The DQ Barrier: Improving Organ Allocation Equity Using HLA-DQ Information

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Background. The United Network for Organ Sharing algorithm for deceased-donor kidney allocation considers only the human leukocyte antigen (HLA)-A, HLA-B, and HLA-DR loci. Although HLA-DQ serologic specificities can be entered as unacceptable antigens, they are assigned only by the identity of the DQβ chain, disregarding the role of the similarly polymorphic α chain. DQα/β combinations result in unique antigenic epitopes, which serve as targets to different antibodies. Therefore, the presence of HLA antibodies to one DQα/β combination should not preclude negative crossmatch (XM) against another combination. In this retrospective analysis, patients were allowed XM against a particular donor if they had antibodies to some, but not all, DQα/β allele combinations with the donor serologic HLA-DQ antigens.

Methods. HLA antibody signature was obtained using solid-phase Luminex-based antibody analysis. Results were captured at the high-resolution level (as provided by the positive beads). Potential donors were typed to include information on both HLA-DQA and HLA-DQB alleles.

Results. Of the 1130 flow XM assays performed, 147 patients had antibodies to donor serologic HLA-DQ antigens. Thirty-five of those patients had antibodies to an allelic DQα/β combination within the donor serologic DQ specificity that were different from the donor's DQα/β, leading to negative flow XM results (24%). Virtual XM, accounting for donor DQα/β combinations, successfully predicts more than 98% of XM outcomes.

Conclusions. In patients with allelic DQα/β antibodies, denying the opportunity for XM based on serologically defined unacceptable antigens can disadvantage the patient. Larger cohort studies are required to substantiate our observation. Introducing DQα/β combination information may increase virtual XM accuracy and organ allocation equity.

Keywords: HLA-DQα, HLA-DQβ, Organ equity, Epitope.

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Human leukocyte antigen (HLA) class II antibodies have not been considered a contraindication to transplantation for many years. Even with the increased understanding of the significance of a positive B-cell crossmatch (XM), the main targets of interest were antibodies to HLA-DR. The significance of other HLA class II antibodies (HLA-DQ and HLA-DP) has only recently come to light (*1–4*). Initial reports have documented the incidence of donor-specific HLA-DQ antibodies (*5–7*). More recently, several groups have reported that HLA-DQ antibodies were the most common donor-specific antibodies (DSA) developed de novo after transplantation and concluded that these antibodies likely contribute to inferior graft outcome (*8–10*). Importantly, Walsh and colleagues (*11*) found that the most frequent antibodies associated with late antibody-mediated rejection were HLA-DQ DSA.

The United Network for Organ Sharing (UNOS) algorithm for deceased-donor kidney allocation still prioritizes candidates with a zero antigen mismatched donor, considering only the HLA-A, HLA-B, and HLA-DR loci for this purpose (*12*). Although HLA-DQ serologic specificities can be entered as unacceptable antigens, these specificities are assigned only by the identity of the DQβ chain, completely disregarding the role of the other half of the HLA-DQ molecule—the α chain (see **Figure S1, SDC**, http://links.lww.com/TP/A753).

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Moreover, allele-level differences between some of the DQβ specificities (for example, alleles of HLA-DQ5 such as *DQB1*05:01* and *DQB1*05:02*) are ignored using the current approach.

Whereas some centers have considered antibodies directed specifically at the DQα chain (*13, 14*), our group has shown previously that HLA-DQ molecules are recognized by antibodies as unique combinations of DQα and DQβ chains (*15, 16*). These two chains are encoded by distinct genes at the molecular level but are expressed as a unique, single protein on the cell surface where they are accessible to antibodies or T-cell receptors (TCR). HLA-DR molecules are also formed by combinations of DRα and DRβ chains; however, because the DRα molecule is not polymorphic, it does not contribute to allelic variation between the different DR antigens. Thus, the current practice of typing only for the DRβ chain is sufficient.

In this retrospective study, we tested the impact of assigning HLA-DQ antibodies using the conventional UNOS system (serologic equivalents) versus using the complete HLA-DQ information (molecular definition of the combined DQα and DQβ chains) as it affects patients' access to transplantation. In our region, Gift of Hope (GOH) organ procurement organization laboratory has a mandate to perform XM assays for all deceased-donor kidney offers. The individual transplant centers enter into UNet the HLA specificities that are considered unacceptable. Patients are awarded calculated panel reactive antibody (cPRA) points based on these assignments but are prohibited from being crossmatched with donors expressing these HLA antigens. The Northwestern Comprehensive Transplant Center is willing to consider accepting potential donors with low to moderate immunologic risk depending on patient specific criteria; therefore, only strong HLA antibodies are considered unacceptable. Given our experience with HLA-DQ antibodies, our center has chosen not to report strong HLA-DQ antibodies as unacceptable unless the complete serologic specificity (all DQα/β allele combinations within this antigen assignment) is positive. For example, if some, but not all, HLA-DQ8 alleles are strongly positive, HLA-DQ8 will not be reported as an unacceptable antigen. Our center maintains an internal database with all antibody specificities and their strength to perform real-time virtual XM when a donor opportunity arises. This database is shared with the GOH HLA laboratory on a monthly basis. This arrangement provided us with the unique opportunity to test the impact of entering unacceptable HLA-DQ specificities using the serologic assignment, as it is currently performed in UNet, versus our approach that assigns specificities based on the complete HLA-DQα/β combinations, and assess its potential impact on accessibility to organ transplantation.

RESULTS

Patient Population

Between 1 January 2011 and 31 December 2011, the Northwestern Comprehensive Transplant Center HLA laboratory performed 2037 assays using solid-phase HLA flow PRA tests. Of these, 1173 (58%) patients were sensitized and continued analysis using the single antigen bead (SAB) assay. As shown in Figure 1, 9% of sensitized patients had antibodies against HLA class II antigens alone and additional 48% had antibodies against both class I and II specificities. Antibody signature analysis revealed that 56% of these patients had antibodies directed at HLA-DQ targets; of those, 31% had antibodies against HLA-DQ only, 25% had antibodies directed at both HLA-DR and HLA-DQ, and 44% had antibodies against HLA-DR, HLA-DQ, and HLA-DP. The lower portion of Figure 1 presents a graphic distribution of HLA-DQ antibodies by the relative strength as determined in our center. As can be seen, the vast majority of patients who possessed DQ antibodies had at least one specificity that was assigned as strong (46%; unacceptable) or moderate (39%; relative high risk).

Crossmatch Assays

During calendar year 2011, GOH HLA laboratory performed 1130 XM assays for Northwestern Comprehensive Transplant Center (Table 1). Assays were performed against a total of 259 potential donors. In retrospective analysis, it was found that, of the positive XM assays, 112 were positive due at least in part to donor-specific HLA-DQ antibodies (10% of all assays). In 64 of 112 (57%) assays, in addition to a moderate to strong donor-specific HLA-DQ antibodies, other HLA DSA may have contributed to the positive B-cell flow XM. These patients were not excluded from the match-run because they had only some antibodies to the donor-specific serologic HLA-DQ specificity, but not to all DQα/β allele combinations within this antigen assignment, and therefore, per our algorithm, were not considered unacceptable. In three of the cases (two patients), the donor reactivity might have been explained by a pattern of antibody responses currently considered as a "DQA antibody". In 35 additional cases, however, although the patients had moderate to strong antibodies against the donor-specific DQ serologic specificity, no antibodies were detected against the specific donor HLA-DQα/β combination; in other words, no antibodies to the donor-specific HLA-DQ allele were present. These 35 XM assays were negative and would have enabled these patients to receive an organ from that donor. Thus, using serologic HLA-DQ assignment, a total of 147 patients (112+35) would have been screened out of the XM tray against a specific donor. Using our allele-level approach, 35/147=24% were able to proceed and obtain a negative XM. It is important to state that using the complete donor DQ typing (α/β) and the complete antibody signature, the accuracy of prediction of the virtual XM in these 147 cases was more than 98%. Table 2 provides demographic information specific to this group of patients. All but one patient had at least one previous transplant in their history, with 41% having more than one prior transplant. Their sensitization level was quite high as indicated by the peak and current PRA values for HLA class I and II (65% and 91% for peak PRA and 48% and 69% for current PRA, respectively).

A Representative Case

A 67-year-old Hispanic male received a kidney transplant in 2003 from his haplotype-matched sibling. The transplantation failed, and in 2009, the patient underwent a transplant nephrectomy. The patient was placed on the deceased-donor waiting list with class I and II PRA values of 87% and 90%, respectively. The pertinent details regarding a potential kidney offer in 2011 are presented in Table 3.

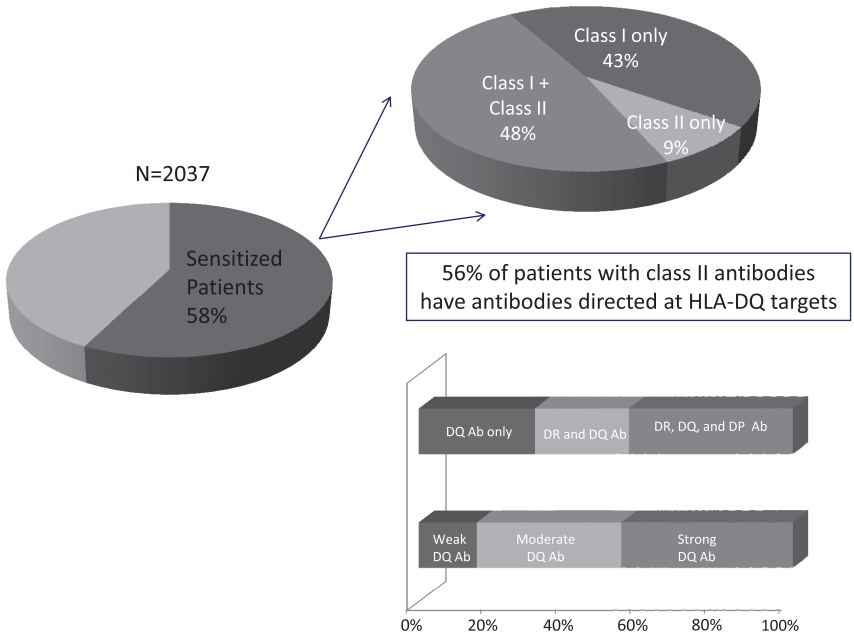

FIGURE 1. HLA antibody signature of patients awaiting kidney transplantation at the Northwestern Comprehensive Transplant Center. Between 1 January 2011 and 31 December 2011, 2037 patients were tested for the presence of HLA antibodies in our laboratory. Fifty-eight percent of patients were sensitized with the relative distribution as presented in the pie chart on the top right-hand side. Of the patients that exhibit class II specificities, 56% had antibodies to HLA-DQ. The antibody make-up and strength of antibodies are presented in the lower portion as bar charts. HLA, human leukocyte antigen.

Solid-phase single antigen Luminex analysis revealed that, in addition to many other antibody specificities, three of the five beads carrying the serologic DQ2 specificities and two of the five beads carrying the serologic DQ7 specificities were strongly positive (mean fluorescence intensity [MFI] values of >10,000). Following our center's philosophy—that only specificities in which all DQ alleles are positive are listed as unacceptable antigens—DQ2 and DQ7 were not listed as unacceptable antigens for this patient, leading to his eligibility for XM with the specific donor (donor HLA typing is provided in Table 3). After evaluating the complete donor DQα/β combinations (typed by the GOH HLA laboratory as *DQA1*05:01/DQB1*02:01* and *DQA1*05:01/DQB1*03:01*), it was clear that the patient had no antibodies to the donor-specific HLA-DQα/β combination and thus had no DSA. Predictably, the T-cell and B-cell XM results were negative. The three-dimensional structure of the HLA-DQ molecule, indicating the specific area in which the positive DQ2 and DQ7 allelic antibodies differ from the negative DQ2 and DQ7 allelic antibodies, is presented in Figure 2. This area/epitope is accessible to antibody binding as well as for TCR recognition. Without the ability to take into consideration the HLA-DQα/β information, this highly sensitized patient would have been denied the offer from this donor and would have missed an opportunity to find a compatible kidney. Our approach increased accessibility to transplant for this patient and for 24% of patients with serologic, but not allelic, DQ DSA.

DISCUSSION

One of the major contributions to the field of histocompatibility in recent years was the introduction of the solid-phase single antigen Luminex assay (*17, 18*). Other than the increased sensitivity that the assay provides, the ability to test for individual HLA targets allowed us to overcome limitations in determining the exact locus contributing to the positive response. This is due in part to the strong linkage disequilibrium between the different HLA loci and is even more noticeable when trying to distinguish between antibodies against HLA class II molecules—separating antibodies against HLA-DQ or HLA-DP from antibodies targeting HLA-DR. Between the two vendors of Luminex-based SAB assays, there are currently 43 different alleles of HLA-DQ specificities. Those represent a total of seven serologic DQ antigens (HLA-DQ2, HLA-DQ4, HLA-DQ5, HLA-DQ6, HLA-DQ7, HLA-DQ8, and HLA-DQ9), providing multiple alleles for each serologic specificity. A list of these combinations is provided in Table S1 (see **SDC,** http://links.lww.com/TP/A753). Therefore, the information obtained by the SAB assay is significantly more informative than the one entered into the UNet unacceptable antigen database. Moreover, most

TABLE 1. XM assays performed by GOH for Northwestern Comprehensive Transplant Center between 1 January 2011 and 31 December 2011

Total XM assays performed	1130
Total potential donors	259
Number of positive XM assays due to HLA-DQ DSA[a]	112
Number of negative XM assays with serologic HLA-DQ DSA	35

[a] All positive B-cell flow XM assays that could have been due to HLA-DQ DSA were considered as such, even if additional loci DSA were present.

TABLE 2. Patients associated with serologic HLA-DQ DSA but not allelic HLA-DQ DSA (n=22)

Mean age, yr		50±10.5
Male		8
Race	Caucasian	7
	African American	8
Prior sensitization	Prior transplant	12
	>1 Prior transplant	9
Median class I PRA, %		48
Median class II PRA, %		59
Median peak class I PRA, %		65
Median peak class II PRA, %		91
Blood group	O	7
	A	8
	B	2
	AB	5

DSA, donor-specific antigen; HLA, human leukocyte antigen; PRA, panel reactive antibody.

laboratories that use Luminex-based sequence-specific oligonucleotide probe hybridization as a method to type donors should have the complete DQα/β information available to them. Thus, no additional investment, other than time to actually capture the information in a usable format, is required to obtain this information. We have demonstrated in this study that using the complete information provided by the SAB assay for each individual patient, rather than assigning unacceptable antigens based on HLA-DQ serologic specificities, can increase the likelihood of obtaining a negative XM assay (due to allelic HLA-DQ antibodies) by approximately 25%.

The overall structure of HLA class I and II molecules is very similar. Both antigens have four extracellular domains of which two are more proximal and two are more distal from the cell membrane. The distal domains are the more polymorphic domains and are considered more critical for TCR and antibody recognition (for illustrative purposes, a schematic diagram is presented in **Figure S1, SDC,** http://links.lww.com/TP/A753). In HLA class I molecules, both distal domains, named α1 and α2 (as well as one of the proximal domains α3), are encoded by a single gene. Thus, the protein product of this one gene is considered as one allele with polymorphic α1 and α2 domains. Class II molecules are encoded by two separate genes, each contributing one of the distal domains α1 and β1 (as well as one of the less polymorphic proximal domains α2 and β2). However, because each of the DQ genes is typed separately, the protein products of each gene are currently viewed as separate targets to antibodies, although both chains, α1 and β1, form the two distal domains of a single HLA-class II molecule. This misconception leads to the practice of viewing antibodies directed against DQA separate from those directed against DQB, although, on the cell surface, these two parts of the DQ chain relate to each other the same way as the α1 and α2 chains of an HLA class I molecule. This practice has not been detrimental for the assignment of HLA-DR antibodies given that only the DRβ1, but not the DRα1, are polymorphic and contributing to TCR and antibody recognition. The situation is different for HLA-DQ and HLA-DP molecules as both distal domains are polymorphic.

A conceptual change in how we view HLA class II molecules is required. There are multiple examples of HLA class I molecules in which two antigens are identical in one of the proximal domains, α1 or α2, but not the other. This should provide compelling evidence to how the immune system views HLA antigens. For instance, HLA-A25 and HLA-A26 share the same exact sequence of the α2 domain (which is the equivalent of the HLA class II β1 domain). They differ only in short sequences within the α1 domain. In fact, this is the reason why A25 and A26 were originally identified as one antigen—A10. With time, more serologic typing reagents became available, allowing for the "split" of A10 into A25 and A26, which is based on the differences in the α1 domain. Currently, these HLA-A antigens have different serologic names and are considered as different immunogenic entities. There should be no reason to treat class II molecules differently. Thus, class II molecules with polymorphisms in the α1 domain, in the presence of the same β1 domain, should be considered different antigens (or alleles). The nomenclature hierarchy of the HLA system is quite convoluted and is based on historic events. This should not deter us from

TABLE 3. A representative case

Patient	A2, 24; B35, 39; C10, 12; DR8, 14; DR52; DQ4, 5 (*DQA1*04:02*/DQB1*04:02, *DQA1*01:04*/DQB1*05:03)
Patient's Antibody signature (among others, moderate to strong):	DQ2 (*DQA1*02:01*/DQB1***02:01**) DQ2 (*DQA1*03:01*/DQB1***02:01**) DQ2 (*DQA1*02:01*/DQB1***02:02**) DQ7 (*DQA1*02:01*/DQB1***03:01**) DQ7 (*DQA1*03:01*/DQB1***03:01**)
Donor	Al, 2; B8, 44; C5, 7; DR1, 17; DR52; **DQ2, 7** (DQB1*02, DQB1*03:01); DPB1*01:01, *04:02
Donor DQ2: *DQA1*05:01*/DQB1*02:01	Negative results in solid-phase assay
Donor DQ7: *DQA1*05:01*/DQB1*03:01	Negative results in solid-phase assay

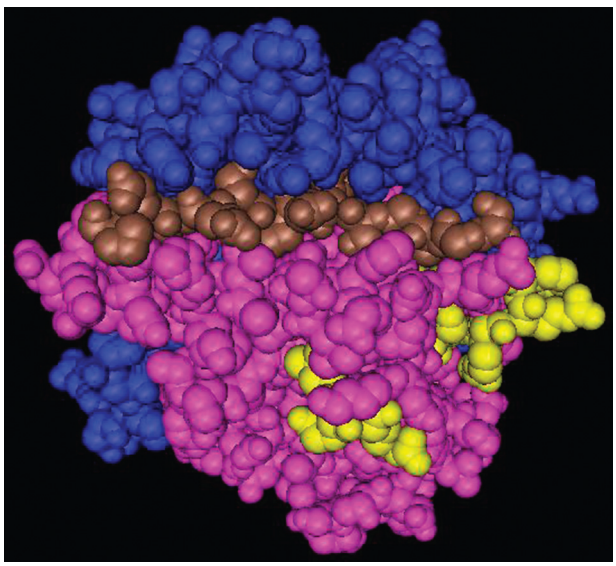

FIGURE 2. A three-dimensional structure of HLA-DQ2 indicating in yellow the epitopes/amino acid sequences that differ between *DQA1*05:01/DQB1*02:01* (against which antibodies were not present) and the other HLA-DQ2 alleles against which SAB were positive. DQα chain is pink, DQβ chain is blue, and the peptide is brown. The yellow amino acids are the epitopes accessible for antibody binding; therefore, changes in these amino acid sequences may lead to differences in antibody recognition. These specific amino acids include GR at positions 40 to 41 and LRQ at positions 51 to 53. HLA, human leukocyte antigen; SAB, single antigen bead.

recognizing the importance of understanding the nature of HLA-DQ (and HLA-DP) antigen–antibody interactions.

The introduction of cPRA as a measure of patients' sensitization level represents a fundamental change in the transplant society's commitment to accessibility and equity in kidney transplantation (*19*). The inception of this concept stemmed from the understanding that different techniques to measure HLA antibodies have different sensitivity and specificity abilities as well as the need to make specific transplant centers accountable for the way they report and use these results in making decisions about candidate eligibility for organs. It has been shown that the use of cPRA and virtual crossmatching resulted in a significant increase in the percentage of broadly sensitized patients that were transplanted as well as a significant reduction in the number of kidney offers that were declined due to an unexpected positive XM (*20, 21*).

Given the information provided in this report, there is a need to reevaluate how we assign cPRA points based on HLA-DQ antibodies. For example, patients with antibodies to only HLA-DQ7 will receive a cPRA of 46% using the existing system. As shown in Table S1 (see **SDC,** http://links.lww.com/TP/A753), currently, reagents are available to identify seven different alleles of the DQ7 antigen. All alleles carry the DQ7-β chain (*DQB1*03:01*) but different DQα chains. For the following exercise, we used data from the National Marrow Donor Program that provides frequency data for HLA-DRB1/HLA-DQB1 alleles in different ethnic populations. For the purpose of this demonstration, we used data specific for the Caucasian population. There are multiple HLA-DR alleles that are in linkage disequilibrium with DQ7. Among the more common ones, there are several DR4 alleles (some of the DRB1*04:01 and most of the *DRB1*04:07* and *04:08) that are usually associated with the *DQA1*03:02/DQB1*03:01* allele of DQ7. On the other hand, most of the DR11, DR12, and some DR13 alleles (*DRB1*11:01, *11:04, *12:01, *13:03, *13:04,* and **13:05*) are associated with a different DQ7 allele, which that is *DQA1*05:01/DQB1*03:01*. The two alleles of DQ7 in this example differ in their DQα chain only. Using the frequency of these DRB1-DQB1 associations from the National Marrow Donor Program, one can estimate that the frequency of the DQ7 allele associated with DR4 (carrying the *DQA1*03:02*) is about one half of the frequency of the DQ7 allele associated with the DR11/12/13s (carrying the *DQA1*05:01*). Therefore, a patient that has antibodies only to *DQA*03:02/DQB1*03:01* allele and not to *DQA1*05:01/DQB1*03:01* allele will have antibodies to only one-third of all DQ7, roughly speaking. This will then translate to a cPRA of only one-third of the 46%. Failing to recognize the specific alleles of the DQ7, and thus failing to assign accurate cPRA accordingly, leads to unfair inflation in cPRA calculations for some of the patients while disadvantaging other patients who carry antibodies against all alleles of the DQ7 (in this example). The same is true for other HLA-DQ specificities. Ultimately, using the accurate cPRA information, patients will be seen as compatible with more potential donors.

Currently, to the best of our knowledge, there is no good source of HLA-DRB1-DQA1-DQB1 association frequencies in the different ethnic groups. Such a database will be very useful to derive the appropriate percent distribution for cPRA calculations. We urge UNOS and the histocompatibility committee to address this issue to incorporate new evidence regarding the manner in which antibodies "view" HLA-DQ molecules and by that improve cPRA definition, leading to higher equity and efficiency in organ allocation.

This new appreciation of the structural relationship between epitopes of the HLA-DQ molecules and antibodies can potentially explain why it was previously accepted that HLA-DQ antibodies are not relevant for transplant outcome, yet many laboratories have noted increased expression of HLA-DQ antibodies in patients with failed allografts. This "conflicting" information may be resolved by verifying whether the so-called DQ DSA was indeed against the donor HLA-DQα/β combinations. For example, a patient with antibodies against HLA-DQ2 (*DQA1*02:01/DQB1*02:01*) should not be considered as having HLA-DQ DSA if the donor DQ2 is *DQA1*05:01/DQB1*02:01*.

In conclusion, a greater appreciation of the antigenic determinants for unique DQα/β combinations is emerging. UNOS is attempting to address this issue by introducing a method to report DQA antibodies separate from DQB antibodies. By accepting the terminology of "DQA antibodies," we accept the misconception that the DQA and the DQB are two separate molecules (targets). Although indeed some of the DQ antibodies recognize structural epitopes that are restricted to DQα amino acids, this is the exception, not the rule, and still both chains form one single target. Thus, the simple entry of the DQA alleles into the UNOS database will not be sufficient to permit clear identification of

unacceptable DQα/β allele combinations. We believe the approach presented in this work has the potential to improve organ allocation efficiency and equity. Larger cohort studies are required to substantiate our data.

MATERIALS AND METHODS

For HLA antibody reactivity testing, new patients listed for kidney transplantation at the Northwestern Comprehensive Transplant Center underwent testing of two consecutive (monthly) serum samples to identify potential presence of HLA antibodies using solid-phase assays. The algorithm includes initial testing by flow PRA. For patients with a positive PRA, the antibody signature is then characterized by the SAB assay. SAB testing alternates between kits from two commercial vendors: One Lambda (Canoga Park, CA) and Gen-Probe, Life Codes (Stamford, CT). Assays are performed according to the manufacturer's recommendations and the presence and strength of antibodies is documented. Generally speaking, MFI value of more than 1000 is considered positive, but exact cutoffs are tailored based on patient-specific information. Using similar criteria, antibodies are considered to be moderate or strong when MFI values are greater than 3000 to 4000 or 10,000 MFI, respectively.

Each patient's antibody signature is captured in HistoTrac (HLA laboratory management software system) and is transmitted electronically each day to the Organ Transplant Tracking Record clinical transplant database. Antibodies are listed according to their relative strength (strong, moderate, or weak), with the complete molecular typing information available for the positive SAB. This approach allows us to capture allele-level antibodies as well as the complete high-resolution HLA-DQα/β and HLA-DPα/β information. Each month, a report of all patients tested is generated and sent to the GOH HLA laboratory indicating which specificities were entered as unacceptable in UNet and which allelic specificities are positive, providing the relative strength for each.

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