Transplant Immunology 25 (2011) 210–216

Contents lists available at SciVerse ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

EpHLA: An innovative and user-friendly software automating the HLAMatchmaker algorithm for antibody analysis

Luiz Cláudio Demes da Mata Sousa [a,b], Herton Luiz Alves Sales Filho [a], Cristina de Queiroz Carrascosa Von Glehn [c], Adalberto Socorro da Silva [a,*], Pedro de Alcântara dos Santos Neto [b], José Adail Fonseca de Castro [a], Semíramis Jamil Hadad do Monte [a]

[a] Immunogenetics and Molecular Biology Laboratory, Federal University of Piauí, Teresina, Campus Ministro Petrônio Portela SG-16, Teresina, PI, CEP: 64049-550, Brazil
[b] Department of Informatics and Statistics, Federal University of Piauí, Campus Ministro Petrônio Portela SG-9, Teresina, PI, CEP: 64049-550, Brazil
[c] Immunogenenetics Laboratory, Pontifícia Universidade Católica do Paraná, Guabirotuba Street, Technology Park - 2nd Floor – Blook IV, Curitiba, PR, CEP: 80000-215, Brazil

ARTICLE INFO

Article history:
Received 15 July 2011
Received in revised form 29 August 2011
Accepted 29 August 2011

Keywords:
Software
HLA
Antibody
Epitope

ABSTRACT

The global challenge for solid organ transplantation programs is to distribute organs to the highly sensitized recipients. The purpose of this work is to describe and test the functionality of the EpHLA software, a program that automates the analysis of acceptable and unacceptable HLA epitopes on the basis of the HLAMatchmaker algorithm. HLAMatchmaker considers small configurations of polymorphic residues referred to as eplets as essential components of HLA-epitopes. Currently, the analyses require the creation of temporary files and the manual cut and paste of laboratory tests results between electronic spreadsheets, which is time-consuming and prone to administrative errors.

Results: The EpHLA software was developed in Object Pascal programming language and uses the HLAMatchmaker algorithm to generate histocompatibility reports. The automated generation of reports requires the integration of files containing the results of laboratory tests (HLA typing, anti-HLA antibody signature) and public data banks (NMDP, IMGT). The integration and the access to this data were accomplished by means of the framework called *eDAFramework*. The *eDAFramework* was developed in Object Pascal and PHP and it provides data access functionalities for software developed in these languages. The tool functionality was successfully tested in comparison to actual, manually derived reports of patients from a renal transplantation program with related donors.

Conclusions: We successfully developed software, which enables the automated definition of the epitope specificities of HLA antibodies. This new tool will benefit the management of recipient/donor pairs selection for highly sensitized patients.

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1. Introduction

The interaction among HLA molecules and antibodies has been in the limelight among researchers and clinicians in the history of organ transplantation. Patel and Terasaki showed with lymphocytotoxicity cross-match tests [1] a correlation between donor-reactive antibodies and poor graft survival, and this made this test a mandatory pre-transplant evaluation [2]. Subsequently, issues were raised about the sensitivity and specificity of the complement dependent lymphocytotoxicity assays (CDC), and this led to the development of the solid phase assay methods (SPA) which are now used on a worldwide basis. Especially single allele panels have been useful to test for HLA antibodies [3]. This technique has also been used to predict cross-matches in sensitized candidates and to monitor the development of clinically relevant HLA antibodies post-transplant.

A new outlook of the HLA–antibody interaction in the transplantation context was reported when Rene Duquesnoy reasoned that the antibody interacts not with "HLA antigens", but with structurally defined epitopes called eplets, present in the HLA molecules. According to this hypothesis, different HLA molecules will be recognized by the same antibody if such HLA molecules have one or more eplets in common recognized by that antibody [4]. Characterizing eplet-specific

Abbreviations: SPA, Solid Phase Assay; AMM, Acceptable Mismatches; GUI, Graphical user interface; CDC, Complement-dependent Cytotoxicity; LIB, Immunogenetics and Molecular Biology Laboratory; UFPI, Federal University of Piauí; SSOPH, Sequence-specific Oligonucleotide Probe Hybridization; PRA, Panel of Reactive Antibodies; NMDP, National Marrow Donor Program; MFI, Median-Fluorescence Intensity; IMGT, the ImMunoGeneTics program; CREG, Cross Reactive Group.

* Corresponding author. Tel.: +55 86 32155691; fax: +55 86 32155690.
E-mail addresses: claudio.demes@ufpi.edu.br (L.C.D.M. Sousa), hertonluiz@hotmail.com (H.L.A.S. Filho), c.glehn@pucpr.br (C.Q.C. Von Glehn), adalbertosocorro@gmail.com (A.S. da Silva), pasn@ufpi.edu.br (P.A.S. Neto), castro@ufpi.br (J.A.F. de Castro), libpi@ufpi.br (S.J.H. Monte).

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doi:10.1016/j.trim.2011.08.008

antibodies is useful to identify acceptable mismatches (AMM). In this sense, AMM are HLA antigens which differ from the patient's own HLA antigens, but they do not have antibody-eplets. Realizing that establishing AMM increases the transplantation chances in highly sensitized patients, Duquesnoy and collaborators developed HLA-Matchmaker, a donor–recipient compatibility algorithm based on eplets that may react with antibodies [5]. This algorithm, validated by the Eurotransplant group, increases the rate of transplantation among highly sensitized recipients with a shorter waiting time. In fact, every highly sensitized recipient entering the AMM Program has a 43% chance of receiving a transplant within 12 months, or 58% within 21 months. The follow-up of these recipients showed that the graft survival at two years is 87%, the same result as that observed for non-sensitized recipients transplanted in the same period [6]. These results, which were confirmed by other groups [7–9], point to AMM Program as an alternative for transplantation of highly sensitized recipients against HLA antigens.

Data Input for HLAMatchmaker algorithm is a set of data resulting from the screening for the presence of HLA antibodies in the recipient's serum (SPA Results). Data output from HLAMatchmaker is a set of eplets that permits an expert laboratory personnel working in the HLA field to identify AMM. Unfortunately, both input data into HLAMatchmaker and output data analyses are manually performed with labor-intensive Microsoft Excel programs, which limit applying the eplet - concept in the clinically oriented HLA laboratory. Currently, there is no software automating the input and output data analysis for HLA-Matchmaker. A computerized tool and a centralized relational database would reduce potential analyses errors, increasing reproducibility of histocompatibility studies, facilitating the data management and making data analysis less labor-intensive and more clinically applicable. The EpHLA software has been developed to carry out HLAMatchmaker in HLA laboratories that serve clinical transplant programs. It provides searches with a non-redundant and structured local database managed through a graphical user interface (GUI).

2. Objectives

The purpose of this work is to describe and test the functionality of the EpHLA software, a program that automates the analysis of acceptable and unacceptable HLA epitopes on the basis of the HLAMatchmaker algorithm.

3. Materials and methods

3.1. Execution

EpHLA is built in the Object Pascal programming language and uses an MS-Access (http://office.microsoft.com/pt-br/access/default.aspx) [10] or MySQL (http://www.mysql.com/) [11] database to store clinical and genetic data. In order to ease data integration between HLAMatchmaker, Solid Phase Assay (SPA) results and web repositories, we developed the easy Data Access framework (*eDAframework*). This framework was developed in Object Pascal (http://delphi.com/) [12] and PHP (Hypertext Preprocessor — http://www.php.net/) [13] programming languages and provides import, data access and export functionalities. The import functionality allows the importing of data from different file formats (FASTA, text files, comma separated values and Excel spreadsheet — http://office.microsoft.com/pt-br/excel/default.aspx [14]) to laboratory local databases, releasing them to access at only one repository. Such data can be accessed through eDAframework and used for processing through the EpHLA software. The results of this processing are exported as Excel spreadsheets using the export functionality.

The EpHLA software uses the HLAMatchmaker algorithm to find acceptable and unacceptable mismatches for HLA sensitized recipients. The input data to the HLAMatchmaker algorithm are: donor and recipient's HLA alleles, serum date, cutoff value and the SPA results. However, if high resolution HLA alleles are not available, allele frequencies databases can be queried in order to define the most likely allele for each case. The HLAMatchmaker algorithm works by comparing eplets found in donor and recipient's HLA molecules, generating a list of matches and mismatches for each other. The reports generated by EpHLA program allow laboratory personnel to divide potential donors into three different categories: (i) full HLA match; (ii) acceptable mismatches, and (iii) unacceptable mismatches. Note that if donor and recipient HLA molecules are identical, their eplets are identical too, and the transplant is acceptable. On the other hand, if organ donor/recipient HLA molecules are not identical, two cases are possible: (i) The recipient has preformed antibodies against donor eplets; (ii) The recipient does not have antibodies against donor eplets. In the first case, there is a higher risk associated with the transplantation, and in the second one, there is a lower risk [2, 15].

3.2. Using the system

The EpHLA program runs without complex setup procedures: the user has only to copy its files to drive C on a computer executing the Windows or MAC operational system (using a virtual machine). The EpHLA software consists of an executable program (EpHLA.exe), a relational database and auxiliary directories, as shown in the directory tree of Fig. 1, [A].

The EpHLA program's workflow consists of five steps: 1. Preparation of CSV files with the SPA results; 2. The processing of one or more CSV files; 3. The inclusion of the HLA alleles from recipient and donor; 4. Definition of cutoff value of SPA results; and 5. Generation of the Histocompatibility Map report.

Preparation of CSV files is related to transferring CSV files to the *input* directory of the EpHLA program's directory tree. The CSV files copied to the *input* directory are shown in the form Available *CSV files in directory* (Fig. 1, [B]). Using this form, one or more files can be selected and processed (workflow's second step). The EpHLA software uses information available in the HLAMatchmaker program's spreadsheets ([5] http://www.hlamatchmaker.net), including class of HLA and lot number of SPA kits (obtained from the manufacturer — Fig. 1, [C]). The result of the processing is available in the EpHLA — *Local repository* form. This form contains information on the recipient and his/her SPA results. Thus, one must access the *Local repository* form of the EpHLA software and type in the class I and class II HLA alleles of the recipient and donor.

The next step is to determine the cutoff value. The standard value of the EpHLA program is 500 of Median-Fluorescence Intensity (MFI). However, the laboratory personnel can define the value or alter to the suggested value in section *Calculated Cutoff*, according to Rene Duquesnoy [16] (Fig. 2). In the last step, the EpHLA program executes the HLAMatchmaker algorithm to generate the Histocompatibility Map report. During this step, the recipient's eplets of the self HLA molecules are removed from the histocompatibility analysis; the remaining eplets (non-self) are shown in the Histocompatibility Map report and classified by the EpHLA program as potentially or weakly immunogenic based on the adopted MFI cutoff value. All alleles of the panel whose MFI is lower than the cutoff established by the laboratory personnel will have its eplets classified as weakly immunogenic in all HLA molecules studied. These eplets are shown in blue. Otherwise, the eplet is considered potentially immunogenic and is typed black or red. A black eplet means that it is not the only eplet responsible for immunogenicity of the HLA molecule. On the other hand, a red eplet stands for a unique eplet responsible for immunogenicity in at least one HLA molecule for the tested serum whose MFI value is larger than the cutoff.

The Histocompatibility Map report from the EpHLA program contains two tabsheets: (i) Eplets Map and (ii) Eplet's Report. Eplets Map contains five predictable tabs groupings: Acceptable Mismatches, No

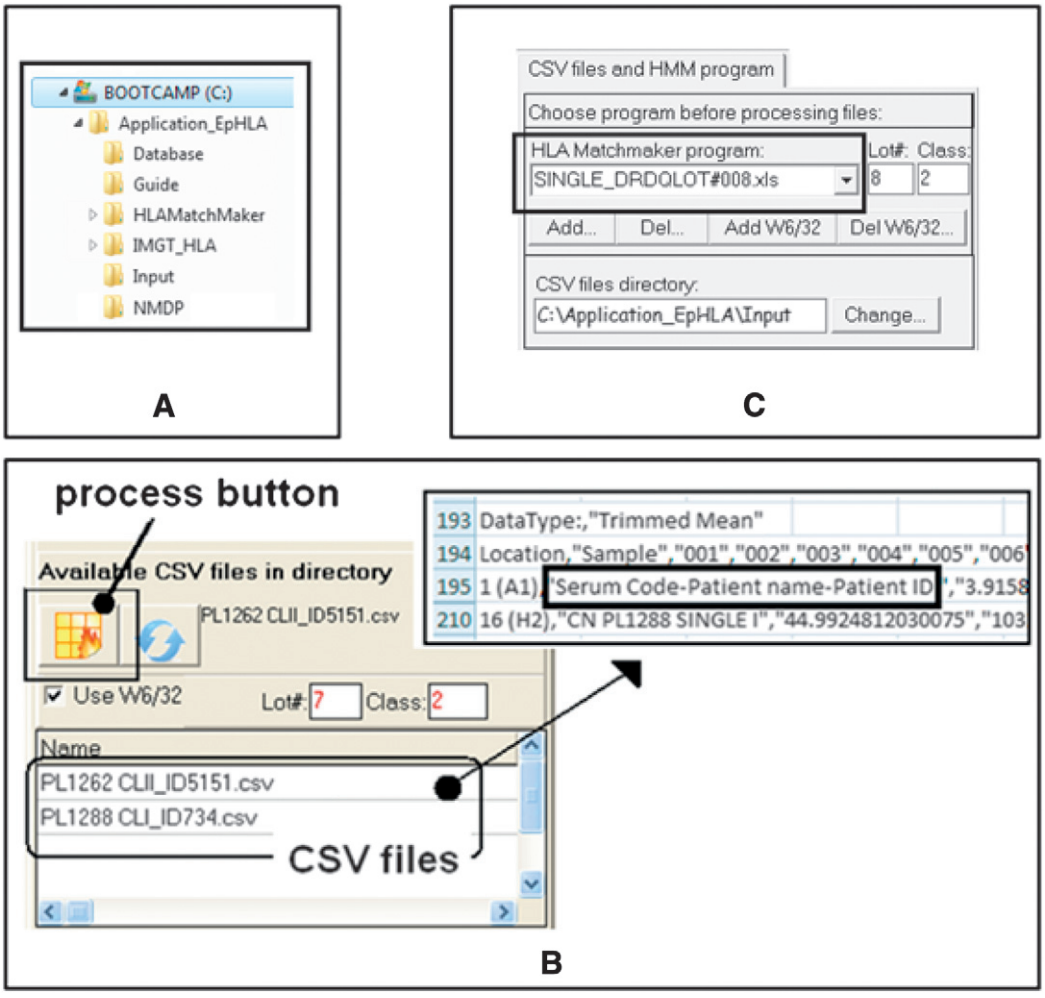


Fig. 1. EpHLA's screenshots.

Mismatches, Recipient × Donor, Unacceptable Mismatches and All Mismatches (Fig. 3). These tabs allow the laboratory personnel to visualize, to order and to group HLA alleles so as to improve the histocompatibility study of the recipient/donor pair.

The Recipient × Donor tab shows the donor's HLA antigens and his/her eplets easing the immunological risk definition associated to the recipient/donor pair in the study. In this tab, the laboratory personnel by analyzing the class II HLA eplets will be able to visualize the eplets of

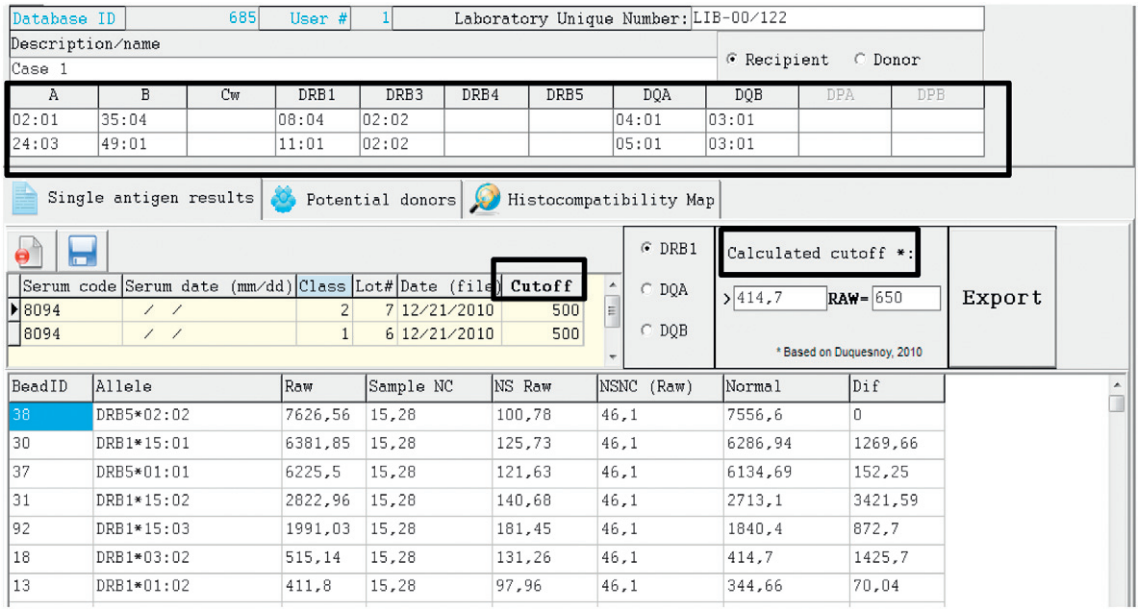


Fig. 2. Local repository form with recipient's HLA alleles, Solid Phase Assay results and cutoff value.

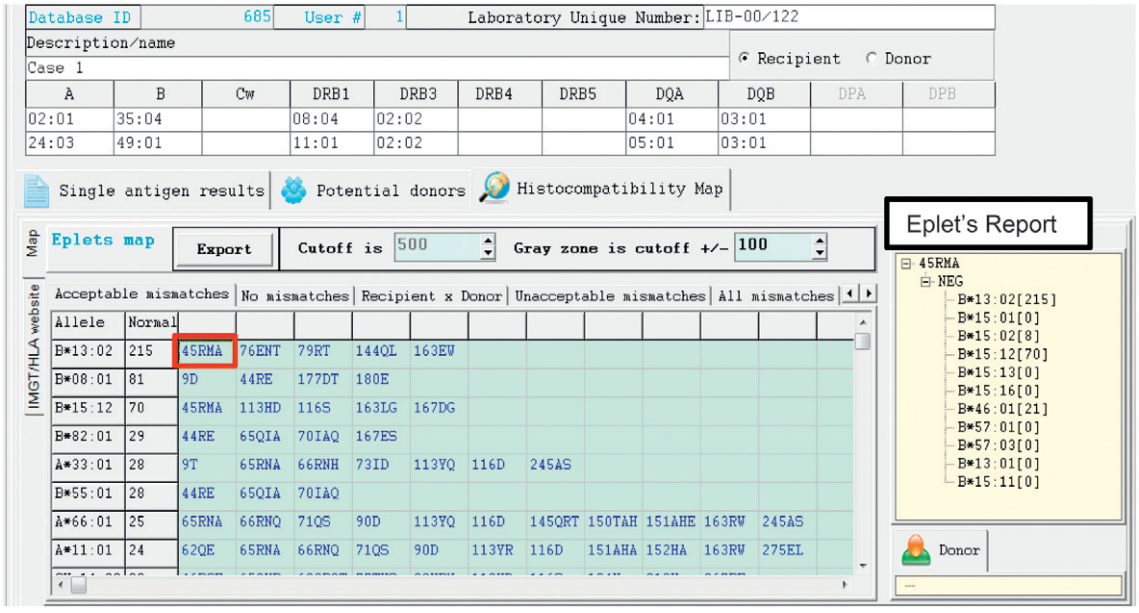


Fig. 3. EpHLA software's Histocompatibility Map report.

the subunits DQA1* and DQB1* separately. It is important to point out that this tab was idealized to allow only the visualization of eplets. For that reason, in the Recipient × Donor tab the EpHLA Software shows zero for the MFI value of the subunits DQA1* and DQB1* shown separately in the columns "Normal" (Figs. 5 and 6). The actual MFI values associated to the beads of the panel containing the subunits DQA1* and DQB1* studied can be visualized in the remaining tabs.

The Histocompatibility Map report also shows in the upper right corner the Eplet's Report tab, where the laboratory personnel can easily verify if an eplet plays a potential role in allosensitization and observe, quickly, if a certain eplet appears only in positive molecules or also in negative ones (Fig. 3).

In order to carry out the post-transplant follow-up or to study the potential donors for a certain recipient, the EpHLA program allows registering for donor on the Local repository form. It is only necessary to register the following data: name, laboratory unique number and the HLA alleles, represented by the fields A, B, Cw, DRB1, DRB3, DRB4, DRB5, DQA and DQB. One or more registers of potential donors can be associated to a recipient registration — using the Potential Donors tab accessible on the Local repository form. For each recipient/donor pair, the EpHLA program generates a report showing the donor's alleles and their respective non-self eplets, as previously shown.

3.3. Case reports

To test the tool's functionalities, the EpHLA software was used to determine the antibody profile of two sensitized recipients from the renal transplant program studied at the Federal University of Piauí's Immunogenetics and Molecular Biology Laboratory (LIB-UFPI). The first recipient exhibited a positive CDC assay with B-lymphocytes due to IgG antibodies, and the second recipient had a negative CDC assay with a current serum and a positive CDC assay with historical serum.

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, Cw, DRB1, DQB1. HLA alleles were inferred using the NMDP codes and the allele frequency tables available at http://bioinformatics.nmdp.org/ [17].

The HLA alleles of the *loci* DRB345 and DQA1 were generated on the basis of their linkage with the DRB1 allele, using the HLAMatchmaker software (DRDQ Allele Antibody Screen) — available at http://www.hlamatchmaker.net/ [5].

In this study we used the following MFI cutoff values to classify antibody–antigen reactions: strong reaction — MFI higher than 3,000; moderate reaction — MFI between 500 and 3,000, and weak or negative reaction — lower than 500.

In order to obtain the calculated PRA we used the public program cPRA, available at Organ Procurement and Transplantation Network's website: http://optn.transplant.hrsa.gov/resources/professionalResources.asp?index=78[18], using as input data the HLA antibody specificities, which were considered Unacceptable Mismatches.

The study was approved by the Research Ethics Committee of UFPI with the number 0153.0.045.000-10, and informed consent was obtained from recipients and relatives prior to inclusion, according to the Declaration of Helsinki.

4. Results

4.1. Case 1

A 55-year-old man with CDC assay negative received a kidney transplant from his mother (Table 1). Eight years after transplantation he lost the kidney by chronic rejection. A serum screen of this recipient using single class I and II allele SPA Luminex panels (Labscreen; OneLambda, Canoga Park, CA) revealed the presence of anti-class II donor specific antibodies (anti-DRB5*02:02) as well as non-donor specific antibodies (Fig. 4).

Table 1
Recipient 1 and donor HLA type.

Locus	A*	B*	DRB1*	DRB3*	DRB5*	DQA1*	DQB1*
Recipient	02:01, 24:03	35:04, 49:01	08:04, 11:01	02:02	–	04:01, 05:01	03:01, 03:01
Donor (mother)	02:01, 24:03	15:04, 35:04	08:04, 16:01	–	**02:02**	04:01, 01:02	03:01, 05:02

* There are preformed antibodies against DRB5*02:02, this mismatch is shown in bold.

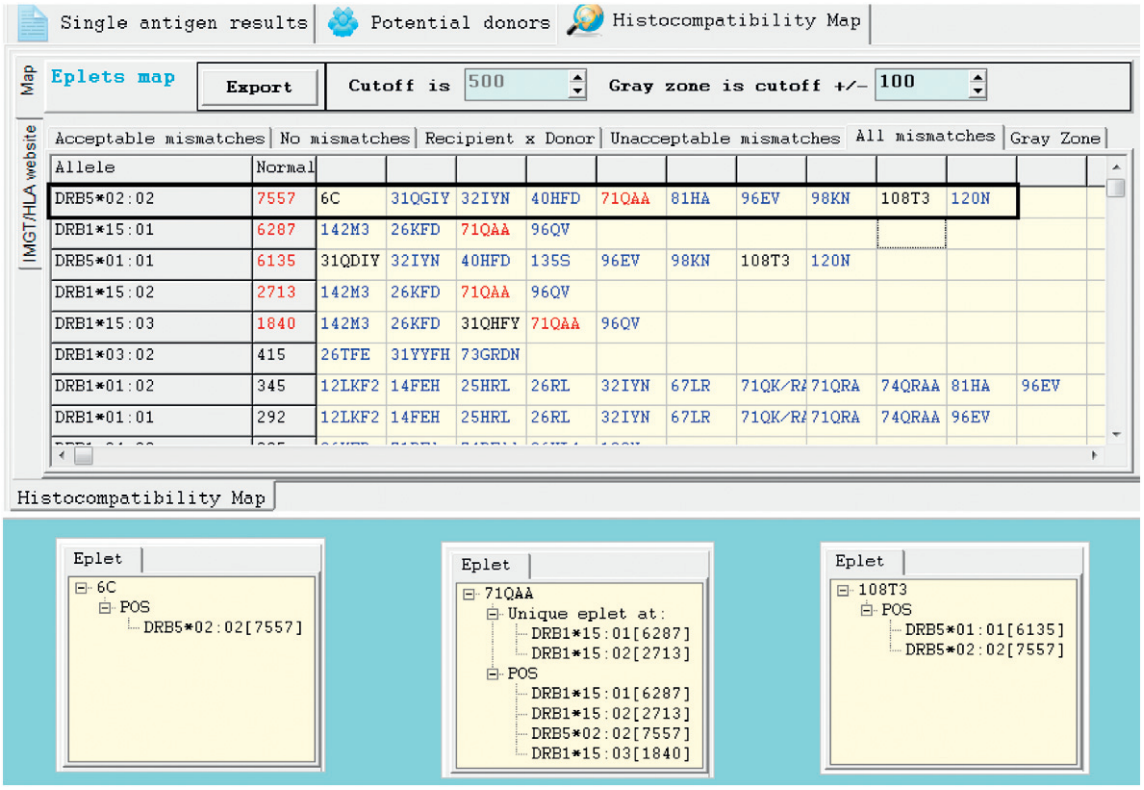


Fig. 4. Histocompatibility Map report generated by the EpHLA software from case 1.

We asked why the recipient developed antibodies against antigens to which he was never exposed. In order to solve this problem we used the EpHLA software. A closer view of results in the EpHLA's Histocompatibility Map report showed that all HLA molecules to which the recipient developed antibodies share eplets with DRB5*02:02 from the immunizer. Interestingly, the DRB5*02:02 molecule has three potentially immunogenic eplets: 6C, 71QAA, 108T3. We noted that while 6C eplet appears only on DRB5*02:02 molecule, the 71QAA eplet is shared by molecules of serum group DR15 (DRB1*15:01, DRB1*15:02, DRB1*15:03) and the 108T3 eplet is shared by DRB5*01:01 (Fig. 4). Thus, we were able to identify the epitopes targeted by the recipient's HLA antibodies using the EpHLA software, and the alleles DRB5*02:02, DRB5*01:01, DRB1*15:01, DRB1*15:02, DRB1*15:03 are the unacceptable mismatches for this case; they are associated to a 28% cPRA.

4.2. Case 2

A 35-year-old female in chronic hemodialysis, enrolled in the related renal transplantation program with two potential donors (brothers). The donors were typed as identical HLA each other and distinct as regards the recipient (Table 2). The result of the T and B CDC assays were positive to both donors. Four years later, the CDC assays performed with the same donors were negative and flow cytometry crossmatches were positive for T and B cells.

The SPA results using current serum showed preformed antibodies directed to a myriad of different class I and II HLA antigens (cPRA = 91%). The possible immunization events were blood transfusions and three gestations from the same husband, whose HLA typing is shown in Table 2. A closer examination of the SPA results revealed: (i) specific antibodies against the husband's HLA mismatches, including allele A*02:01 (MFI = 8,994), and (ii) antibodies against potential donors' HLA antigens, including allele A*68:02 (MFI = 12,353). Because A*02:01 and A*68:02 alleles belong to the same CREG, we reasoned that such alleles could share the same eplet targeted by the recipient's HLA antibodies.

We tested our hypothesis using the EpHLA software analyzing recipient versus immunizer, and then versus potential donors (Figs. 5 and 6). We found that the recipient HLA antibodies recognize the pair of eplets 142MT + 145KHA. Such eplets are shared by the HLA allele A*02:01 from the immunizer and allele A*68:02 from potential donors (Figs. 6 and 7). As showed in Table 2, the immunizer and potential donors share the same beta subunit in the HLA DQ molecule (DQB1*03:01), however combined to different alpha subunits. Such beta subunit presents an only potentially immunogenic eplet: 45EV. Nevertheless, as the MFI value of the HLA heterodimer DQA1*02:01–DQB1*03:01 of the potential donors is 921, the immunological risk is low for class II HLA. Contrariwise, we were able to detect a strong reactivity against A*68:02, representing a high immunological risk for antibody-mediated rejection.

As there is no agreement upon current CDC assay with the flow cytometry crossmatch and SPA results, we believe that the recipient has a mixture of antibodies with a prevalence of non-fixing complement isotypes, or the titles of the fixing complement antibodies present in the current serum were not enough to activate the classic pathway of the complement system.

Thus, the potential donors' allele A*68:02 is considered one of the unacceptable mismatches for this recipient. As the calculated PRA was 91%, this case exemplifies the importance of using the Acceptable Mismatch approach.

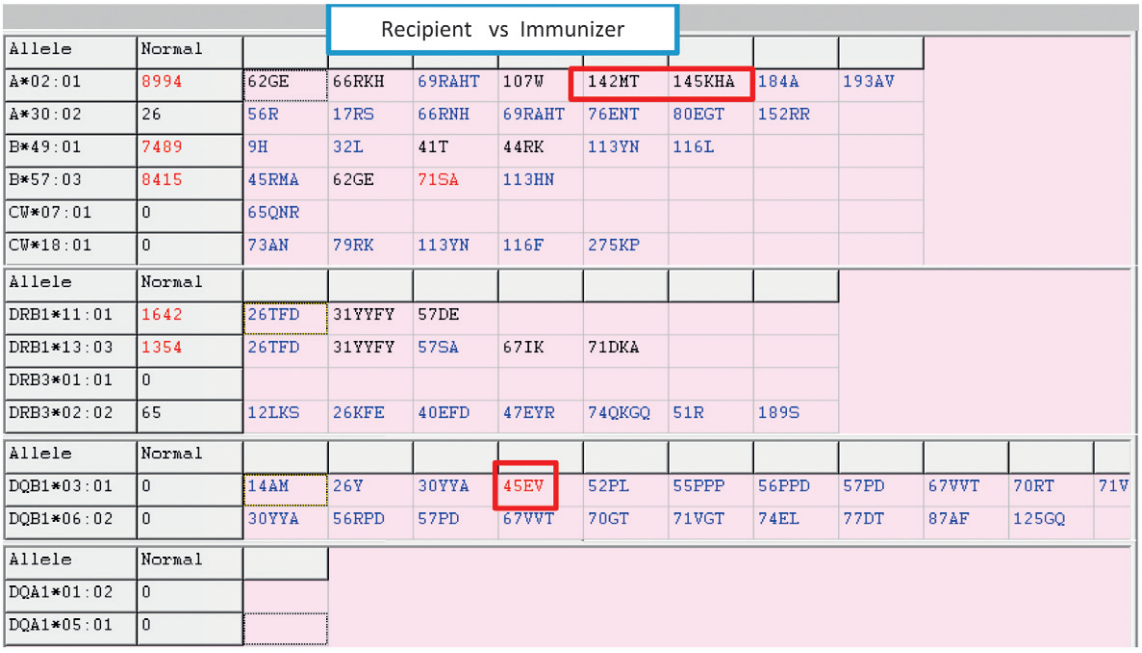
5. Discussion

The implementation of the EpHLA program will allow a simple and automated analysis of antibody data using the HLAMatchmaker algorithm and prevent the many laborious manual steps used in the current analyses. Based on the HLA types of the recipient/donor pair and the SPA result, it is possible to generate reports automatically which will support the transplantation team to define the risk of developing antibody-mediated

Table 2
HLA type from recipient 2, donors and potential immunizer.

Locus	A*	B*	Cw*	DRB1*	DRB3*	DRB4*	DRB5*	DQA1*	DQB1*
Patient	03:01, 24:02	07:02, 35:01	03:04, 07:02	03:01, 15:01	01:01	–	01:01	05:01, 01:02	02:01, 05:02
Potential Donors	23:01, **68:02**	15:18, 15:10	03:04, 03:04	07:01, 08:04	–	01:01	–	02:01, 04:01	02:01, **03:01**
Immunizer	**02:01,** 30:02	**49:01, 57:03**	**07:01, 18:01**	11:01, 13:03	02:02, 01:01	–	–	01:02, 05:01	06:02, **03:01**

* There are preformed antibodies against the mismatches shown in bold.



Allele	Normal											
A*02:01	8994	62GE	66RKH	69RAHT	107W	142MT	145KHA	184A	193AV			
A*30:02	26	56R	17RS	66RNH	69RAHT	76ENT	80EGT	152RR				
B*49:01	7489	9H	32L	41T	44RK	113YN	116L					
B*57:03	8415	45RMA	62GE	71SA	113HN							
CW*07:01	0	65QNR										
CW*18:01	0	73AN	79RK	113YN	116F	275KP						
Allele	Normal											
DRB1*11:01	1642	26TFD	31YYFY	57DE								
DRB1*13:03	1354	26TFD	31YYFY	57SA	67IK	71DKA						
DRB3*01:01	0											
DRB3*02:02	65	12LKS	26KFE	40EFD	47EYR	74QKGQ	51R	189S				
Allele	Normal											
DQB1*03:01	0	14AM	26Y	30YYA	45EV	52PL	55PPP	56PPD	57PD	67VVT	70RT	71V
DQB1*06:02	0	30YYA	56RPD	57PD	67VVT	70GT	71VGT	74EL	77DT	87AF	125GQ	
Allele	Normal											
DQA1*01:02	0											
DQA1*05:01	0											

Fig. 5. Histocompatibility Map report generated by the EpHLA software from case 2 versus immunizer.

rejection. The automated analysis is important to increase the efficiency in generating results with at least the same degree of accuracy [19].

Automation will certainly decrease the incidence of administrative errors and facilitate the information management within the organization [20].

6. Conclusions

In conclusion, the EpHLA program integrates SPA results and the HLAMatchmaker algorithm in an automated histocompatibility analysis. The program will certainly benefit the donor selection and risk assessment for HLA sensitized recipients.

Acknowledgments

The work was supported by the Immunogenetics and Molecular Biology Laboratory from UFPI. Thanks to CNPq for the scholarship granted to Herton Luiz Alves Sales Filho. The authors also acknowledge João Batista de Oliveira Silva Jr. for his corrections of the English version in preparation of the manuscript.

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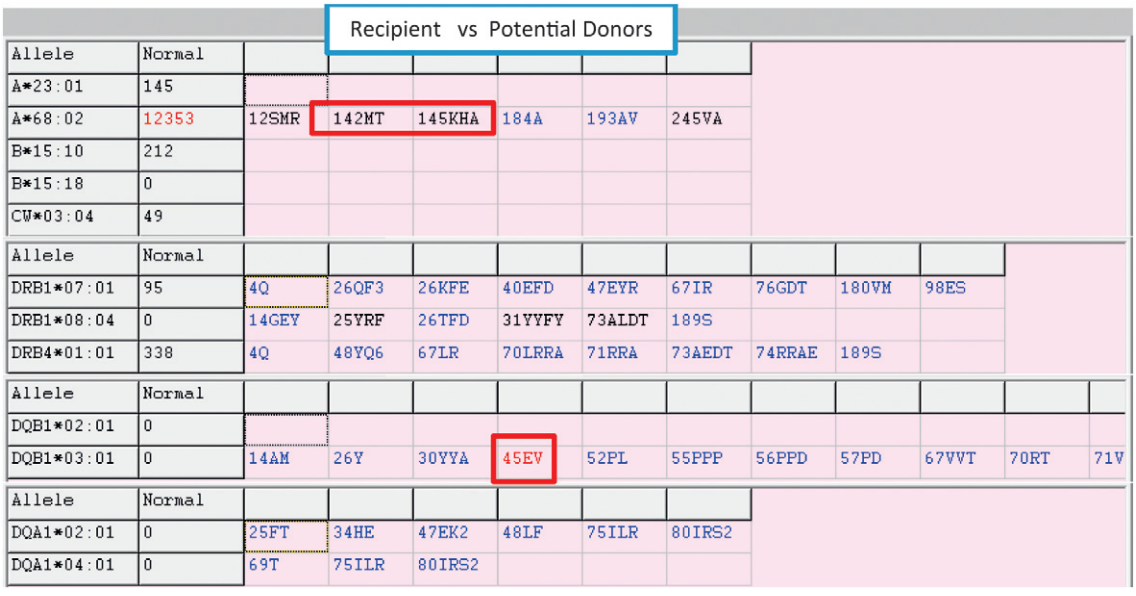

Allele	Normal										
A*23:01	145										
A*68:02	12353	12SMR	142MT	145KHA	184A	193AV	245VA				
B*15:10	212										
B*15:18	0										
CW*03:04	49										
Allele	Normal										
DRB1*07:01	95	4Q	26QF3	26KFE	40EFD	47EYR	67IR	76GDT	180VM	98ES	
DRB1*08:04	0	14GEY	25YRF	26TFD	31YYFY	73ALDT	189S				
DRB4*01:01	338	4Q	48YQ6	67LR	70LRRA	71RRA	73AEDT	74RRAE	189S		
Allele	Normal										
DQB1*02:01	0										
DQB1*03:01	0	14AM	26Y	30YYA	45EV	52PL	55PPP	56PPD	57PD	67VVT	70RT	71V
Allele	Normal										
DQA1*02:01	0	25FT	34HE	47EK2	48LF	75ILR	80IRS2				
DQA1*04:01	0	69T	75ILR	80IRS2							

Fig. 6. Histocompatibility Map report generated by the EpHLA software from case 2 versus potential donors.

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