Recommended Histocompatibility Guidelines

The following guidelines are the consensus of the UNOS Histocompatibility Committee regarding practices that should be required in laboratories supporting clinical transplantation. They are intended to encourage consistent standards of practice among histocompatibility labs. The Committee believes that implementation of these guidelines is vital to ensure expedient placement of donor organs and to minimize organ wastage. These guidelines are meant as recommendations only and are not intended to be standards at this time.

Background and Significance
HRSA has charged the OPTN/UNOS to significantly decrease organ cold ischemic time and concomitant organ wastage; and, significantly reduce the number of deaths of highly sensitized patients waiting for transplantable organs during this contract period. Achieving these two goals requires a complete review of all organ allocation algorithms and changing them, when necessary. A significant contributor to increased cold time and management of the sensitized patient is the histocompatibility portion of the allocation algorithms. The use of regional organ placement trays (ROP trays, high reactor trays) to screen highly sensitized patients and the inconsistent method by which HLA-specific antibodies are identified in these patients play a major role in the inefficient dispersing of organs to patients.

The executive leadership of the OPTN/UNOS has challenged the histocompatibility community to develop protocols and strategies that would lead to more uniform antibody characterization. Such protocols would obviate ROP trays in favor of a more standardized approach to identifying unacceptable antigens in the sensitized patient. This would, in turn, improve organ allocation by ensuring the high probability of a negative crossmatch when an organ was shipped for a specific recipient. In essence, allocation would be made as a result of a prediction of a high probability of successful organ placement via a “virtual crossmatch” based upon a more uniform listing of unacceptable antigens for potential recipients.

Technological advances now permit a level of evaluation of histocompatibility antigens/antibodies between recipients and donors that surpasses what was achievable with older techniques, making this concept of a “virtual crossmatch” more feasible than it ever has been.

The following guidelines reflect the consensus of the OPTN/UNOS Histocompatibility Committee regarding state-of-the-art practices that will serve the best interest of patients and help the OPTN/UNOS achieve its goals of expediting organ placement and minimizing organ wastage. It is the Committee’s intent that these guidelines not only serve as an attempt to provide some initial thoughts on how the scientific community can help resolve these two critical issues in organ placement, but also to make the histocompatibility community aware that it has been urged by our clinical colleagues to develop a system of identifying acceptable and unacceptable antigens that will become a standard of practice.

Proposed Best Practice Guidelines for OPTN/UNOS Histocompatibility Laboratories
The responsibility of the histocompatibility laboratory is to provide an evaluation of histocompatibility data and pertinent patient immunologic risk factors that will allow the clinician and patient to decide which approaches to transplantation are in the patient’s best interest, such as standard criteria donor (SCD), expanded criteria donor (ECD) or donation after cardiac death (DCD) donor wait lists, desensitization, or potential paired donation exchanges. In addition to accurate HLA typing and evaluation, this information may include the following from patient history and histocompatibility test results:

The Histocompatibility Committee recognizes that the entire patient history is not always available and recommends that the laboratory make every effort to work with clinicians and healthcare providers to secure as accurate a sensitization record as possible.

The patient’s level of immunologic risk for transplantation, defined by:

a. Sensitization history
b. Detection and characterization of HLA specific antibodies
c. The titers of the various HLA-specific antibodies, as appropriate
d. Repeat mismatches
e. Aggressiveness of response to previous transplant
f. Numbers of pregnancies and age of youngest child

g. Antibody trend - decreasing or increasing
h. Donor relationship - husband to wife or child to mother

An assessment of the above factors should provide a list of HLA antigens that would be unacceptable in a donor.

The only new practice for some laboratories will be the listing of unacceptable HLA antigens defined by historic or current presence of HLA specific antibodies. Such definition is now routinely performed in many labs, but unacceptable antigens are not always listed for allocation purposes.

The Committee recognizes that the determination of PRA is a basic component of the current allocation system. However, current technology permits more sensitive detection and identification of HLA specific antibodies. Consequently, it is the consensus of this Committee that detection, identification, and listing of unacceptable antigens will contribute to more equitable organ allocation.

1. UNOS Policy 3.5.11.3, approved by the OPTN/UNOS Board of Directors at their June 2005 meeting, requires listing of unacceptable antigens for patients to be eligible for receipt of additional points for PRA of 80 or greater. This proposal will go into effect when the necessary computer programming can be accomplished within the latter part of 2006. The unacceptable antigens may be defined by identification and characterization of HLA-specific antibodies and, as appropriate for individual transplant center protocols, by other criteria that would be unacceptable in a donor, such as repeat mismatches, or exposure through pregnancy.

2. Monthly serum samples should be obtained for all patients on the renal OPTN wait list and it is recommended that similar samples be obtained from patients waiting for other solid organ transplants. It is highly recommended that sera from known sensitized patients be tested for identification of anti-HLA specific antibodies at least quarterly to detect any changes in antibody levels or specificity.

3. Antibody screening should be performed following notification of a potentially sensitizing event. In addition to transfusion, transplantation and pregnancy, such events may include trauma, infection, vaccination, or any condition that provokes an inflammatory response.

4. If a change in antibody titer and/or specificity is detected, the patient's listing of antibody defined, unacceptable antigens should be updated in UNET.

Guidelines for Characterization of Patient Sensitization
Current technology is available to permit thorough antibody characterization and recommended methods are listed below.

A. Methods for HLA Typing, Detection of anti-HLA Antibodies, and Crossmatching

1. HLA typing

All laboratories supporting solid organ transplantation must be CLIA certified/UNOS approved and must type for A, B, Bw4, Bw6 and DR antigens as defined as listed in the current policy appendix (3A), HLA Antigen Values and Split Equivalences, to the UNOS Policy 3: Organ Distribution. It is highly recommended that typing also include HLA-Cw, DRw51, 52, 53 and DQ antigens. For HLA-Cw, DRw51, 52, 53, and DQ, at least the serologically-defined antigens should be identified. This level of typing is required to maximize the probability of a negative crossmatch for donor organs used locally or shared regionally or nationally since transplant centers differ in their protocols for listing unacceptable antigens.

- Acceptable methods for typing include any that meet UNOS Standards. All ambiguous antigen assignments must be resolved by additional testing.
- The HLA phenotype of shared donors must be verified by repeat typing in the recipient center laboratory.

2. Antibody Screening and Identification

Laboratories must use a <u>combination of techniques</u> to meet the following conditions:

1. Test sensitivity sufficient to detect low levels of existing antibody <u>against HLA class I and II antigens.</u> The OPTN/UNOS Histocompatibility Committee recognizes that antibody identification is improved through the use of solid phase immunoassays, and therefore highly <u>recommends that at least one solid phase immunoassay be used</u> for antibody screening and antibody identification<u>.</u>

2. Determination of HLA specificity and IgG and IgM isotype, when relevant to crossmatch interpretation.

3. Ability to identify when antibodies are not HLA specific and, therefore, may not be detrimental to a transplant, e.g. auto-antibodies.

4. Monitoring for changes in antibody levels at least quarterly and periodic identification of antibody specificity must be performed for patients active on the wait list.

Methods: Test methods may vary, depending upon individual transplant center protocols, but the techniques used for antibody screening and identification MUST be at least as sensitive as the crossmatch technique used as the criterion for transplant eligibility. Furthermore, antibody detection methods should be routinely validated by correlation with crossmatch results.

Acceptable methods include:

1. Complement Dependent Cytotoxicity (CDC) An enhanced sensitivity assay, e.g., Antiglobulin or three wash, must be used. Heat inactivation or treatment with a reducing agent such as dithiothreitol should be used to detect the presence of IgM antibodies.

2. Flow cytometric crossmatches with appropriate donor or surrogate target cells.

3. Solid phase immunoassays using soluble HLA antigens. Antigens employed may include: pooled, soluble antigens from multiple phenotypes for screening purposes; soluble antigens from single full HLA phenotypes, or single HLA antigens. No one solid phase immunoassay can provide a complete identification of all HLA-specific antibodies and laboratories should employ a combination of assays. Methods may include ELISA based assays or micro-particle based flow cytometric or suspension array assays.

Samples used for listing of antibody defined, unacceptable antigens:
Because individual transplant center protocols may vary with regard to the emphasis given to historic versus current sensitization, the OPTN/UNOS Histocompatibility Committee does not think it is appropriate to dictate the use of current over historic samples. However, it is imperative that the listing of antibody defined, unacceptable antigens be consistent with crossmatch practices. Therefore, if historic definition of unacceptable antigens is used for listing, appropriate historic sera must be used for final crossmatch decisions.

3. Crossmatch Techniques

The crossmatch is the test of the recipient's serum against the donor antigens. This becomes particularly important considering that it may not be possible to define all antibody specificities for some patients. The techniques used for crossmatching may vary depending on a transplant center's protocols, but, as noted above, must be as sensitive as the techniques used for antibody identification. Techniques may include:

- Complement Dependent Cytotoxicity (CDC) with or without modifications
- Flow cytometry
- If antibodies to both Class I and Class II antigens have been listed than both T and B lymphocyte crossmatches must be performed.

Solid phase capture crossmatch assays using solubilized donor antigens may be used as an adjunct to the above methods, but as these methods have not yet been extensively validated, they should not be used as the only crossmatch technique.

4. Any of the above and/or new methods may be used provided that they have been thoroughly validated by the laboratory and shown to be clinically relevant.

5. Referral Laboratories

For listing of unacceptable antigens, in lieu of implementing any of the recommended solid phase immunoassays, samples may be sent to a referral laboratory provided that the laboratory is CLIA certified/UNOS approved and has both validated and documented expertise in the appropriate test methods.

B. Quality Assurance:

1. Recipient center histocompatibility laboratories should be responsible for determining the cause of any discrepant typing results and/or unexpected positive crossmatches. If an unexpected positive crossmatch is found to result from the presence of HLA-specific antibody, the recipient laboratory must update the unacceptable antigen list for that patient. If the crossmatch cannot be accounted for by HLA specific antibody, the laboratory should determine the nature of the non-HLA specific reactivity and take steps to prevent any future false-positive crossmatches.

   Appropriate analyses and precautions may include:

   - Repeating crossmatch for verification (surrogate cells can be used if necessary)
   - Re-screening and/or re-analysis of anti-HLA antibodies in the recipient crossmatch sample
   - Recipient auto-crossmatch
   - Autologous absorption of recipient sera prior to crossmatching

2. The OPTN/UNOS Histocompatibility Committee will monitor and review the incidence of discrepancies, repeated instances of positive crossmatches, cases where a planned transplant was aborted, and other relevant quality assurance data. As needed, the Committee may recommend appropriate policy or practice changes.