**Tutorial (June 2016)**

**HLAMatchmaker ABC antibody analysis (Version 02)**

**Introduction**

HLAMatchmaker is an algorithm to predict HLA epitopes by molecular structural modeling and amino acid sequence comparisons between HLA alleles. It considers each HLA allele as a series of small configurations of polymorphic residues referred to as eplets as essential components of HLA epitopes. The website-based International Registry of HLA Epitopes ([http://www.epregistry.com.br](http://www.epregistry.ufpi.br)) describes the repertoires of HLA-ABC, -DRDQDP and -MICA eplets. An important question is which eplets correspond to actual epitopes specifically recognized by HLA antibodies.

Recent publications describe 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 whereas the remaining alleles in the panel are non-reactive. 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 between all antibody-reactive alleles. Such epitopes are described by so-called eplet pairs. The repertoires of antibody-verified epitopes must be considered incomplete.

This new Excel program has been designed to analyze HLA antibody reactivity patterns with single allele panels. Reactive alleles carry epitopes which are studied in two steps. First, we determine the presence of antibody-verified epitopes (eplets and eplet pairs) that are mismatched for the antibody producer. Second, we determine if reactive alleles in the panel have other epitopes which have not (yet) been antibody-verified in the HLA Epitope Registry. The updated antibody analysis version has features that permit a quick assessment of epitope specificities and the interpretation of allele mismatch acceptability.

We recommend keeping a master copy of this HLAMatchmaker program on your computer and creating a working copy to use for actual analysis.

This ABC antibody analysis demo has 14 sheets to show how to perform this epitope analysis.

1 Panel

This sheet has five columns to describe the composition of the single allele kit. There are 100 rows to enter the panel information. The actual program has sheets describing lot numbers of One Lambda (ThermoFischer) and LifeCodes (Immucor) kits, copy the selected kit and paste it on the Panel sheet. The latest lots can be added to the OL and LC sheets.

This sheet shows the epitopes after pasting the HLA information of OL “lot xyz”; columns C, D and E show bead numbers, QC information and the list of alleles. The program automatically generates the repertoires of antibody-verified eplets (columns F-DA), antibody-verified pairs (DC-GX) and “other” eplets recorded in the Registry (GY-KT). Please note that updated versions of the antibody analysis program will include newly antibody-verified epitopes and they will be posted on the HLAMatchmaker website.

2 Enter

The program has copied the HLA information of from the Panel sheet and columns L, M and N show the antibody-verified eplets, the antibody-verified pairs and the “other” eplets. The eplets are listed sequentially, with no breaks between the names. We must of course know which ones are mismatched for the antibody producer.

3 HLAinfo

This sheet has four examples of Luminex data with class I antibodies. All of them have HLA types of antibody producer and immunizer and the cPRA values are very high. Let’s select case #217. For training purposes, you can try out the other cases at another time.

4EnterpHLA

After recording the HLA type of the antibody producer (this must be done at the 4-digit high-resolution level), the program automatically determines which epitopes in columns L, M and N are mismatched. Note that the number of epitopes in each column is reduced. Now enter the MFI values for the Luminex panel.

5MFIcsv

The easiest way for entering the MFI values is to go to the csv files of the Luminex software programs (you may need some instructions from the manufacturer). Row 22 of this sheet has the trimmed mean values for case #217. Copy the horizontally located values and use the paste-special-transpose command (the shortcut click: alt E, S, E) to enter the MFI values in column J starting on row 12 of the Enter sheet.

6EnterMFI

The next step is the determinations of the cut-off MFI value must for positive and negative reactions. Box G10 shows the mean MFI value of self-alleles of the antibody producer; it appears to reflect a true non-reactivity. Any other allele in the SAB panel with a MFI more than three standard deviations above the mean value with self can be statistically considered as being significantly higher.

You can see in column G that each allele of the antibody producer of Case #217 had an extremely low MFI value, and we determined a cut-off value of 100.

7Enter Cut-off

After entering the cut-off value, the epitopes reappear on the sheet and column I has a “NEG” annotation for each allele that has an MFI below the cut-off value, in this case 100. Moreover, the program removes all the epitopes expressed by the negative alleles from the entire panel. The reactive alleles show the remaining epitopes. You will see that most of them have small numbers of antibody-verified epitopes.

8Enter ImHLA

We must raise the question which of these epitopes would be specifically recognized by serum antibodies. Information about the immunizing event will offer an answer. Case #217 was a post-pregnancy serum and the paternal haplotype of the child was recorded on Row 5 of the Entry sheet.

Columns L, M and N show the Abver Eplets, AbverPairs and OtherEplets on the reactive alleles in the panel. The next step of this analysis is to determine which epitopes are immunizer-specific or “third-party”.

9SortBefore

The Sort Ep sheet shows has Columns K, L and M for the immunizer-specific epitopes and columns N, O and P have the third-party epitopes. You may see that most reactive alleles have one or few antibody-verified eplets or pairs but you have to scroll up and down to see which ones are involved. A dedicated sort command can be used for better visualization of the data.

Highlight Rows 12-111 and under the Data tab click on the Sort Command which uses a certain sequence within the various columns.

10SortAfter

The sorted data for case #217 show that the antibodies react with a limited repertoire of immunizer epitopes. The immunizing B\*07:02 has 65QIA, 70IAQ, 163EW and 180E. You may notice that the 163EW carrying HLA-C alleles have very low MFI values suggesting that the 163EW-defined epitope needs another configuration shared between HLA-B and A\*66:02 (note that A\*66:01 has a much lower MFI value). The immunizing A\*03:01 has 161D and one or two epitopes that correspond with antibody-verified pairs. Several weakly reacting alleles share 45EE with the immunizing B\*07:02, this epitope has not (yet) been antibody-verified. You may also note that a group of HLA-B alleles with very low MFI values share a third-party 44RT epitope. Although the clinical significance of low MFI values might be considered questionable it is interesting to note that some of them may reflect weakly reacting epitope-specific antibodies.

The major purpose of serum analysis for specific antibodies is the determination mismatch acceptability of donor alleles. This HLAMatchmaker program makes this determination

11AccMm

This sheet shows the information about mismatched epitopes on class I alleles including many that are not in single allele panels. Columns B-G list the immunizer-specific alleles whereas columns H-M list all epitopes shared with reactive alleles. Row 5 has a filter command to select alleles with certain numbers of mismatched epitopes. Depending on the determination of the cut-off value in the Luminex test and the interpretation which epitope is clinically important, the program can readily determine which alleles are acceptable mismatches.

Summary

Now try out the analysis of the other three cases listed in this demo. Let us know if you have any questions and suggestions.

**Please Note:**

The new antibody analysis versions have additional features aimed to identify antibodies against new epitopes defined by amino acid residues. The Manual of the programs provides detailed instructions.