University of Chicago Medicine
Transplant Immunology and Immunogenetics Laboratory

HLA Testing Criteria for the Kidney/Kidney-Pancreas Transplant Program

This agreement adheres to the United Network for Organ Sharing (UNOS), the American Society for Histocompatibility and Immunogenetics (ASHI), 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 will be submitted to the TIIL at the time of initial work-up. The transplant (TX) program is encouraged to send samples at least every three months after initial work-up and UNetSR listing
   - Allo-crossmatch:
     i. Recipient: 1 red top tube
     ii. Living donor: 3 yellow top tubes (ACD-Solution A) as well as 1 red top tube
     iii. Deceased 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 for typing recipients and donors: 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 are routinely performed: HLA-DPB1, DPA1 and/or DQA1
   - If typing for HLA-DPB1, DPA1 and/or DQA1 is 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
   - Starting on 02-04-2019, for patients listed in UNetSR waitlist with serological HLA typing, HLA molecular typing could be performed by the TIIL in the following circumstances:
     o To determine antigen split typing, in cases where only broad antigen typing was performed
     o When HLA antibodies are identified to loci not typed by the external HLA laboratory
     o When potential HLA antibodies are identified to a self HLA antigens
     o Or 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-TIILab@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.
   - The laboratory should be informed (minimally) on a weekly basis of all new patients entered into UNOS via e-mail from the recipient’s coordinator. Additionally, the TIIL performs an audit of UNOS monthly to identify all new listings and/or removals.
   - Upon receiving notification of a new UNOS listing, laboratory staff updates patient HLA typing in UNET and if the patient is sensitized, enters unacceptable antigens (see below).
   - All UNOS transactions are reviewed by two technologists for accuracy.

5. **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 the accuracy of transferring of results and documents 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.

6. **Turnaround time from receipt of sample to reporting of results to the transplant program:**
   - HLA typing
     - Routine (high resolution): 7 business days (BD)
     - STAT (low resolution): 2 BD
     - Same-day STAT (low resolution): 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

**NOTE:** for STAT testing, the transplant program should send a request to the TIIL by email at #HLA-TIILlab@uchospitals.edu or by pager #8722.
7. **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 transfusion, 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]

8. **Frequency of periodic sample collection:** Following the initial work-up, and once added to the UNOS waitlist, specimens are collected from patients as described below, and submitted to the TIIL on a monthly basis.

9. **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.
   - The TX program is encouraged to send serum samples from recipients in the National Kidney Registry (NKR) at least every three months for solid-phase testing, including SAB assay for specificity identification.
   - In cases of major changes in antibody status between samples, the TIIL may request 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.

10. **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 pre- and peri-transplant crossmatching:**
    - HLA crossmatching is performed using a flow cytometric assay.
    - Based on antibody profile, flow crossmatching may be performed on multiple serum dates with titers.
    - Patients known to be HIV-positive, with other viral infections, or patients with history of false positive crossmatch are crossmatched against pronase-treated and non-treated donor cells.
    - If specimens are available, recipient and donor auto-crossmatches are performed as needed 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.

11. **Criteria for determining and listing unacceptable antigens used during organ allocation:**
    - HLA class I antibodies classified as strong (antibodies with MFI values predicted to yield a positive flow-cytometric crossmatch); present in current patient serum.
    - HLA class II antibodies, of any strength. This policy went into effect June 6th, 2017, and will be followed indefinitely unless otherwise indicated by the renal transplant program.
      i. HLA class II antibodies will be listed the first time they are detected.
      ii. HLA class II antibodies will be removed if they are not confirmed with a second sample.
iii. Any HLA class II antibodies detected on any two samples will be listed.

- 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 list as unacceptable antigens.
- In some cases, due to medical reasons, antigens corresponding to moderate and weak HLA antibodies present in patient serum may also be listed in UNet $^{58}$ as unacceptable.
- UNet $^{58}$ updates are performed and documented by the TIIL.

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

12. 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 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.
- 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 should be established in the agreement between the Transplant Program and Gift of Hope HLA laboratory.*

13. Protocol for monitoring antibody levels pre and post-desensitization treatment: in order to maximize efficacy of antibody monitoring, the TX program is encouraged to follow this sample collection and testing regimen:

- Pre-desensitization protocol and post-desensitization protocol
- If plasma exchange (PE) is performed, immediately pre- and post- each PE treatment, as well as within the first week after completion of PE treatment
- Post-PE treatment, biweekly for the first three months, and monthly for the following three months.

*NOTE:* It is highly recommended that the program indicates type of desensitization treatment and timing with each sample sent to the lab for testing.

14. Criteria for crossmatching:

- A prospective crossmatch is required prior to kidney transplant, even if in combination with other organ transplant
- For patients with living donors: a physical crossmatch is performed prior to transplant.
- The TX program may request retrospective T-cell and B-cell flow crossmatch testing, if cadaveric donor samples are available. (Please note, GOH HLA laboratory does not perform long-term storage of donor cells.)
- It should be noted that if a retrospective crossmatch is requested by the TX program due to concerns of a potential false positive crossmatch reported by the OPO-HLA laboratory and the TIIL should use the same sera tested by GOH, double billing of crossmatches using same serum cannot be performed.
- 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 formats with the OPO to ensure appropriate testing for patients on the UNOS waitlist. 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.

15. 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).
- If previous transplant donor typing and/or previous HLA testing information is available to the TIIL, the presence of repeat mismatches and/or historic donor-specific antibodies (DSA) may be noted on the vXM report.
- All patients evaluated by vXM will be followed by a physical retrospective XM performed by the TIIL, assuming sufficient donor material has been provided.
- 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.
- Patient 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 from the TIIL will be used to perform a virtual crossmatch; the TIIL is an ASHI and CAP accredited laboratory.

16. 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.
- If previous transplant donor typing and/or previous HLA testing information is available to the TIIL, the presence of repeat mismatches and/or historic donor-specific antibodies (DSA) may be noted on the vXM report.
- All patients evaluated by vXM will be followed by a physical retrospective XM performed by the TIIL, assuming sufficient donor material has been provided.
- 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.
- Patient 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 from the TIIL will be used to perform a virtual crossmatch; the TIIL is an ASHI and CAP accredited laboratory.

17. 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.
18. **Protocol for post-transplant monitoring of antibody levels:**

   It is the intent of the kidney transplant program to draw post-transplant specimens of patients the program deems to be highly sensitized according to the following schedule:
   - Year 1: week 2, months 1, 2, 3, 4, 6, 9, and 12.
   - Years 2 and 3: every three months.

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

   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 if deemed appropriate) may be performed.
   - Due to potential changes in HLA antibody levels, the program is encouraged to notify the TIIL when patient is treated with plasmapheresis. It is recommended that blood drawn for patients treated with plasmapheresis are performed by Transfusion Medicine personnel.

19. **National Kidney Registry (NKR)**

   - When a recipient is newly listed in the NKR system, the TIIL enters all current and class II historic antibodies regardless of relative strength. Previously listed historic class I antibodies which are no longer detected will be removed and the program will be notified. Class II antibodies will follow the rules set forth in section 11. Original testing reports will be uploaded, when necessary.
   - Recipients listed in the NKR will have their antibody profile reviewed by the TIIL at least every 90 days, as required by the NKR.
   - NKR active recipients are to be collected at least every 90 days.
   - NKR active recipients are to be tested by Luminex methods at least every 90 days.
   - When a donor is newly listed in the NKR system the program will notify the lab via email at #HLA-TIILLab@uchospitals.edu. The TIIL will enter the HLA typing results within 1 business day. Original typing reports will be uploaded, when necessary.
   - When UCM NKR active recipient has a swap offer, the NKR informs the laboratory via email. The laboratory reviews sensitization history, performs a donor-recipient Virtual Antibody Assessment, determines if additional testing is needed, and communicates findings to the transplant program.

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**Signature Section**

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Appendix A

Information of potential interfering factors for HLA antibody testing

Patient Name: ___________________________ MRN: ___________________________
Completed by: ___________________________ Date: ___________________________

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.