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# EpHLA software: A timesaving and accurate tool for improving identification of acceptable mismatches for clinical purposes

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ABSTRACT

The HLAMatchmaker algorithm, which allows the identification of "safe" acceptable mismatches (AMMs) for recipients of solid organ and cell allografts, is rarely used in part due to the difficulty in using it in the current Excel format. The automation of this algorithm may universalize its use to benefit the allocation of allografts. Recently, we have developed a new software called EpHLA, which is the first computer program automating the use of the HLAMatchmaker algorithm. Herein, we present the experimental validation of the EpHLA program by showing the time efficiency and the quality of operation. The same results, obtained by a single antigen bead assay with sera from 10 sensitized patients waiting for kidney transplants, were analyzed either by conventional HLAMatchmaker or by automated EpHLA method. Users testing these two methods were asked to record: (i) time required for completion of the analysis (in minutes); (ii) number of eplets obtained for class I and class II HLA molecules; (iii) categorization of eplets as reactive or non-reactive based on the MFI cutoff value; and (iv) determination of AMMs based on eplets' reactivities. We showed that although both methods had similar accuracy, the automated EpHLA method was over 8 times faster in comparison to the conventional HLAMatchmaker method. In particular the EpHLA software was faster and more reliable but equally accurate as the conventional method to define AMMs for allografts.

*Conclusion:* The EpHLA software is an accurate and quick method for the identification of AMMs and thus it may be a very useful tool in the decision-making process of organ allocation for highly sensitized patients as well as in many other applications.



## 1. Introduction

The presence of anti-HLA antibodies in sera of solid organ transplant recipients remains a well-documented risk factor for transplantation [1]. Because of this, the development of methods to detect the presence of anti-HLA antibodies has been a guiding motif for research since the beginning of clinical transplantation. As a result of this effort, several methods have been developed including complement-dependent cytotoxicity assay (CDC) [2], flow cytometry crossmatching [3], as well as many solid phase assays (SPAs) [4]. One of the solid phase assays uses multicolor beads, each coated with a single class I or II HLA protein, to test previously sensitized patients' sera

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to identify: (I) allelic HLA specificities of preformed antibodies; and (II) the relative reactivity patterns of these antibodies to define their clinical importance [4]. While the high sensitivity of such methods to detect very small quantities of anti-HLA antibodies seems very attractive, the clinical interpretation of their impact on allograft survival remains open. This is an especially pressing issue with the rise in numbers of highly sensitized patients on waiting lists [5]. The actual challenge is to find for each sensitized patient a matching donor with acceptable HLA alleles (against which patient has no preformed antibodies). To accomplish this goal, we need to identify a list of unacceptable (with strong reactivity) and acceptable (with weak or no reactivity) HLA alleles for each sensitized patient. Overall, the objective is to increase the number of transplants for highly sensitized patients without compromising the graft survival [6].

Another solution in the search for acceptable donors is the adoption of a concept of acceptable mismatches (AMMs), which have been extensively discussed elsewhere [7]. Indeed, the concept of AMMs follows the assumption that the recognition of epitopes on HLA molecules by antibodies occurs in discreet areas of the HLA molecules and some of these epitopes are identical on different HLAs [8]. Furthermore, since the patient's immune system is tolerant to self-HLA molecules, all their epitopes may be designated as safe when they are expressed on potential donor HLAs. Duquesnoy and his collaborators have described the sequences of polymorphic amino acid residues in the areas of class I and II HLA molecules, defining functional epitopes and named them eplets [9,10]. This work has resulted in the development of the HLAMatchmaker algorithm [11], which has been validated by the Eurotransplant group and other centers [12–14]. This program has resulted in an increased transplantation rate among highly sensitized patients and a decreased waiting time without compromising graft survival [15]. Such encouraging results support a new paradigm, in which the search for epitope compatibility helps in the search for HLA molecules in the context of transplantation.

The HLAMatchmaker algorithm is a powerful tool for determining AMMs. However, despite this benefit it is not universally used. A limiting factor for using this tool is the difficulty in handling and interpretation of often complex results. This is at least partly due to the fact that many of the processing stages must be performed manually, which is not only time-consuming but it increases the likelihood of errors. We believe that the new paradigm of finding epitope-based compatibility for highly sensitized patients needs to be developed as a user-friendly tool that pinpoints strongly immunogenic as well as weak and non-immunogenic epitopes on the HLA alleles. This would enable to define better the immunological risk of transplantation. With this objective in mind, we have developed the EpHLA software which automates many of the functions of the HLAMatchmaker algorithm [16].

In the presented work we tested the ability of the EpHLA software to determine HLA acceptable mismatches, in a timesaving way, regardless of the user's background in immunogenetics. As it is the case for every new automation tool, the EpHLA software was tested for the minimum features that attest to software quality as required by the ISO/IEC 9126-1 International Standard (Information Technology-Software product quality-Part 1: Quality model; June/1998). The tested features were those that are easily perceptible by the users (e.g., functionality, reliability, usability, and efficiency). Herein, we report an experimental validation aimed at testing the capacity of the EpHLA software in fulfilling these perceptible qualities.

## 2. Objectives

To validate the EpHLA software by: (i) successfully categorizing HLA molecules as AMMs or Unacceptable Mismatches (UMMs); and (ii) to show the analysis is done with higher functionality, reliability, usability, and efficiency in comparison to the HLAMatchmaker algorithm in its current Microsoft Excel format.

## 3. Methodology

### 3.1. Description of the EpHLA software and its functions

The EpHLA automation software (NIT 000083/2011, INPI Brazil) was developed in the Object Pascal language. Its architecture enables the automatic execution of the HLAMatchmaker algorithm; additionally, it integrates public and private databases and reports to the user the non-self eplets, AMMs, and UMMs for the analyzed recipient. Further, the EpHLA software provides the calculated Panel of Reactive Antibodies (cPRA) and the virtual cross-match results for the recipient/donor pair. The input data for EpHLA include HLA allele typing, the file with the SPA test data, and the cutoff MFI value [16].

### 3.2. Users who tested single antigen results

Eleven users with different expertise in HLAMatchmaker were invited to evaluate single antigen results from 10 different HLA sensitized patients waiting for a kidney transplant. All patients enrolled in this study presented either class I or class II PRA higher than 61%, a finding confirmed by cPRA (ranging from 61% to 100%, obtained by means of the Organ Procurement and Transplantation Network tool (OPTN) [17]. Sera were tested using single antigen beads (One Lambda, Canoga Park, CA) on the Luminex platform, according to the manufacturer's instructions.

The HLA typings were carried out at medium-resolution using sequence-specific oligonucleotide probe hybridization—SSOPH (One Lambda, Canoga Park, CA, USA)—for the loci A, B and DRB1. HLA alleles were inferred using the NMDP codes and the allele frequency tables available at http://bioinformatics.nmdp.org/. The HLA alleles of the loci DRB345, DQA1 and DQB1 were generated on the basis of their linkage with the DRB1 alleles, using the HLAMatchmaker software (DRDQ Allele Antibody Screen)—available at http://www.hlamatchmaker.net/.

The users were divided according to their backgrounds in a conventional HLAMatchmaker analysis into two groups: the first experienced group was composed of four technicians from Pontifical Catholic University of Paraná with a modest amount of experience using HLAMatchmaker during the last two years; the second non-experienced group was composed of seven undergraduates from Federal University of Piauí without any previous experience with HLAMatchmaker or tissue typing training.

For the execution of this study, users from the experienced group received additional training with the EpHLA software while users from non-experienced group received training with the conventional HLAMatchmaker algorithm (implemented on an Excel electronic spreadsheet) as well as in EpHLA software. Both groups were trained by the same instructor and all users were asked to evaluate the same 10 single antigen results using the HLAMatchmaker and EpHLA methods.

### 3.3. Analysis stages with the conventional and the automated methods

We provided users the same 10 Comma Separated Values (*CSV*) files selected for experimental validation. A panel with Luminex beads, each coated with different recombinant HLA molecules (97 alleles for class I with 1758 eplets and 91 alleles for class II with 2026 eplets), was represented in each CSV file. A full list of eplets are available at http://www.hlamatchmaker.net [18]. As previously explained, it is important to emphasize that self-eplets were removed for each patient from the eplets list for both conventional and automated HLAMatchmaker analysis as soon as the user enters the patient's HLA alleles. HLA alleles and number of non-self eplets for each patient are shown in Tables 1 and 2.