University of Chicago Medicine
Transplant Immunology and Immunogenetics Laboratory

HLA Testing Agreement with the Lung Transplant Program

This agreement adheres to the United Network for Organ Sharing (UNOS) guidelines under Appendix C, Section C.2.C and the American Society for Histocompatibility and Immunogenetics (ASHI) guideline D.2.5.1 (guidance copied from the OPTN bylaws), College of American Pathologists (CAP) guidelines, and the OPTN bylaws.

1. Sample requirements
   - HLA typing:
     i. 3 yellow top tubes (ACD-Solution A)
     ii. Specimens are submitted to the Transplant Immunology and Immunogenetics Laboratory (TIIL) at the time of initial work-up
   - Antibody testing:
     i. 2 red top tubes (serum)
     ii. Specimens are submitted to the TIIL at the time of initial work-up. The transplant program is encouraged to send samples at least every three months after initial work-up and UNet® listing
   - Allo-crossmatch:
     i. Recipient: 1 red top tube
     ii. Donor: 3 yellow top tubes (ACD-Solution A), spleen, and/or lymph nodes stored in appropriate medium as well as 1 red top tube.
   - Auto-crossmatch:
     i. Recipient: 3 yellow top tubes (ACD-Solution A) and 1 red top tube

2. HLA loci and level of resolution typing: HLA high- or low-resolution molecular typing for HLA-A, B, C, DRB1, DRB3/4/5 (DR52/53/51), and DQB1 is performed.
   - Additional testing for the following loci is routinely performed: DPB1, DPA1, and DQA1.
   - If typing for DPB1, DPA1, and DQA1 are not obtained, these loci will not be repeated or reported, unless the recipient has antibodies to these loci and/or typing is otherwise clinically relevant.
   - If STAT typing is requested, HLA-A, B, C, DRB1, DRB3/4/5 (DR52/53/51), and DQB1 will be performed at low-resolution.
   - If not already performed, HLA-DPB1, DPA1 and/or DQA1 typing is performed on recipients and donors when recipient antibodies are detected to these loci.

3. Starting on 02-04-2019, for patients listed in UNet® waitlist with serological HLA typing, HLA molecular typing could be performed by the TIIL in the following circumstances:
   - To determine antigen split typing, in cases where only broad antigen typing was performed
   - When HLA antibodies are identified to loci not typed by the external HLA laboratory
   - When potential HLA antibodies are identified to a self HLA antigens
   - Or if requested by the transplant program

4. To request extended HLA typing: the transplant program should send a request to the Transplant Immunology and Immunogenetics Laboratory (TIIL) by email at #HLA-TIILLab@uchospitals.edu or by pager #8722.

02-21-2019
5. **A. Process for reporting HLA typing results to the OPTN Contractor:**
   - All new solid organ recipients are initially entered into the UNOS system by the nurse coordinators or other members of the transplant team.
   - The laboratory receives updates of all new patients entered into UNOS via the Heart-Lung Active Patient List distributed by the thoracic transplant team.
   - Upon receiving notification of a new UNOS listing, laboratory staff updates patient HLA typing in UNET and if the patient is sensitized, enter unacceptable antigens (see below).
   - All UNOS transactions are reviewed by two technologists for accuracy

**B. Process for reporting HLA testing results to Gift of Hope (GOH):**
   - HLA testing results are reported to GOH-HLA laboratory electronically on a monthly basis and by fax on a case by case basis as needed.
   - Each time there is a change in the reporting system; the TIIL verifies for accuracy of transferring of results and document this process.

*It is recommended that verification of accuracy in transferring of results by GOH-HLA laboratory should be included in the agreement between the Transplant Program and GOH-HLA laboratory.*

6. **Process for resolving HLA typing discrepancies and errors:** When a typing result is found to be different from that reported by another Histocompatibility testing laboratory, the following steps are taken:
   - The TIIL reviews typing, and if necessary, typing will be repeated using re-extracted DNA. This decision will be made for each case individually by the manager or director.
   - After testing has been reviewed, the other HLA laboratory is contacted to discuss the discrepancy and compare results to resolve the discrepancy.
   - This information is documented on the Quality Assurance: Corrective Action form with the appropriate documentation by the TIIL.

7. **Turnaround time from receipt of sample to reporting of results to the transplant program:**
   - HLA typing (high resolution): 7 business days (BD)
   - HLA typing (low resolution)
     - Routine: 4 BD
     - STAT: 1-2 BD
     - Same-day STAT: End of day, if sample received in lab before 9am
   - Antibody testing
     - Routine: 7 BD
     - STAT: 1-2 BD
     - Same-day STAT: End of day, if sample received in lab before 11am
   - Flow Crossmatch
     - Routine: 4 BD
     - STAT: 1 BD
     - Same-day STAT: End of day, if sample(s) received in lab before 11am

**NOTES:**
- For STAT testing, the transplant program should send a request to the TIIL by email at #HLA-TIILLab@uchospitals.edu or by pager #8722.

02-21-2019
8. **Process to obtain sensitization history for each patient:** The TX program is encouraged to provide the TIIL with sensitization history for each patient at the time of initial work-up and/or when a known immunization event (blood transfusions, pregnancy, or transplant) has occurred by:
   - Filling out the question/comment section associated with orders transmitted through Epic
   - E-mail or phone, when applicable
   - Completing a “Information of Potential Interfering factors for HLA antibody testing” form [Appendix A]

9. **Frequency of periodic sample collection:** Following the initial work-up, and once added to the UNOS waitlist, specimens are collected from patients as described above, and submitted to the TIIL. The program is encouraged to send samples at least every three months after initial work-up and UNET listing.

10. **Frequency of pre-transplant antibody screenings:**
   - The TX program is encouraged to send serum samples from recipients on the UNOS wait list at least every three months for testing using solid-phase assays. Recipients with PRA 1% or greater are tested using single antigen beads (SAB) at least once a year.
   - In cases of major changes in antibody status between samples, the TIIL may request an additional specimen(s) to confirm results.
   - The transplant program is encouraged to send new samples 14 days after any potential immunizing event: blood transfusion, pregnancy, or transplant to document the sensitizing event as well as to re-test patient sensitization status.

11. **A. Assay format for antibody screening:** HLA class I and class II antibody testing is performed by solid-phase assays. Based on antibody profile, antibody titers may be performed.

   **B. Assay format for peri-transplant crossmatching:**
   - Prospective crossmatches are performed by Gift of Hope HLA laboratory.
   - Retrospective crossmatches are performed using the flow cytometric assay.
   - Based on antibody profile, flow crossmatching may be performed on multiple serum dates with titers.
   - HIV-positive patients treated with antiretroviral drugs are crossmatched against pronase-treated and non-treated donor cells.
   - If specimens are available, recipient and donor auto-crossmatches are performed to test for presence of autoantibodies or when specimens have high background on the solid-phase antibody tests.
   - If flow cytometric crossmatch is positive in the absence of any detected donor specific antibodies (DSA), recent recipient and donor blood transfusions, medications and other interfering substances should be investigated. In order to facilitate test interpretation, the TX program is encouraged to send a completed “Information of Potential Interfering factors for HLA antibody testing” form [Appendix A] to the TIIL at the time of crossmatch and/or antibody test ordering.
   - If requested by the TX program, a virtual antibody assessment of a donor will be performed.
   - Due to low level of sensitivity and specificity, complement-dependent cytotoxicity (CDC) crossmatch testing has been discontinued by the TIIL, but may be outsourced if necessary.

12. **Criteria for determining and listing unacceptable antigens used during organ allocation:**

   02-21-2019
• Strong antibodies (antibodies with MFI values predicted to yield a positive flow-cytometric crossmatch) present in current patient serum.
• Current or previously detected antibodies which are not classified as strong but represent repeated mismatched antigen(s) from previous transplant(s).
• Current or previously detected antibodies of any strength which have caused a positive crossmatch when performed by the TIIL only.
• Current or previously detected antibodies of any strength which have caused a positive crossmatch when performed by GOH will not be listed as unacceptable antigens.
• In some cases, due to medical reasons, antigens corresponding to moderate and weak HLA antibodies present in patient serum, could also be listed in UNet as unacceptable.

UNET updates are performed by the TIIL.

Final algorithm for deceased donor selection should be established in the agreement between the Transplant Program and GOH-HLA laboratory.

13. Duration for which specimens need to be stored for repeat or future testing:
• Serum specimens collected from recipients are stored frozen in the TIIL for a minimum of 6 months.
• If possible, serum specimens collected from deceased donors are stored frozen in the TIIL indefinitely.
• Isolated cells collected from deceased donors are stored for a minimum of one year. Storage beyond one year may not provide viable cells for future crossmatching.
• If possible, buffy coats and DNA specimens collected from recipients and donors (living and deceased) are stored frozen in the TIIL for a minimum of 5 years.

Duration of deceased donor specimen storage should be established in the agreement between the Transplant Program and Gift of Hope HLA laboratory.

14. Protocol for monitoring antibody levels pre and post-desensitization treatment: This is determined on a case by case basis between the Transplant Program and the TIIL.

15. Criteria for crossmatching:
• Retrospective T-cell and B-cell flow crossmatch testing is performed by the TIIL if cadaveric donor samples are available. (Please note GOH HLA laboratory does not perform long-term storage of donor cells.)
• For unexpected crossmatch results, based on patient HLA antibody status and donor HLA type, the TIIL will request information on recent donor transfusion events, by providing the program with “Request for information of potential interfering factors for HLA antibody testing” form [Appendix A].

Prospective crossmatches against deceased donors are performed and interpreted by GOH-HLA laboratory. The Transplant Program should establish crossmatch assay format with the OPO to ensure appropriate testing for patients on the UNOS waitlist. Patient’s status of HIV infection needs to be communicated to GOH so that appropriate crossmatches can be proactively performed. It is recommended that the Transplant Program requests the OPO perform prospective crossmatches with and without pronase treatment for patients with 0% PRA and a history of false positive crossmatches.

02-21-2019
16. **Process for blood type verification according to Policy 3.1.4: Waiting List:** The TILL does not register candidates for the Transplant Program, nor performs ABO typing. Blood type verification is performed by the Transplant Program.

17. **Protocol for post-transplant monitoring of antibody levels:**
   Class I and class II Donor Specific Antibody (DSA) identification and strength testing is performed using solid-phase assays.
   - C1q testing is performed for all patients with DSA.
   - Based on antibody profile (MFI ≥20,000), antibody titers (1:10 serum dilution or other dilutions as needed) may be performed.

   Antibody testing may be performed at the following times:
   - When acute allograft rejection is suspected.
   - If immunosuppressive therapy changes (e.g., treatment of rejection episodes, desensitization therapy).
   - At the time of graft biopsy and C4d staining.
   - Per request of the program.

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Appendix A

Information of potential interfering factors for HLA antibody testing

<table>
<thead>
<tr>
<th>Patient Name:</th>
<th>MRN:</th>
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<tbody>
<tr>
<td>Completed by:</td>
<td>Date:</td>
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PRE-TRANSPLANT and/or CROSSMATCH TESTING

**RECIPIENT**

Infections
- Human Immunodeficiency Virus (HIV) YES NO
- Hepatitis C Virus (HCV) YES NO

Autoimmune diseases
- Systemic lupus erythematosus (SLE) YES NO
- Type 1 diabetes mellitus (Type 1 DM) YES NO
- Other (Sjogren’s, etc.) YES NO

Blood transfusion(s) in the last 7 days YES NO

Treatments
- Rituxan (rituximab) YES NO
- Antithymocyte globulin (ATG) YES NO
- Alemtuzumab (campath) YES NO
- Intravenous immunoglobulin (IVIG) YES NO

DONOR

Blood transfusion(s) in the last 7 days YES NO

POST-TRANSPLANT SOLID-PHASE ANTIBODY TESTING

**RECIPIENT**

Infections
- Human Immunodeficiency Virus (HIV) YES NO
- Hepatitis C Virus (HCV) YES NO

Autoimmune diseases
- Systemic lupus erythematosus (SLE) YES NO
- Type 1 diabetes mellitus (Type 1 DM) YES NO
- Other (Sjogren’s, etc.) YES NO

Blood transfusion(s) in the last 7 days YES NO

Intravenous immunoglobulin (IVIG) YES NO

Please fill out this form (pre- or post-transplant section) and return to the Transplant Immunology and Immunogenetics Laboratory (TIIL) at HLA-TIILLab@uchospitals.edu or fax: (773)-834-5573.

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