HLA Testing Agreement with the Heart 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, New York State Department of Health (NYSDOH) 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 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 low-resolution or high-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, DQA1, and DPA1.
   - If typing for DPB1, DQA1, and DPA1 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 and sufficient DNA is available.
   - Beginning 3/1/2019, for patients listed in the UNet® waitlist with serological HLA typing performed by an outside laboratory, HLA molecular typing may 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 self HLA antigens
     - If requested by the transplant program

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

4. 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.

08/06/2019
• The laboratory receives updates of all new patients entered into UNetSR via the Heart-Lung Active Patient List distributed by the thoracic transplant team and/or e-mail notification from the nurse coordinators.
• All unacceptable antigens are listed by the heart transplant program.
• All UNetSR updates are performed by the heart transplant program. However, HLA testing results are checked by TIIL for accuracy and additional typing data is added as needed.

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.

5. 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 by the TIIL management team, if possible, the other HLA laboratory will be contacted and informed of the typing results.
• This information is documented as part of the Quality Assurance system as an Incident Report and if appropriate, as a Corrective Action.

6. Turnaround time from receipt of sample to reporting of results to the transplant program:
• HLA typing
  o Routine (high resolution): 7 business days (BD)
  o STAT (low resolution): 2 BD
  o 24 hr. STAT (low resolution): results available within 24 hr. following sample receipt
    o Samples should be received in the TIIL before 9am
    o Page must be sent to pager #8722 indicating STAT status
• Antibody testing
  o Routine: 7 BD
  o STAT: 2 BD
  o 24 hr. STAT: results available within 24 hr. following sample receipt
    o Samples should be received in the TIIL before 11am
    o Page must be sent to pager #8722 indicating STAT status
    o 24 hr. STAT testing will require Luminex single antigen classes I and II testing concurrently with phenotype beads
• Flow Crossmatch
  o Routine: 4 BD
  o STAT: 2 BD
  o 24 hr. STAT: results available within 24 hr. following sample receipt
    o Samples should be received in the TIIL before 11am
    o Page must be sent to pager #8722 indicating STAT status
    o 24 hr. STAT testing may require testing Luminex phenotype, single antigen class I, and single antigen class II concurrently with the flow crossmatch

NOTES:
• When the TIIL is notified of adult patients with status of 1, 2, 3, or 4, or pediatric patients with status of 1A, testing is considered STAT.

08/06/2019
For all other STAT testing, the transplant program should send a request to the TIIL by email at #HLA-Tiillab@uchospitals.edu or by pager #8722. All requests made for 24 hr. STAT testing should be made by paging #8722.

7. Process to obtain sensitization history for each patient: The sensitization history for each patient is to be provided by the nurse coordinator, physician, or other member of the transplant team at any point in time; in particular at the time of initial work-up, when a known immunization event (blood transfusions, pregnancy, or transplant) has occurred, or when ordering post-transplant monitoring by:
   - Filling out the question/comment section associated with orders transmitted through Epic
   - By completing attached Appendix A via email #HLA-Tiillab@uchospitals.edu

8. Frequency of periodic sample collection: Following the initial work-up, and once added to the UNetSR waitlist, specimens are collected from patients as described in section 1 above, and submitted to the TIIL. The program is encouraged to send samples at least every three months after initial work-up and UNetSR listing.

9. Frequency of pre-transplant antibody screenings:
   - Serum samples from recipients on the UNetSR wait list are tested at least every three months, unless otherwise requested, using solid-phase assays. Recipients with PRA 1% or greater are tested using single antigen beads at least once a year.
   - In cases of major changes in antibody status between samples, the TIIL will request an additional specimen 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.

10. Criteria for determining unacceptable antigens used during organ allocation:
    - All unacceptable antigens are listed by the heart transplant program.
    - All UNetSR updates are performed by the heart transplant program. However, HLA testing results are checked by TIIL for accuracy and additional typing data is added as needed at time of listing and/or when notified that changes have been made.

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

11. 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.

12. Virtual crossmatch and peri-transplant crossmatching:
    - Prospective crossmatches are performed by Gift of Hope HLA laboratory.
    - Retrospective flow cytometric crossmatches will be run by the TIIL if adequate donor material and an appropriate recipient serum sample is available. Due to factors related to deceased donors, sufficient donor cells are not always available for retrospective crossmatch. However, the probability of obtaining sufficient cells increases with the amount of donor material provided to the TIIL. Based on antibody profile, flow crossmatching may be performed on multiple serum dates with titers.
    - If notified of diagnosis, 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 are investigated to evaluate potential interference. The TIIL may contact the transplant program to 08/06/2019
obtain this information by requesting that the Information of potential interfering factors for HLA antibody testing form be completed (see Appendix A).

- Published data supports the use of virtual crossmatch in the presence of a false positive crossmatch.
- 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.

**Prospective final crossmatches with deceased donors are performed and interpreted by GOH HLA laboratory. Crossmatch test is not a standardized test and there may be discrepancies in results between laboratories that could affect test interpretation. It is recommended that the Transplant Program establishes crossmatch assay format with the OPO to ensure appropriate testing for patients on the UNetSR waitlist. A high degree of false-positive crossmatch results has been observed at GOH.**

**A. Virtual Crossmatch of 0% PRA Kidney and multi-organ transplant patients (excluding liver)**

- Prospective compatibility for kidney transplant patients with 0% PRA may be determined by virtual crossmatch (vXM).
- Assuming sufficient donor material is available to the TiIL, all patients evaluated by vXM will be followed by a physical retrospective XM performed by the TiIL.
- The most recent sample, ≤ 90 days, available and tested by the TiIL by either FPRA or Luminex phenotype beads and if appropriate, by single antigen beads (SAB), will be used for vXM evaluation.
- Specific circumstances may require a physical XM. These include, but not limited to, the following: when necessary antibody testing has not been completed, when testing results are unclear, when molecular typing is incomplete, and/or when requested by the transplant physicians.
- Only antibody test results obtained from testing performed by the TiIL will be used to perform a virtual crossmatch.
- The TiIL is an ASHI and CAP accredited laboratory.

**B. Virtual Crossmatch of multi-organ transplant patients that include liver:**

- The transplant program(s) must notify the TiIL by email (#HLA-TiILLab@uchospitals.edu) of all patients listed in UNet for kidney transplant combined with any other organ transplant.
- Prospective compatibility for combined transplant patients will be determined by virtual crossmatch (vXM). Physical XM may be requested based on individual cases and per surgeon discretion.
- Assuming sufficient donor material is available to the TiIL, all patients evaluated by vXM will be followed by a physical retrospective XM performed by the TiIL.
- The most recent sample, ≤ 90 days, available and tested by Luminex phenotype beads and if appropriate, by single antigen beads (SAB), will be used for vXM evaluation. If the patient is 0% PRA, Flow PRA will also be acceptable if ≤ 90 days.
- Specific circumstances may require a physical XM. These include, but not limited to, the following: when necessary antibody testing has not been completed, when testing results are unclear, when molecular typing is incomplete, and/or when requested by the transplant physicians.
- Only antibody test results obtained from testing performed by the TiIL will be used to perform a virtual crossmatch.
- The TiIL is an ASHI and CAP accredited laboratory.

13. **Duration for which specimens need to be stored for repeat or future testing:**

- If possible, 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 for 3 to 6 months. 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.

08/06/2019
• If possible, buffy coats and DNA specimens collected from recipient and donors (living and deceased) are stored frozen in the TIIL for a minimum of 5 years.

*Duration of deceased donor specimen storage at Gift of Hope 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.

**NOTE:** It is highly recommended that the program indicates type of desensitization treatment used and timing in relationship to treatment, e.g. pre-plasmapheresis or post-plasmapheresis; with each sample sent to the lab for testing.

15. **Process for blood type verification according to Policy 3.1.4: Waiting List:** the TIIL does not register candidates for the Transplant Program, nor performs ABO typing. Blood type verification is performed by the Transplant Program.

16. **Protocol for post-transplant monitoring of antibody levels:**

   - It is the intent of the heart transplant program to draw post-transplant specimens according to the following schedule:
     - Year 1: months 1, 2, 3, 4, 6, 8, 10, and 12.
     - Years 2 and 3: every three months.
     - Year 4 and after: every 6 months.
     - Also, 2 weeks after CMV or any infection requiring hospitalization.
     - Specimens are tested by the TIIL as they are received.
   - Class I and class II DSA identification and strength testing is performed using solid-phase assays. Based on antibody profile (MFI ≥20,000), antibody titers (1:10 serum dilution) may be performed.
   - If DSA positive, C1q testing antibody assay is performed.
   - Due to potential changes in HLA antibody levels, the program is encouraged to notify the TIIL when patient is treated with plasmapheresis.

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08/06/2019
Appendix A

Information of Potential Interfering Factors for HLA Antibody Testing

Patient Name: ______________________

MRN: ______________________________

Completed by: ______________________ Date: ________________

RECIPIENT  (please circle)

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

Autoimmune diseases:
- Systemic lupus erythematosus (SLE) NO YES
- Type 1 diabetes mellitus (Type 1 DM) NO YES
- Other (Please add): __________________

Treatments:
- Anti-CD20 monoclonal antibodies NO YES (current -- last month -- last 3 months -- last year)
  (includes Rituximab, Ocrelizumab, Veltuzumab, Obinutuzumab, Ofatumumab)
  Entyvio (Vedolizumab) NO YES (current -- last month -- last 3 months -- last year)
  Antithymocyte globulin (ATG) NO YES (current -- last month -- last 3 months -- last year)
  Alemtuzumab (campath) NO YES (current -- last month -- last 3 months -- last year)
  Intravenous immunoglobulin (IVIG) NO YES (current -- last month -- last 3 months -- last year)
  Immunosuppressive treatment NO YES (current -- last month -- last 3 months -- last year)
  Therapeutic Plasma Exchange (TPE) NO YES (current -- last month -- last 3 months -- last year)

Prior Sensitizing Events:
- Blood transfusion(s) NO YES
  (within the last 7 days -- last month -- last 3 months -- earlier)
  Dates (if known): __________________
- Pregnancy(ies) NO YES (living -- deceased)
  Donor ID (if known): __________________
  Date (if known): __________________

DONOR  (please circle)

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

List medications in the last 7 days: ____________________________

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

08/06/2019