80:01

Table 11
CT Matching Level Requirements for HLA-B

Serological Requirements				Molecular Typing Requirements		
B7	B8	B44	B45	B*07:02-68	08:01-38	13:01-23
B13	B64	B65	B62	14:01-09	18:01-32	27:01-45 15:01-99 15:101-153
B63	B75	B76	B77	35:01-94	37:01-14	38:01-17
B38	B39	B57	B58	39:01-46	40:01-94	41:01-08
B18	B49	B50	B54	42:01-09	44:02-65	45:01-09
B55	B56	B27	B35	46:01-18	47:01-05	48:01-19
B37	B51	B52	B53	49:01-05	50:01-04	51:01-63
B78	B59	B60	B61	52:01-14	53:01-16	54:01-17
B41	B42	B46	B47	55:01-34	56:01-24	57:01-19
B48	B67	B71	B72	58:01-20	59:01-04	
	B73			67:01-02	78:01-06	81:01-04N
				73:01	82:01-02	83:01

Table 12

CT Matching Level Requirements for HLA-C

Serological Requirements	Molecular Typing Requirements
Cw1 Cw2	C*01:02-22 C*02:02-23
Cw4 Cw5	C*04:01-37 C*05:01-23
Cw6 Cw7	C*06:02-19 C*07:01-65
Cw8	C*08:01-19
Cw9 Cw10	C*03:02-50
	C*12:02-21 C*14:02-11
	C*15:02-21 C*16:01-12
	C*17:01-05 C*18:01-03