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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.

Table 1
Class I HLA alleles and number of class I non-self eplets of 10 different HLA sensitized patients.

Patient	HLA-A*	HLA-B*	Nº non self eplets
1	02:01, 11:01	35:01, 39:01	614
2	24:02, 30:01	35:01, 42:01	630
3	01:01, 02:01	18:01, 40:02	741
4	02:01	39:02, 40:01	861
5	02:01, 30:02	15:03, 40:02	658
6	03:01, 32:01	07:02, 44:03	558
7	02:01, 74:01	48:01, 58:01	557
8	02:01, 11:01	07:02, 51:01	549
9	25:01, 31:01	15:01, 35:01	741
10	02:01, 31:01	27:03, 39:03	719

The remaining eplets (non-self eplets) were then counted and categorized either as reactive or non-reactive based on the cutoff value of the median fluorescence intensity (MFI) value (herein calculated as 500). Non-reactive eplets (assigned blue) were those appearing in HLA alleles of the panel, which had an MFI value lower than the cutoff value. In contrast, reactive eplets (assigned black) were those appearing only in HLA alleles which had an MFI value higher than the cutoff value. Overall, eplets categorized in this way were used for the classification of the HLA alleles into AMMs and UMMs. Users considered all non-self HLA molecules composed of non-reactive eplets and self-eplets as AMMs.

The next step was to compare the results of the conventional and automated approaches. Just one eplet, with the same color, should fill correspondent positions in both results. When this rule is broken, there is a disagreement in eplet categorization. A supportive program was created to identify the number of these eplet disagreements between the conventional and automated analyses for each CSV file. It filtered all of the agreeing eplets and showed the number of eplets, AMMs and disagreements in those variables. When disagreements were found, the instructor was invited to critically review the case in order to define whether the error leading to disagreement occurred in the analysis of the single antigen results performed by the conventional or automated method.

3.4. Quality features of the software verified in its validation

The four major perceivable features (functionality, reliability, usability and efficiency) were tested to evaluate the quality of the EpHLA software. Functionality reflects the accuracy in accomplishing the tasks for which the software was designed. Reliability refers to the lack of failures in the software. Usability is an expression of use adequacy as the software must be adequate to the type of user for which it was designed. Thus, it is important that the user can easily understand the concept and application of the program and can learn how to use, operate, and control the tool. Efficiency expresses the capacity of the software to obtain results quickly while using few computer resources.

Table 2
Class II HLA alleles and number of class II non-self eplets of 10 different HLA sensitized patients.

Patient	HLA-DRB1*	HLA-DRB3*	HLA-DRB4*	HLA-DRB5*	HLA-DQA1*	HLA-DQB1*	Nº non self eplets
1	03:01, 04:07	02:02	01:01	–	05:01, 03:02	02:01, 03:01	398
2	01:01, 13:01	02:02	–	–	01:01, 01:03	05:01, 06:03	550
3	04:04, 15:01	–	01:01	01:01	03:01, 01:02	03:02, 06:02	364
4	04:04, 11:01	02:02	01:01	–	03:01, 05:01	03:02, 03:01	325
5	11:01, 11:02	02:02	–	–	05:01	03:01	679
6	01:01, 15:01	–	–	01:01	01:01, 01:02	05:01, 06:02	581
7	01:01, 09:01	–	01:01	–	01:01, 03:02	05:01, 03:03	457
8	15:01, 16:01	–	–	01:01, 02:02	01:02	06:02, 05:02	643
9	08:01	–	–	–	04:01	04:02	906
10	08:02, 15:01	–	–	01:01	04:01, 01:02	04:02, 06:02	439

3.5. Statistical analysis

Differences in the time spent for the achievement of results using conventional and automated HLAMatchmaker analysis was measured using Student's *t*-test and the Mann–Whitney non-parametric test. The disagreements analysis of the numbers of eplets and AMMs among the users were evaluated using the likelihood ratio test after Poisson distribution (H_0; lambda<0.1 vs. H_1; lambda ≥ 0.1). The significance levels for all of the tests were established at $p<0.05$.

4. Results

4.1. The time for training and the analysis time of the single antigen results

The non-experienced group required 60 training hours to be able to analyze single antigen results with HLAMatchmaker using Microsoft Excel format. After this stage, experienced and non-experienced groups required 10 and 20 training hours, respectively, to be able to use the EpHLA software.

The comparison of the average time spent in obtaining results from HLAMatchmaker using the conventional and automated methods revealed that the EpHLA software was almost 6 times faster when used by manual analysis experts (experienced group) and over 10 times faster when used by users with low analysis experience (Table 3, *t*-test, $p<0.0001$). The class II HLA analysis required a longer average time to perform for both conventional (Table 3; *t*-test, $p<0.002$) and automated (Table 3; Mann–Whitney, $p<0.0001$) programs when compared to the class I HLA analysis.

4.2. Assignment of the total numbers of eplets, AMMs, and disagreements

No difference in the number of non-self eplets was reported by users after both types of analyses: it was counted a total of 72,908 non-self eplets in HLA class I and 58,762 non-self eplets in HLA class II. However, disagreements were observed with respect to the categorization (colors) given to some eplets between the conventional and automated methods. In fact, there was one disagreement for HLA class I and eleven disagreements for HLA class II eplets. These twelve eplets were classified as reactive (black) in the conventional analysis and as non-reactive (blue) in the automated analysis. As a consequence of such eplet categorization, twenty-one HLA alleles were considered UMMs, when using the conventional analysis, whereas they were classified as AMMs when using the automated analysis. Due to these 21 AMMs' disagreements, the number of HLA alleles considered AMMs in the conventional approach was 10,737, however in the automated approach 10,758 HLA alleles were considered AMMs.

Table 3
Time expressed in minute spent for the analyses of results obtained by single antigen assays with conventional versus automated methods.

Testers' groups	Method					
	Conventional			Automated (EpHLA		
	HLA Class I	HLA Class II	HLA Class I and II	HLA Class I	HLA Class II	HLA Class I and II
EXPG ($n=40$)	30.0 ± 10.1	27.9 ± 8.7	57.9 ± 13.6	**4.5 ± 1.5**[a]	**6.0 ± 2.1**[a]	**10.5 ± 3.2**[a]
NEXPG ($n=70$)	37.7 ± 14.2	49.8 ± 18.2	87.4 ± 28.8	**3.2 ± 1.5**[a]	**5.2 ± 2.2**[a]	**8.4 ± 3.3**[a]
All testers ($n=110$)	34.9 ± 13.3	**41.8 ± 18.7** [b]	76.7 ± 28.2	**3.7 ± 1.6**[a]	**5.5 ± 2.2**[a]	**9.2 ± 3.4**[a]

Data are shown as average ± standard deviation. Bold face represents significant values.
[b]comparison between HLA inter class (Student *t* test) McDonald, J.H. 2009. Handbook of Biological Statistics (2nd ed.). Sparky House Publishing, Baltimore, Maryland.
EXPG—experienced group
NEXPG—non-experienced group
[a] Comparison between HLA intra classes (Student *t* test).

A closer examination of the above reported results revealed that there were errors in eplets' categorization when using the conventional HLAMatchmaker analysis. In particular, Fig. 1 shows a case with disagreements due to human error in conventional analysis. The revised analysis permitted the correct categorization of eplets as non-reactive and the respective HLA molecules as AMMs.

Fig. 1 shows screenshots of categorization eplets' disagreements between conventional and automated HLAMatchmaker analysis.

The assigned cutoff was 500, alleles in bold were assigned was AMMs. The eplets 57PS and 125SH should be blue in conventional analysis (panel 1A), because they are present on bead 47 with negative reaction of MFI = 67 as shown by automated analysis (panel 1B). Also, the allele DQB1*05:02 in conventional analysis should be in bold (panel 1A), because it is an AMM with blue non-self eplets as shown in automated analysis (panel 1B).

4.3. EpHLA software and HLAMatchmaker algorithm produce similar outcomes in single antigen results analyses

All disagreements identified in this study occurred due to human errors made by the non-experienced group during the conventional HLAMatchmaker analysis. However, the comparison between two methods showed no statistically significant difference for these variables (class I eplets, $p = 0.99$; class I AMMs, $p = 0.85$; class II eplets, $p = 0.42$ and class II AMMs, $p = 0.14$). Thus, the EpHLA software accuracy was achieved in executing the HLAMatchmaker algorithm as it did not make any system errors. The same results suggest that although there was no statistical difference between two methods, even rare human errors in manual analysis can reduce the recipients' chance of transplantation or expose them to an unforeseen risk.

4.4. The EpHLA software fulfills required quality of perceivable features for the user

As previously shown, the EpHLA software was capable of automatically executing the HLAMatchmaker algorithm as accurately as the conventional manual method on an Excel spreadsheet. Therefore, the EpHLA software fulfilled the functionality requirements because it accomplished the task to which it was designed with no errors in applying the algorithm. During a period of 3 months, the EpHLA software was continuously used by 11 different users to perform analysis of 110 single antigen results. During this validation period there were no errors due to EpHLA software failures. Therefore, the automation tool enabled the performance of reliable histocompatibility analyses.

The emerging results of this study make it evident that users with minimal knowledge of the fundamentals about HLAMatchmaker are able to easily operate the EpHLA software. It is noteworthy that the automation of manual steps enabled the user to have a higher productivity in analyzing single antigen results. The decrease in the average time for this analysis was evidenced when users improved their skills with the EpHLA software (Table 3).

The EpHLA program does not need a computer with any special configuration in order to run. An adequate efficiency can be obtained when running on low-performance machines. During its validation, the EpHLA software was used in Core 2 Duo machines with 2 GB of RAM. In these machines the response for each input applied to the EpHLA software

1A Conventional analysis: DQB1 locus's patient 3

Bead ID	DQB1 - DQA1	MFI	DQB1 non-self eplets
39	DQB1*02:01 - DQA1*02:01	1677	,,,,,45GE5,,,,56LPA,,,66DI,,,,,77DR,,,,,,,,,,
40	DQB1*02:01 - DQA1*03:01	938	,,,,,45GE5,,,,56LPA,,,66DI,,,,,77DR,,,,,,,,,,
41	DQB1*02:01 - DQA1*05:01	6419	,,,,,45GE5,,,,56LPA,,,66DI,,,,,77DR,,,,,,,,,,
42	DQB1*02:02 - DQA1*02:01	1221	,,,,,45GE5,,,,56LPA,,,66DI,,,,,77DR,,,,,,,,135G,,,
43	**DQB1*04:01** - DQA1*02:01	576	,,23L,26G,,,,,,,57LD,,66DI,,70ED,,74SV,,,,,,,,,,,
44	**DQB1*04:02** - DQA1*02:01	552	,,,26G,,,,,,,57LD,,66DI,,70ED,,74SV,,,,,,,,,,,
45	**DQB1*04:02** - DQA1*04:01	1547	,,,26G,,,,,,,57LD,,66DI,,70ED,,74SV,,,,,,,,,,,
46	**DQB1*05:01** - DQA1*01:01	0	,14GL,,26G,30HYV,,57PV,,,,70GA,,74SV,77DR,,,87AY,,116I,125SQ,,,,,
47	DQB1*05:02 - DQA1*01:02	67	,14GL,,26G, 30HYV,,57PS,,70GA,,74SV,77DR,,,87AY,,116I,125SH,
48	**DQB1*06:01** - DQA1*01:03	10	3P3,14AM,,26Y,,,,,,,,,66DI,,,,,,,,,,,,,,167HG,
49	**DQB1*06:02** - DQA1*01:02	26	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
50	DQB1*03:01 - DQA1*03:01	2356	,14AM,,26Y,,45EV,,,,56PPD,,,,,,,,,,,,,,,,,167HG,
51	DQB1*03:01 - DQA1*02:01	3606	,14AM,,26Y,,45EV,,,,56PPD,,,,,,,,,,,,,,,,,167HG,
52	DQB1*03:01 - DQA1*06:01	7086	,14AM,,26Y,,45EV,,,,56PPD,,,,,,,,,,,,,,,,,167HG,

Self antigens Negative reactions Positive reactions

1B Automated analysis: DQB1 locus's patient 3

Eplets Map - All mismatches, Patient 3

Allele	MFI										
DQB1*02:01-DQA1*02:01	1677	45GE5	56LPA	66DI	77DR						
DQB1*02:01-DQA1*03:01	938	45GE5	56LPA	66DI	77DR						
DQB1*02:01-DQA1*05:01	6419	45GE5	56LPA	66DI	77DR						
DQB1*02:02-DQA1*02:01	1221	45GE5	56LPA	66DI	77DR	135G					
DQB1*04:01-DQA1*02:01	576	23L	26G	57LD	66DI	70ED	74SV				
DQB1*04:02-DQA1*02:01	552	26G	57LD	66DI	70ED	74SV					
DQB1*04:02-DQA1*04:01	1547	26G	57LD	66DI	70ED	74SV					
DQB1*05:01-DQA1*01:01	0	14GL	26G	30HYV	57PV	70GA	74SV	77DR	87AY	116I	125SQ
DQB1*05:02-DQA1*01:02	67	14GL	26G	30HYV	57PS	70GA	74SV	77DR	87AY	116I	125SH
DQB1*06:01-DQA1*01:03	10	3P3	14AM	26Y	66DI	167HG					
DQB1*06:02-DQA1*01:02	26	SELF									
DQB1*03:01-DQA1*03:01	2356	14AM	26Y	45EV	56PPD	167HG					
DQB1*03:01-DQA1*02:01	3606	14AM	26Y	45EV	56PPD	167HG					
DQB1*03:01-DQA1*06:01	7086	14AM	26Y	45EV	56PPD	167HG					

Fig. 1. 1A Conventional analysis: DQB1 locus' patient 3. 1B Automated analysis: DQB1 locus patient 3.

was as fast as observed with the HLAMatchmaker algorithm run on an Excel spreadsheet (a few milliseconds). Thus, the EpHLA software may perform all necessary operations on standard computers.

5. Discussion

In spite of the ability of the HLAMatchmaker algorithm to improve the allocation of solid organs for highly sensitized patients [15], the widespread use of this tool is limited by the manually demanding and time consuming intermediate steps. To solve this problem, we have developed a new software called EpHLA, which fully automates the functional steps of the HLAMatchmaker algorithm [16]. The present study has shown that the EpHLA software facilitates the identification of AMMs in a considerably shorter time while maintaining the same level of accuracy when using the conventional HLAMatchmaker algorithm.

Since the EpHLA program is saving time and it is easy to use, we predict that it will have a significant impact on the applicability of epitope-based histocompatibility matches of donors for sensitized recipients. The EpHLA program is also very useful to interpret antibody-mediated rejections by identifying immunogenic epitopes. For these reasons, the speed of generating results and their accuracy have gained great importance [19]. The expectation is that the EpHLA software will not only reduce the necessary time for case analysis, but also its user friendly nature will popularize the clinical application of the HLAMatchmaker algorithm. Hence, the time optimization obtained with EpHLA program will allow for strategies similar to the Acceptable Mismatch Program of Eurotransplant to be applied in other transplant programs. This will benefit the steadily growing numbers of highly sensitized patients (PRAs $>85\%$) enrolled in multiple transplant programs.

Another advantage for using the EpHLA software is the elimination of human errors. The results of this study demonstrate that infrequent disagreements between two methods occur due to errors in the manual application of the algorithm, especially for less-experienced users. Therefore, a computerized tool and a centralized database can significantly reduce the potential for errors, increase reproducibility of calculated values and histocompatibility choices, facilitate data management, and make data analysis less labor-intensive; thus, all these benefits make EpHLA program more clinically applicable [16].

It is expected that HLAMatchmaker analysis automation will improve the ability to accurately determine AMMs. We believe that the selection of accurate AMMs will increase the number of acceptable donors to choose for highly sensitized patients waiting for kidney transplants. Identification of matching donor/recipients pairs based on eplets-based analysis may be the best cost–benefit option for improving organ transplantation practice because the use of EpHLA program is fast, easy and inexpensive.

In summary, we have performed an experimental evaluation of the EpHLA software for automated use of the HLAMatchmaker algorithm. Our results demonstrate that the software is functional, reliable, and efficient, with very good usability. Hence, we propose that the EpHLA program can be incorporated into the daily clinical routine of kidney and heart transplant programs to facilitate the decision-making process especially for highly sensitized patients.

6. Conclusion

The EpHLA software is an efficient tool for the identification of acceptable mismatches for highly sensitized patients. This program is superior to the manual use of the HLAMatchmaker algorithm with respect to accuracy and speed of the analysis.

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References

[1] Terasaki PI. Humoral theory of transplantation. Am J Transplant 2003;3:665–73.
[2] Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969;280:735–9.
[3] Bryan CF, Baier KA, Nelson PW, Luger AM, Martinez J, Pierce GE, et al. Long-term graft survival is improved in cadaveric renal retransplantation by flow cytometric crossmatching. Transplantation 1998;66:1827–32.
[4] Tait BD. Solid phase assays for HLA antibody detection in clinical transplantation. Curr Opin Immunol 2009;21:573–7.
[5] Claas FH, Doxiadis II. Management of the highly sensitized patient. Curr Opin Immunol 2009;21:569–72.
[6] Zachary AA, Leffell MS. Barriers to successful transplantation of the sensitized patient. Expert Rev Clin Immunol 2010;6:449–60.
[7] Claas FH, De Meester J, Witvliet MD, Smits JM, Persijn GG, Doxiadis II. Acceptable HLA mismatches for highly immunized patients. Rev Immunogenet 1999;1:351–8.
[8] Duquesnoy RJ. Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. Curr Opin Immunol 2008;20:594–601.
[9] Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. Hum Immunol 2006;67:847–62.
[10] Duquesnoy RJ, Askar M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. V. Eplet matching for HLA-DR, HLA-DQ, and HLA-DP. Hum Immunol 2007;68:12–25.
[11] Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. Hum Immunol 2002;63:339–52.
[12] Valentini RP, Nehlsen-Cannarella SL, Gruber SA, Mattoo TK, West MS, Lang C, et al. Intravenous immunoglobulin, HLA allele typing and HLAMatchmaker facilitate successful transplantation in highly sensitized pediatric renal allograft recipients. Pediatr Transplant 2007;11:77–81.
[13] Campos ER, Doxiadis IIN, Temin J, Plothow A, Bellintani EC, Miyamoto Y, et al. Proposal for a program to enhance renal transplantation opportunity for highly sensitized patients. J Bras Transplantes 2010;13:1251–5.
[14] Haririan A, Fagoaga O, Daneshvar H, Morawski K, Sillix DH, El-Amm JM, et al. Predictive value of human leucocyte antigen epitope matching using HLAMatchmaker for graft outcomes in a predominantly African-American renal transplant cohort. Clin Transplant 2006;20:226–33.
[15] Claas FH, Witvliet MD, Duquesnoy RJ, Persijn GG, Doxiadis II. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. Transplantation 2004;78:190–3.
[16] Sousa LCDM, Sales Filho HLA, Von Glehn CQC, da Silva AS, Neto PAS, de Castro JAF. EpHLA: an innovative and user-friendly software automating the HLAMatchmaker algorithm for antibody analysis. Transpl Immunol 2011;25:210–6.
[17] CPRA calculator: organ procurement and transplantation network; 2011.
[18] HLAMatchmaker 2012.
[19] Pumarola T. Influence of new technologies in modern microbiology. Enferm Infecc Microbiol Clin 2010;28(Suppl. 3):59–62.