

Technology Reports: Application of HLA Epitope-Based Matching in the Clinical Transplant Setting

Part II: Pre-Transplant Serum Analysis for Epitope-Specific Antibodies

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Part I addressed the interactions of HLA epitopes with monospecific antibodies. The HLA Epitope Registry (<http://www.epregistry.ufpi.br>) has a record of antibody-verified epitopes for each locus but the list is still incomplete. Very recently, the website has included a downloadable PDF file, "EpiPedia of HLA," which describes the antibody-verifications in detail and which will be updated on a regular basis. With the help of participating HLA laboratories that might have interesting serum antibody reactivity patterns, we will continue our investigations to identify new epitopes. The educational section of the www.HLAMatchmaker.net website has now a downloadable Excel document, "Five Maps of HLA Epitopia," which describe the sequence locations of antibody-verified eplets and polymorphic residues as potential candidates defining additional epitopes. These maps can be used in navigating the continents of HLA Epitopia while searching for newly antibody-defined epitopes.¹

HLA Antibodies in Sera from Sensitized Patients

Allosensitized patients have HLA antibodies that can be induced by a transplant, transfused blood, or during pregnancy. Most sera from sensitized patients have mixtures of antibodies and although the reactivity patterns are generally limited to a few specificities, there are additional features that can make epitope-based interpretations quite challenging. They include unexpected ("natural" antibody or unexplained) reactivities of certain alleles in SAB panels, differences between competing antibody characteristics, including Ig subtypes, and the presence of non-specific blocking factors including the prozone effect. Many sera from highly sensitized patients have antibodies reacting with high-frequency (i.e., >80%) epitopes that make detections of antibodies against lower frequency epitopes more difficult unless these antibodies are separated through absorption-elution studies with selected alleles.

Technique-dependencies of serum reactivity may also affect the interpretation of epitope specificities especially for highly sensitized patients who have several antibody populations in different concentrations and affinities that affect their reactivity with HLA panels. Again, absorption-elution studies with selected alleles might dissect these serum reactivity patterns so that an epitope analysis can be more readily done.

In the clinical setting, the primary purpose of the serum HLA antibody analysis of transplant candidates is to identify potential donors whose mismatched HLA antigens are acceptable. The

traditional approach has been to identify serum-reactive antigens to be considered as unacceptable mismatches. Since HLA antibodies react specifically with epitopes it is now apparent that mismatch acceptability must be determined at the epitope level. Accordingly, any antigen that carries an epitope recognized by patient's antibodies can be considered an unacceptable mismatch.

Programs for Epitope Specificity Determinations of HLA antibodies

The HLA Matchmaker website has three downloadable antibody analysis programs in Excel format: HLA-ABC, HLA-DRDQDP, and MICA. The latest 02 versions focus on antibody-verified epitopes recorded so far in the HLA Epitope Registry. All of them correspond to eplets and there are two patterns. First, a specific antibody reacts with all alleles carrying a given eplet. In these cases, an eplet describes the epitope specifically recognized by antibody. Second, an epitope is defined by the combination of an eplet and another polymorphic residue configuration (eplet) uniquely shared by a group of antibody-reactive alleles. Such epitopes are referred to as eplet pairs. The antibody analysis programs also include "other" theoretical eplets that might become experimentally verified if informative antibodies are identified. The HLA Matchmaker website has a downloadable instruction manual for the epitope analysis of HLA antibodies tested in assays with single alleles.

The antibody analysis programs have two sheets on which the following data need to be entered: First, the HLA information of up to 120 alleles in the panel. Second, the MFI values with the panel; this can be done manually but the easiest way is to copy the numbers from the csv files of the Luminex software programs. Third, the four-digit allele HLA type of the patient; this provides information which eplets on the panel alleles are mismatched. Fourth, the HLA alleles of the immunizer (for instance, a previous transplant or in case of a pregnancy, the paternal allele(s) of a child) will identify the mismatched epitopes the patient has been exposed to. HLA Matchmaker shows also so-called third-party epitopes on reactive alleles. Such epitopes may reflect reactivity with antibodies induced during another sensitization event, and it is possible to rule them out from the analysis of this patient's immunizer-specific serum reactivity (e.g., determining the acceptability of a child as a potential living donor). For many sera, the laboratory has no HLA information about immunizers; in such cases all epitopes on reactive alleles are designated as third-party and all antibody reactivity should be considered as relevant. Information about the HLA sensitization history will facilitate interpretations of epitope specificities.

Table 1. Examples of Residue Differences Between Alleles Corresponding to Antigen Groups

A*02:01	9F	43Q	95V	149A	152V	156L	DRB1*01:01	67L	70Q	71R	85V	86G
A*02:02	9F	43R	95L	149A	152V	156W	DRB1*01:02	67L	70Q	71R	85A	86V
A*02:03	9F	43Q	95V	149T	152E	156W	DRB1*01:03	67I	70D	71E	85V	86G
A*02:05	9Y	43R	95L	149A	152V	156W						
A*02:06	9Y	43Q	95V	149A	152V	156L	DRB1*03:01	26Y	28D	47F	86V	
							DRB1*03:02	26F	28E	47Y	86G	
A*24:02	166D	167G										
A*24:03	166E	167W					DRB1*04:01	57D	67L	70Q	71K	86G
							DRB1*04:02	57D	67I	70D	71E	86V
A*30:01	70Q	76V	77D	152W			DRB1*04:03	57D	67L	70Q	71R	86V
A*30:02	70H	76E	77N	152R			DRB1*04:04	57D	67L	70Q	71R	86V
							DRB1*04:05	57S	67L	70Q	71R	86G
A*33:01	171H	186R										
A*33:03	171Y	186K					DRB4*01:01	135S				
							DRB4*01:03	135G				
A*66:01	90D	163R										
A*66:02	90A	163E					DRB5*01:01	6R	30D	37D	38L	67F
							DRB5*02:02	6C	30G	37N	38V	67I
B*07:02	69A	70Q	71A									
B*07:03	69T	70N	71T				DQB1*0201	135D				
							DQB1*0202	135G				
B*27:03	59H	77D	80T	82L	83R							
B*27:05	59Y	77D	80T	82L	83R		DQB1*0301	13A	26Y	45E	57D	167H
B*27:08	59Y	77S	80N	82R	83G		DQB1*0302	13G	26L	45G	57A	167R
							DQB1*0303	13G	26L	45G	57D	167R
C*03:02	91G	95L	116S									
C*03:03	91R	95I	116Y				DQA1*01:01	2D	25Y	34E	41R	129Q
C*03:04	91G	95I	116Y				DQA1*01:02	2D	25Y	34Q	41R	129Q
							DQA1*01:03	2D	25F	34Q	41K	129H
							DQA1*01:04	2G	25Y	34E	41R	129Q
C*07:01	66N	95L	99Y	116S	156L	177E						
C*07:02	66K	95L	99S	116S	156L	177E						
C*07:04	66K	95F	99Y	116F	156D	177K						

Table 2A. Example of an HLA Class I Epitope Specificity Analysis

Antibody Producer	A*02:01,A*23:01	B*15:18, B*51:01	C*07:04, C*15:02
Immunizing Haplotype	A*02:01	B*40:02	C*02:02
Allele	MFI	Ep1	Ep2
B*40:02 Immunizer	9647	41T	163EW+66I
B*47:01	5358	41T	163EW+66I
B*40:01	10547	41T	163EW+66I
B*40:06	10787	41T	163EW+66I
B*13:02	8552	41T	163EW+66I
B*13:01	5221	41T	163EW+66I
B*41:01	7579	41T	
B*44:03	6717	41T	
B*44:02	6513	41T	
B*45:01	10001	41T	
B*49:01	8509	41T	
B*50:01	8242	41T	
B*07:02	11608		163EW+66I
B*27:05	7995		163EW+66I
B*27:08	9459		163EW+66I
B*48:01	5159		163EW+66I
B*73:01	8410		163EW+66I
B*81:01	7533		163EW+66I
A*66:02	7976		163EW+66N
A*66:01	84		
C*02:02 Immunizer	430		163EW+66K
C*17:01	67		163EW+66K
Self Alleles	86+39		
72 remaining alleles	168+181		
Positive Control	8285		
Negative Control	0		

Table 2B. Determination of Mismatch Acceptability for Selected Non-Luminex Alleles

Non-Luminex allele	Epitope 1	Epitope 2	Acceptable mismatch?
B*44:04	41T	163TS	No
B*44:05	41T	163LS	No
B*44:06	41A	163LS	Yes
B*44:07	41A	163LS	Yes
B*44:08	41A	163LS	Yes
B*49:02	41T	163LW	No
B*49:03	41A	163LW	Yes
B*49:04	41T	163LW	No
A*66:03	41A	163EW+66N	No
A*66:04	41A	163RW	Yes
A*66:05	41A	163RW	Yes
B*40:05	41T	163LW	No

HLAMatchmaker has several protected sheets with formulas and calculations including a mean MFI for self-alleles that can be used as a guide for establishing a cut-off value for positive reactions. Any MFI value can be entered as a cut-off and HLAMatchmaker automatically deletes the epitopes on alleles with MFI values below the selected cut-off. For sera with wide MFI ranges, we recommend comparing different cut-off values to see how they affect the presence of epitopes on reactive alleles.

On the “Sort Ep” sheet, you can organize the remaining immunizer-specific and third-party epitopes on reactive alleles. On the right side of the sheet is shown for each allele in the panel the polymorphic residues in the amino acid residue sequence; this information might be helpful in identifying new epitopes. This would apply if the positive reactions of certain alleles cannot be explained with the current repertoire of epitopes.

Each HLAMatchmaker program has a “Comparisons” sheet that addresses certain situations whereby Luminex panel alleles within the same two-digit antigen group have opposite reactivities that might be explained by residue differences. Table 1 has some examples for selected class I and class II antigens. Residue differences might reflect an epitope either as a part of mismatched eplet or as a self-configuration that serves as a critical contact site with antibody.

For instance, certain antibodies react with A*02:01, A*02:03, and A*02:06, which share 43Q on the molecular surface and 95V hidden below whereas the non-reactive A*02:02 and A*02:05 have 43R and 95L. This means that residue 43Q and/or 95V are important for the epitope recognized by these antibodies. As another example, an antibody reacts only with A*02:03, which has 149T and 152E, but not with the other A*02 alleles, which have 149A and 152V. The HLAMatchmaker antibody analysis programs have complete sets of residue comparisons.

Three Examples of HLAMatchmaker-Determined Epitope Specificities of Serum Antibodies

The first case is a post-pregnancy serum whereby a B*40:02 mismatch had induced antibodies specific for two epitopes: the 41T eplet and 163EW paired with a residue configuration in sequence

position 66 (Table 2A). B*13:01, B*13:02, B*40:01, B*40:06, and B*47:01 also have both epitopes. Six alleles, B*41:01, B*44:02, B*44:03, B*45:01, B*49:01, and B*50:01, have just 41T and they can be considered informative for reactivity with this epitope. Six HLA-B alleles, B*07:02, B*27:05, B*27:08, B*48:01, B*73:01, and B*81:01, are informative for the 163EW-defined epitope. On the other hand, the 163EW-carrying C*02:02 and C*17:01 were essentially non-reactive; both alleles have residue 66K rather than 66I shared by the 163EW-carrying HLA-B alleles. This suggests that 66I, which is about eight Ångströms from 163EW, is an important component of this epitope called 163EW+66I. It should be noted that 66I is self on the HLA type of the antibody producer.

Although this serum had no HLA-A induced antibodies, the data showed that A*66:02, but not A*66:01, was reactive. These alleles have a distinct residue difference namely 163E versus 163R. This means that A*66:02 has 163EW and A*66:01 has 163RW. The antibody-reactive A*66:02 has 66N rather than 66I present on the reactive 163EW-carrying HLA-B alleles. This suggests that 163EW+66N is serologically cross-reactive with 163EW+66I. On the other hand, the 66K substitution seen on C*02:02 and C*17:01 has a dramatic effect on the epitope recognized by the antibodies in this patient.

Table 3. Example of an HLA-DRB Epitope Specificity Analysis

Antibody Producer: DRB1*01:01,*03:01; DRB3*01:01,-					
Immunizing Haplotype: DRB1*15:01, DRB5*01:01					
DRB Allele	MFI	Epitope 1	Epitope 2	Epitope 3	Epitope 4
DRB1*15:01 Immunizer	8962	142M ₃			
DRB1*15:02	9726	142M ₃			
DRB1*15:03	10559	142M ₃			
DRB1*16:01	12963	142M ₃		70D	
DRB1*16:02	13492	142M ₃		70D	
DRB5*01:01 Immunizer	13021		108T	70D	28H
DRB5*02:02	12887		108T		28H
DRB1*01:03	7608			70D	
DRB1*04:02	8257			70D	
DRB1*07:01	6110			70D	
DRB1*08:01	9254			70D	
DRB1*11:01	9710			70D	
DRB1*11:04	9923			70D	
DRB1*12:01	12545			70D	
DRB1*12:02	10308			70D	
DRB1*13:01	11480			70D	
DRB1*13:03	10080			70D	
DRB1*09:01	294				28H
DRB1*09:02	455				28H
Self alleles	9±14				
Negative Alleles (N=11)	43±52				
Positive Control	6390				

Consistent with the overall goal of determining mismatch acceptability for sensitized patients, we can consider for this patient that all 41T and/or 163EW+66I/N carrying alleles on the Luminex panel are unacceptable mismatches. But what is the mismatch acceptability of other alleles? HLAMatchmaker has a special sheet “Acc Mm” that shows which alleles, including those not tested in the panel, have epitopes reacting with patient’s antibodies. Any allele that lacks such epitopes can be considered as an acceptable mismatch. For instance, the panel has the reactive 41T-carrying B*44:02 and B*44:03 and non-Luminex alleles such as B*44:04 and B*44:05 can be considered as unacceptable mismatches because they also have 41T (Table 2B). On the other hand, B*44:06, B*44:07, and B*44:08 appear to be acceptable mismatches because they have 41A instead of

41T. Similarly, B*49:02 and B*49:04 have 41T but B*49:03 has 41A. On the basis of the 163EW+66I/N epitope, A*66:03 would be unacceptable but A*66:04 and A*66:05 can be considered as acceptable mismatches. All three reactive B40 alleles in the panel are unacceptable. B*40:05, which was not included in this Luminex panel, has 163LW instead of 163EW but this allele still is an unacceptable mismatch because it has 41T.

The second case deals with a reactivity pattern of a post-pregnancy serum with antibodies that reacted well with DRB alleles expressing three epitopes (Table 3). The immunizing DRB1*15:01 had induced antibodies to 142M₃ present on the DRB1*15 and DRB1*16 alleles. The immunizing DRB5*01:01 had induced two well-reacting antibodies. One was specific for 108T, which is also present on the reactive DRB5*02:02. The other antibody reacted with an epitope defined by 70D. It should be noted that this serum reacted with the 70D-carrying DRB1*01:03 but not with DRB1*01:01 (self) and DRB1*01:02 which carry 70Q. Similarly, the 70D-carrying DRB1*04:02 was reactive but the other DR4 alleles on the panel, DRB1*04:01, DRB1*04:03, DRB1*04:04, and DRB1*04:05 carry 70Q and were non-reactive. These findings illustrate how reactivity differences between DR1 and DR4 alleles (see Table 1) can be explained with distinct residues that define epitopes. All eight remaining 70D-carrying alleles were reactive. This serum had also very weak reactivity towards 28H as indicated by the informative DRB1*09:01 and DRB1*09:02.

These findings suggest that in this case the 70D, 108T, and 142M₃ epitopes are the basis of determining mismatch acceptability for DRB. When applied to alleles not tested for antibody reactivity, HLA-Matchmaker will show that alleles such as DRB1*08:02, DRB1*11:02, DRB1*12:03, DRB1*13:02 are unacceptable mismatches because they have 70D whereas the 70D-negative DRB1*01:06, DRB1*04:06, DRB1*11:13, and DRB1*13:09 would be acceptable. Mismatch acceptability cannot be readily done for epitopes in DRB sequence locations 90 and higher because no residue information is available for many alleles.

The third case describes the reactivity pattern of a post-pregnancy serum with DQ-reactive antibodies (Table 4). This serum had no DQA-reactive antibodies; this is not surprising because the patient typed as DQA1*01:01 and the immunizing DQ dimer had the structurally similar DQA1*01:02. The reactive DQ heterodimers shared the 77T-defined epitope with the immunizing DQB1*06:02; the MFI values were high for the DQB1*03 and DQB1*06 alleles (9822±3002) but much lower for the DQB1*04 alleles (1475±734). The highly reactive DQB alleles had nearby residues 74E and 75L whereas the low-reactive DQB alleles shared 74S and 75V. This suggests that this epitope corresponds to 77T+74E+75L and that the combination 77T+74S+75V has a low degree of serological cross-reactivity.

HLA-Matchmaker has also information about which DQ alleles not included in the panel have mismatched epitopes reacting with patient's antibodies. On the basis of the 77T epitope, all DQB1*02 and DQB1*05 alleles (except DQB1*02:05) would be acceptable mismatches. Almost all DQB1*03, DQB1*04, and DQB1*06 alleles have 77T and would be considered as unacceptable mismatches. The 77R-carrying DQB1*06:06 is the only exception. It should be noted that DQB1*03:06 has the 77T+74S+75V combination associated with a lower MFI value.

Table 4. Example of an HLA-DQB Epitope Specificity Analysis

Patient Type	DQB1*02:01	DQB1*05:01	DQA1*01:01	DQA1*05:01
Immunizer	DQB1*06:02	DQA1*01:02		
Allele	Allele	MFI	Epitope	Nearby residues
DQB1*03:01	DQA1*05:03	8222	77T	74E+75L
DQB1*03:01	DQA1*06:01	7268	77T	74E+75L
DQB1*03:01	DQA1*05:05	6823	77T	74E+75L
DQB1*03:01	DQA1*02:01	4784	77T	74E+75L
DQB1*03:01	DQA1*03:01	4729	77T	74E+75L
DQB1*03:02	DQA1*03:02	12583	77T	74E+75L
DQB1*03:02	DQA1*02:01	8646	77T	74E+75L
DQB1*03:02	DQA1*03:01	8607	77T	74E+75L
DQB1*03:02	DQA1*01:01	7361	77T	74E+75L
DQB1*03:03	DQA1*03:02	12701	77T	74E+75L
DQB1*03:03	DQA1*02:01	12469	77T	74E+75L
DQB1*03:03	DQA1*03:01	7520	77T	74E+75L
DQB1*06:01	DQA1*01:03	12822	77T	74E+75L
DQB1*06:02	DQA1*01:01	12793	77T	74E+75L
DQB1*06:02	DQA1*01:02	10285	77T	74E+75L
DQB1*06:03	DQA1*01:03	12662	77T	74E+75L
DQB1*06:04	DQA1*01:02	13382	77T	74E+75L
DQB1*06:09	DQA1*01:02	13146	77T	74E+75L
DQB1*04:01	DQA1*03:03	2570	77T	74S+75V
DQB1*04:01	DQA1*02:01	1160	77T	74S+75V
DQB1*04:02	DQA1*02:01	1159	77T	74S+75V
DQB1*04:02	DQA1*04:01	1009	77T	74S+75V
DQB1*02:01	DQA1*04:01	27		
DQB1*02:01	DQA1*05:01	25		
DQB1*02:01	DQA1*02:01	22		
DQB1*02:01	DQA1*03:01	1		
DQB1*02:02	DQA1*02:01	28		
DQB1*05:01	DQA1*01:01	26		
DQB1*05:02	DQA1*01:02	43		

These three examples illustrate that an epitope specificity analysis can be helpful in the interpretation of serum reactivity and the determination of mismatch acceptability for a sensitized patient. Many publications demonstrate how complex serum reactivity patterns can be explained with epitope specificity analysis^{2,8} and EpiPedia has more examples.

Conclusion

The epitope analysis of serum antibody reactivity of sensitized patients is a useful tool for the identification of potential donors with acceptable mismatches. This approach is useful not only for organ transplantation but also for platelet transfusions of allosensitized thrombocytopenic patients. Eurotransplant has incorporated HLA-Matchmaker in the Acceptable Mismatch program to identify donors for highly sensitized patients.^{9,10}

Epitope specificity analyses might also be useful in desensitization protocols to remove donor-specific antibodies.¹¹ Such protocols are not always uniformly successful but for some patients they may remove some epitope-specific antibodies thereby opening new windows of opportunity regarding the identification of selected allelic mismatches.

The final part III of this series will address the issue how epitope-based matching can be applied to control HLA allosensitization.

See references on page 51

Business Topics

- With the outgoing members of the ARB departing, we welcome six new commissioners to the group: Christina Bishop, Daniel Ramon, Lori Osowski, Maureen Miller, Sam Ho, and Vera Hauptfeld-Dolejssek. John Gerlach will also be returning to the ARB as junior co-chair this September.
- We are happy to announce that ASHI has earned a 0% disparity rating with CMS and has been granted a six-year accreditation renewal by CMS!
- The ARB is seeing a number of reports uploaded to the ASHI application that do not have the FDA disclaimer on patient reports. This is a recurring issue of labs not adding this on their reports whether it is an electronic format or not. It is our belief that lab directors and/or lab managers may be looking at the Cleared 510K Submission list for premarket approval and believing the kit is FDA approved, therefore assuming the disclaimer is not needed. This site is just those submissions from vendors that have cleared the 510K premarket submission process and approval for vendors to market their products to the public, not a list of the 510K Cleared or FDA Approved list of kits or devices. Labs must review the package insert for the FDA statement that the "assay is approved by the FDA." Below is the URL to review the documentation submitted and the FDA supporting documentation.

- We observe other report issues as well. A number of reports uploaded as part of the application packet were noted to be missing components specified in the ASHI Standards. In addition, multiple standards require reporting of HLA using appropriate nomenclature. Many labs have reports that mix molecular and serological designations in a single report. While we recognize that UNOS requires reporting a mixture of molecular and phenotypic assignments, the practice does not align with existing standards. The ARB encourages laboratories to review the relevant standards to ensure compliance.

The ARB does recognize the increasing complexity of HLA typing and reporting. We have begun a project to work more closely with our colleagues on QAS and PT to ensure that standards, compliance assessment, and proficiency testing are aligned and sensible.

- Finally as this is my final quarterly report, I would like to thank all of the ASHI labs on behalf of the ARB. We know that labs strive to meet the standards and understand that some of those are open to interpretation. The ARB structure allows for a multilevel review of labs that we hope engenders a thorough, but fair process. Our lack of discrepancy with CMS points to the professionalism of the inspectors, but also to the support from member laboratories.

FDA Documentation

<http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/SubstantiallyEquivalent510kDeviceInformation/ucm063708.htm>

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