

Taking the Mystery Out of the Match: Histocompatibility Testing and Kidney Transplantation

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Understanding human leukocyte antigen typing,
panel reactive antibody determination, and cross-matching
allows clinicians to provide better care for their patients.

Organ transplantation is unique as a surgical specialty because of the surgeon's relationship with the science of histocompatibility. The two have advanced in tandem over the last 50 years—allowing for remarkable improvements in allograft and patient survival.

The immunologic basis of successful solid organ transplantation can be daunting to the medical professional, yet it is essential for those caring for renal transplant patients to be familiar with the principles of histocompatibility testing if they are to understand transplant candidacy and management. Herein, we describe the performance of common histocompatibility tests and their role in the management of the renal transplant recipient. Human leukocyte antigen (HLA) laboratories universally test for donor and recipient HLA (“tissue typing”) and monitor for the presence of specific HLA antibodies in the kidney transplant recipient (before and after transplantation). In this paper, we will describe the following common tests: HLA typing, panel reac-

tive antibody (PRA) determination, cross-matching, and donor-specific antibody monitoring posttransplant.

THE ROLE OF THE HLA LABORATORY

Renal allograft rejection is a serious complication of renal transplantation, which may result in acute or chronic kidney allograft failure. Rejection is the result of immune recognition of the donor kidney by the recipient. Recognition of the foreign (ie, donor) histocompatibility antigens, HLA by the donor immune system, is the major mechanism by which transplant rejection takes place. Antigens shared between the donor and recipient are less likely to be recognized as “non-self,” and historically, the more antigens that are shared between donor and recipient, the better the kidney allograft survival. Although, in the era of modern immunosuppression, lack of close HLA matching before transplant is less likely to result in poor long-term allograft survival, it still is extremely important that the potential recipient not have an existing immune response to the potential donor's HLA. After transplant, recognition of the donor kidney's “foreign” antigens, unless suppressed, will lead to rejection and allograft failure.¹

Initial immune recognition of a donated allograft occurs via the re-

ipient's cellular immune system. Recipient CD4 T-helper cells recognize donor HLA antigens processed and presented by recipient antigen-presenting cells. As the rejection process proceeds, antibodies are made against donor HLA antigens. Thus, a previous transplantation with subsequent rejection can result in the presence of antibodies against future donor HLA. This is called *sensitization*. Patients can also be sensitized when they manufacture anti-HLA antibodies against tissue antigens “seen” as the result of blood product transfusion or pregnancy.² It is vital to avoid placing a kidney allograft in a recipient previously sensitized to the donor HLA.

Donor-specific antibody (ie, antibody made in the recipient directed against the donor's HLA) binds to allograft tissue and fixes complement, with resultant destruction of the kidney. This is called *antibody-mediated rejection*. The spectrum of antibody-mediated rejection may range from hyperacute (immediate) to acute (days to weeks) to chronic (months to years). In the ideal setting, careful attention to clinical parameters, HLA testing, and proper use of immunosuppressive drugs can forestall the immune response to the transplant allograft, allowing the kidney to function maximally during the lifetime of the recipient. Thus, determination of donor-recipient HLA and monitoring

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for donor-specific antibodies are the mainstay of HLA laboratory activities, regardless of the specific techniques used.

HLA TESTING: THE COMPLEMENT-DEPENDENT CYTOTOXICITY (CDC) METHOD

The most common serologic method for HLA testing is complement-dependent cytotoxicity (CDC) testing technique. Serum, which contains antibodies, is applied to lymphocytes, which have HLA antigens on their surface. If there is a specific anti-HLA antibody in the applied serum, it will bind to the HLA antigen on the lymphocyte cell, with subsequent complement activation and perforation of the lymphocyte cell membrane. This binding allows entrance of a vital dye into the damaged lymphocyte, staining the attacked cell. Cell death can then be assessed visually by microscopy.

The CDC test can be applied to many different situations. Recipient serum can be used to test for the presence of anti-HLA antibodies directed against the lymphocytes of a panel of random lymphocyte donors or against a specific potential kidney donor. Serum, with known anti-HLA antibodies, can be applied to the lymphocytes of the recipient, to “serologically” determine the recipient’s HLA antigens. The same can be done to determine the HLA antigens of a potential kidney donor.

For some variations of the CDC test, specific donor or recipient lymphocytes must be obtained. Adequate quantities of T and B lymphocytes must be separated from whole blood using antibody-coated magnetic beads (Figure 1). In a living donor or recipient, the most readily available source is peripheral blood collected in tubes containing acid citrate dextrose. This solution preserves lymphocyte cells in whole blood for up to 72 hours, while also acting as an anticoagulant. Lympho-

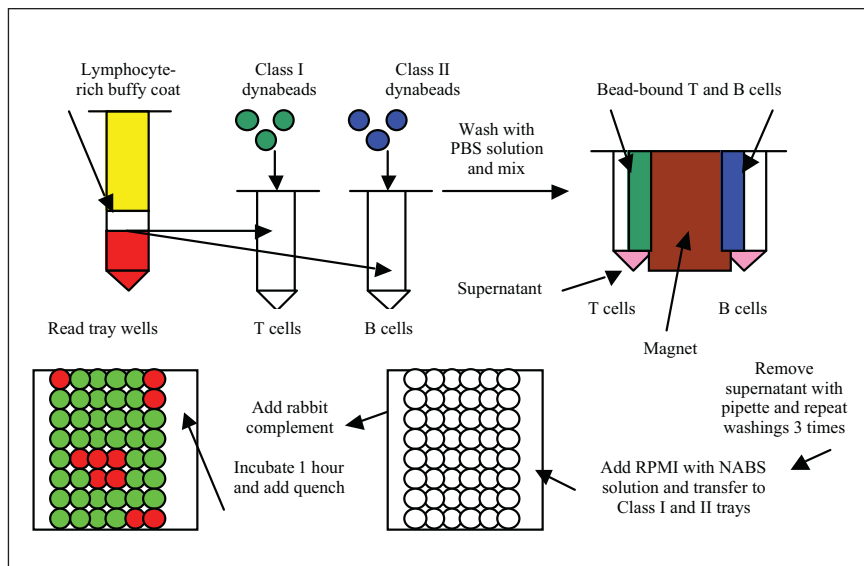


Figure 1. Complement dependent cytotoxicity with magnetic beads.

NABS = Normal group AB “unsensitized” male serum (negative control serum); PBS = phosphate-buffered saline.

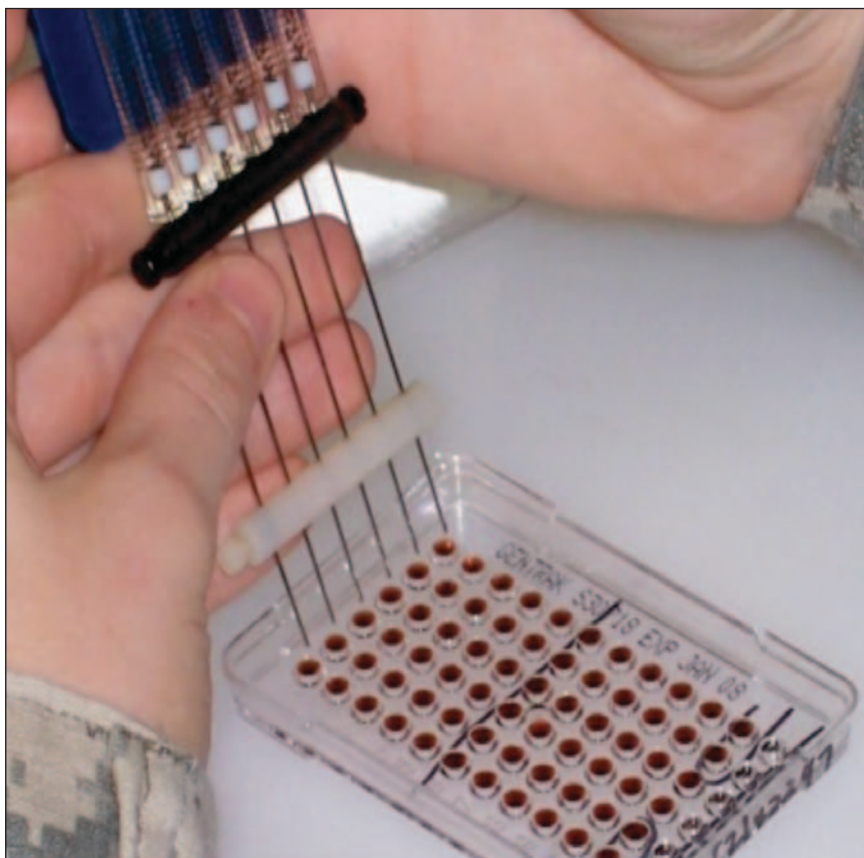


Figure 2. Adding vital stain (quench) to the Terasaki plate.

cytes may also be obtained from lymph nodes or splenic tissue provided at the time of cadaveric donation.

After centrifugation of whole

blood and isolation of the monocyte layer using Ficoll density gradient, separation of lymphocytes into T- and B-cell components is required.



Figure 3: Reading HLA trays with a fluorescent microscope.

HLA Class I antigens (A, B, and C loci) are identified using T cells, and HLA Class II antigens (D/Dr locus) are identified using B cells. The use of antibody-coated magnetic beads is an attractive method for separating T and B lymphocytes, because it is a relatively quick, easy, and inexpensive procedure with high selectivity. Magnetic beads are coated with specific antibodies against Class I and Class II HLA antigens, found on T and B lymphocytes, respectively. The beads are mixed with the lymphocyte cell suspension and a magnet is placed alongside the tube in order to draw the magnetized B or T lymphocytes to the sidewall. After washing, the desired cells are tightly adherent to the sidewall of the tube, against the magnet, retaining lymphocytes while disposing of unwanted platelets, red cells, and other cellular debris.

HLA TYPING

Although HLA A, B, C, and D/Dr antigens can be determined serologically,

only HLA A, B, and Dr antigens are “matched” for purposes of kidney transplantation. Each person inherits 1 “set” of HLA genes from each parent, called a *haplotype*. For the purposes of kidney transplantation, each patient has 6 possible HLA antigens: 2 HLA-A, 2 HLA-B, and 2 HLA-Dr. The recipient and donor HLA are compared at these antigen sites, with a possible “mismatch” of 6 antigens. The optimal situation is for the recipient to “see” an identical HLA on the donor kidney, which in theory would preclude antibody formation to these 6 significant antigens. In situations where the donor is a full sibling, a 2-haplotype “match” occurs 25% of the time. Because of the great variety of HLA antigens in the population, a “no-antigen-mismatch” kidney from an unrelated donor rarely occurs. However, in practice a kidney transplant where the recipient does not have existing antibodies to the donor’s HLA antigens and who shares a compatible blood type with the donor is

clinically acceptable. Historically, the better matched the 2 sets of antigens, the better the immune tolerance to the allograft.

A variation of the CDC test is used to determine which HLA antigens are present on the cells of the donor or the recipient. Lymphocytes are isolated by B- or T-cell type and mixed with serum containing antibodies to known HLA antigens in the wells of a Terasaki plate (Figure 2). The T-cells suspension is plated onto HLA Class I trays (A, B, and C), and mixed with antiserum to known Class I antigens. The B-cell suspension is plated onto HLA Class II trays (Dr, Dp, and Dq) and mixed with antiserum to known Class II antigens. Complement is then added to each well, which attacks cells whose antigens are bound with specific anti-HLA antibodies. A fluorescent dye mixture, called *quench*, is then added to each well, which identifies dead cells (acridine orange turns cells red) vs intact live cells (ethidium bromide turns cells green) (Figures 1 and 2). The microscopist is now able to determine with fluorescent microscopy the ratio of live to dead cells in each well, and because the specificity of the antiserum is known, the HLA antigens present on the donor or recipient cells (Figures 3 and 4). Through this process, hundreds of antibody/antigen interactions are run simultaneously, each with different combinations of HLA antiserum in order to determine all 6 donor or recipient HLA antigens (Figure 4).

PANEL REACTIVE ANTIBODY

While the organ transplant candidate waits for a donor kidney to become available, the PRA is performed monthly or after a blood transfusion or other event that could result in sensitization to foreign HLA. In this test, the potential recipient’s serum is screened to detect the presence or development of anti-HLA

antibodies. The CDC test is used, as described earlier, except the serum is taken from the potential kidney transplant recipient, and the lymphocytes are from a standardized population sample set of HLA antigens. If the recipient makes a new antibody against a known, foreign HLA, it can guide the future selection of a donated kidney. A recipient who has a known antibody against the HLA of a donor kidney would be excluded from receiving that kidney.

PRA is calculated by taking the total number of “positive” wells on the Terasaki plate divided by the total number of wells. Both HLA Class I and Class II plates are run. A positive interaction between the recipient’s serum and the panel lymphocytes is defined as a > 20% cell lysis rate in the well or a score ≥ 4 (Table 1). This number gives an indication of the potential recipient’s overall sensitization to HLA antigens found in the general population and is reported with reference to the historical high. The higher the PRA, the lower the chances that a donated organ will be immunologically compatible with the recipient and the lower the chances of successful transplantation.

Recipients with high PRAs at the time of transplantation have a greater risk of graft rejection.^{1,2} At the time of transplantation, the recipient needs to have a negative current serum cross-match to donor antigens and ideally a negative cross-match to donor antigens with serum saved from their highest historical PRA. Testing the serum from the highest historical PRA screens for HLA antibodies that may have “disappeared” over time from the serum, but for which an immunologic memory response is likely to occur if the recipient is challenged with the HLA antigen again. If these conditions are met, the transplant can occur. Patients with elevated PRA are at a distinct statistical disadvantage in re-

01A	POSITIVE CTRL	05A	A25.A26.A34.A6601.A6602	09A	B51
01B	NEGATIVE CTRL	05B	A26	09B	B51.B78
01C	A1	05C	A26	09C	B51.B52.B78
01D	A1	05D	A26.A34.A6601.A11E	09D	B51.B52.B78
01E	A1.A36	05E	A34	09E	B51.B52
01F	A1.A36	05F	A6602	09F	B7.B73.B81
02F	A2	06F	A11.A25.A26.A6601	10F	B7.B81
02E	A2.A69	06E	A11.A6601:SM	10E	B7.B81
02D	A2.A69.B51	06D	A11	10D	B7.B42.B81
02C	A2.A28	06C	A11	10C	B42
02B	A28	06B	A10.A29.A31.A32.A33.A74.B	10B	B8
02A	A28	06A	A10.A28.A29.A31.A32.A33.A	10A	B8
03A	A3	07A	A29	11A	B8.B59
03B	A3	07B	A29	11B	B8
03C	A3	07C	A30	11C	B44
03D	A3.A11E	07D	A30	11D	B44
03E	A23	07E	A30.A31	11E	B44.B45
03F	A23	07F	A31	11F	B45.B50
04F	A23.A24	08F	A32	12F	B45
04E	A23.A24	08E	A32.A25	12E	B13
04D	A24	08D	A33	12D	B13
04C	A24	08C	A33.A34.A29:E.B11E.A6602	12C	B13.B27.B47
04B	A25	08B	A80	12B	B13.B47.B60.B61.B41.A2
04A	A25	08A	A80	12A	B48.B60.B7E.B81

Figure 4. Typical Class I tray results.

ceiving a donated organ given the limited donor pool of available organs. To account for this, the United Network of Organ Sharing, which is responsible for organ allocation and distribution in the U.S., provides additional points for priority in this subset of patients.

THE CROSS-MATCH

Immediately before any kidney transplantation, a final “cross-match” is performed between the donor and recipient to rule out the existence of donor-specific anti-HLA antibodies in the serum of the recipient. For cadaveric donors, the local organ procurement organization (OPO) offers a potential donor kidney to multiple recipient(s). The OPO provides information about the donor’s medical history, physical condition, pertinent radiologic, pathologic, and serologic testing, and donor-recipient HLA matching. The surgeon then determines whether the recipient is medically fit to undergo transplantation. The highest priority recipient is called to the hospital to facilitate admission, preoperative testing, and the final cross-match. The final cross-match allows assessment of the potential recipient’s immunologic risk of acutely rejecting the transplant kidney.

The recipient’s serum is mixed

Table 1: Standard CDC reaction scores	
Cell death (%)	Score
0-10	1
11-20	2
21-50	4
51-80	6
81-100	8

with B and T cells from the donor, establishing whether the recipient has specific-anti HLA antibody to the donor tissue. The source of donor lymphocytes is either peripheral blood or splenic and lymph node tissue. Lymphocytes are prepared, and B and T cells isolated in the manner already described. Additional blood is also sent for ABO confirmation. Donor T and B cells are incubated with recipient serum obtained within the past 30 days in the presence of complement. If there are specific anti-donor-HLA antibodies present in the recipient’s serum, complement will be fixed, and cell lysis or death will occur. This is a positive cross-match. The absence of dead cells indicates the opposite—a negative cross-match.

If the cross-match is negative, the surgeon proceeds with the transplant.

OTHER METHODS (FLOW CYTOMETRY)

CDC methods are sensitive and specific for the detection of anti-HLA antibodies, but there are circumstances where CDC test results can be inconclusive. Flow cytometry has been described as the most sensitive technique for the detection of HLA-specific antibodies and cross-match testing.³⁻⁵ By using single HLA antigen-coated beads, the laboratory can detect the presence of antibody against a single HLA antigen instead of analyzing mixed serum reactions against cells containing multiple antigens. In flow cytometric methods, recipient serum is mixed with commercial single HLA antigen-coated beads, which bind the antibody. Antihuman immunoglobulin G (IgG) antibody conjugated to phycoerythrin is then added to the beads. It binds to any human anti-HLA antibody bound to the beads, and phycoerythrin fluorescence is used to signal the presence of the anti-HLA antibodies. The intensity of the fluorescence from each bead is measured against a negative control, eliminating the need for operator interpretation. Flow cytometry detection of anti-HLA antibodies is a much simpler process than the deductive reasoning needed to compare multiple reactions with multiple antigens per cell (as on a Terasaki plate), any of which can be responsible for a positive reaction (Figure 4). However, flow cytometry is more expensive, time consuming, and requires technically more complex instrumentation than the CDC method. The sensitivity and specificity gained with flow cytometry allows for a more accurate “virtual cross-match” using the recipient’s stored serum before the organ and the patient are brought to the transplant center. This avoids unnecessary organ

shipping, added cold ischemia time, and patient inconvenience should the cross-match be positive. The increased specificity of flow cytometry correlates with an assured negative cross-match, although the final CDC cross-match is always performed.

POSTOPERATIVE MONITORING

The HLA laboratory also plays a significant role in assisting in the post-transplant care of the transplant recipient. Recipient serum is monitored for the development of donor-specific antibodies. These are antibodies produced specifically against donor HLA, which may not have been present at the final cross-match, or if present, were not detectable at that time. Anti-HLA antibodies, when present, can cause antibody-mediated (humoral) rejection, which is verified by the presence of increased donor-specific antibody, kidney allograft biopsy findings consistent with antibody-mediated rejection, and C4d deposition (a marker for complement pathway activation) found on allograft biopsy. Humoral rejection is caused by anti-HLA antibodies (alloantibodies) directed against the transplant kidney, which activate the inflammatory cytokine cascade to attack the graft’s vascular endothelium. This may or may not overlap with the more traditional notion of rejection caused by lymphocytic infiltration of the graft or cellular rejection. Humoral rejection can be a more subtle diagnosis and requires clinicians to be vigilant to an elevated or serially rising donor-specific antibody level. In addition to clinical triggers such as an elevated serum creatinine or new-onset proteinuria, many centers incorporate regular donor-specific antibody testing into their surveillance practice. Humoral rejection responds best to treatments such as IgG infusion or plasmapheresis and can be differentiated from cellular rejection by the absence of donor-specific antibody and C4d deposition.⁶

CONCLUSION

HLA typing, PRA determination, and cross-matching has led to increased renal allograft survival. Understanding the basic immunologic methods and the rationale behind these tests allows clinicians, caring for kidney transplant recipients and those awaiting transplant, to better care for their patients. ●

Author disclosures

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