University of Chicago Medicine  
Transplant Immunology and Immunogenetics Laboratory

HLA Testing Agreement with the Pediatric and Adult Hematopoietic Cell Transplant Programs

This agreement adheres to the American Society for Histocompatibility and Immunogenetics (ASHI) guideline D.5.3.3), and the National Marrow Donor Program (NMDP) policies P00079, P00080 (updated 2015), and NMDP HLA typing discrepancy review process policy (updated 2008), College of American Pathologists (CAP) guidelines, and New York State Department of Health (NYSDOH) guidelines.

1. Sample requirements
   - HLA typing (Potential Recipients and Unrelated Donors):
     - 1 yellow top tube (ACD-Solution A). Buccal swab specimens (4) are an alternative when blood samples cannot be easily obtained. The clinical program is requested to obtain both blood and buccal samples for potential recipients with WBC counts <4,000, to ensure typing can be successfully performed.
   - HLA typing (Potential Related Donors):
     - 1 yellow top tube (ACD-Solution A) or buccal swabs (4)
     - Specimens are submitted to the Transplant Immunology and Immunogenetics Laboratory (TIIL) at the time of initial work-up.
   - Antibody testing (Recipients):
     - 1 red top tube (serum)
     - It is encouraged that the Hematopoietic Cell Transplant (HCT) program sends specimens to the TIIL prior to donor selection

2. HLA loci and level of resolution typing:
   - Necessary testing includes typing of each HLA locus used for donor selection and/or to which recipient HLA antibodies are directed, on two independent specimens, with at least one typing performed by the TIIL at high resolution. In cases where there is confusion about typing for a particular patient or donor, this guideline will be used to determine appropriate testing.
   - Exceptions to this guideline are for related donor A and DRB1 screening, and cord unit verification typing, which are both performed at low-resolution only.

   **Potential Recipients**
   - **Initial typing:** HLA-A, B, C, DRB1, DQB1, and DPB1 typing is performed at high-resolution
     - Additional testing for the following loci may be performed: DRB3/4/5, DQA1, and DPA1.
     - If typing for DRB3/4/5, DQA1, and/or DPA1 are not obtained, these loci will not be repeated or reported, unless the recipient has antibodies to these loci and/or typing is needed for donor selection.
   - **Verification typing** must be performed on a second independent sample prior to infusion.
     - The HCT program is encouraged to order as follows:
       1. Prior to the unrelated donor search request.
       2. Prior to cord blood unit(s) (CBU) shipment. Ideally, verification typing results should be completed before the time of the CBU shipment request.
       3. Prior to patient conditioning regimen starting.
     - Verification typing of samples previously typed at high-resolution by the TIIL will be resulted at low-resolution, and will be performed on the HLA-A, B, C, DRB1, DQB1, and DPB1 loci.
       1. Additional testing for the following loci may be performed: DQA1 and DPA1.
       2. If typing for DQA1 and/or DPA1 are not obtained, these loci will not be repeated or reported, unless the recipient has antibodies to these loci and/or typing is needed for donor selection.
For patients initially typed at an outside HLA laboratory at any resolution; HLA-A, B, C, DRB1, DQB1, and DPB1 loci, at the minimum, must be typed at high-resolution by the TIIL as verification typing.

If HLA typing from an outside HLA laboratory doesn’t include all required loci (HLA-A, B, C, DRB1, DQB1, and DPB1) an additional sample will be necessary for verification typing; these verification samples will be resulted at low-resolution for all required loci (HLA-A, B, C, DRB1, DQB1, and DPB1). The clinical program will be responsible for ordering these samples, as the TIIL does not have the ability to order testing.

**Potential Donors**

**Related donors**

- **Initial typing**: HLA-A and DRB1 low-resolution typing is performed.
  - If potential donor is either a 4/4 match or a 3/4 match, HLA-A, B, C, DRB1, and DQB1 high-resolution typing is performed.
    - Additional testing for the following loci may be performed: DRB3/4/5, DQA1, DPB1, and DPA1.
    - If typing for DRB3/4/5, DQA1, DPB1, and/or DPA1 are not obtained, these loci will not be repeated or reported, unless the patient has antibodies to these loci and/or typing is needed for donor selection.
  - If potential donor is a 2/4 match, haploidentical testing is only performed per request of the program. In these cases, HLA-A, B, C, DRB1, and DQB1 high-resolution typing is performed.
    - Additional testing for the following loci may be performed: DRB3/4/5, DQA1, DPB1, and DPA1.
    - If typing for DRB3/4/5, DQA1, DPB1, and/or DPA1 are not obtained, these loci will not be repeated or reported, unless the patient has antibodies to these loci and/or typing is needed for donor selection.

- **Verification typing** should be ordered by the HCT program prior to final donor selection, using a second independent sample.
  - Verification typing performed by the TIIL will be resulted at low-resolution, and will be performed on the HLA-A, B, C, and DRB1 loci.
    - Additional testing for the following loci may be performed: DQB1, DQA1, DPB1, and DPA1.
    - If typing for DQB1, DQA1, DPB1, and/or DPA1 are not obtained, these loci will not be repeated or reported, unless the patient has antibodies to these loci and/or typing is needed for donor selection.
  - For samples initially typed at an outside HLA laboratory, HLA-A, B, C, and DRB1 loci, at the minimum, must be typed at high-resolution by the TIIL as verification typing.
  - If HLA typing from an outside HLA laboratory doesn’t include all required loci (HLA-A, B, C, and DRB1) an additional sample will be necessary for verification typing; these verification samples will be resulted at low-resolution for all required loci (HLA-A, B, C, and DRB1). The clinical program will be responsible for ordering these samples, as the TIIL does not have the ability to order testing.

**Unrelated Donors**

- **Initial typing**: Is provided by the National Marrow Donor Program (NMDP).
- **Verification typing**: HLA-A, B, C, DRB1, DQB1, and DPB1 high-resolution typing, serves as verification typing of the NMDP-provided initial typing. The HCT program should send the unrelated donor sample for verification prior to final donor selection.
  - Additional testing for the following loci may be performed: DRB3/4/5, DQA1, and DPA1.
  - If typing for DRB3/4/5, DQA1, and/or DPA1 are not obtained, these loci will not be repeated or reported, unless the recipient has antibodies to these loci and/or typing is needed for donor selection.
For samples initially typed at an outside HLA laboratory (including NMDP laboratories), HLA-A, B, C, DRB1, DQB1, and DPB1 loci, at the minimum, must be typed at high-resolution by the TIIL as verification typing.

If HLA typing from an outside HLA laboratory, including an NMDP laboratory, doesn’t include all required loci (HLA-A, B, C, DRB1, DQB1, and DPB1) an additional sample will be necessary for verification typing; these verification samples will be resulted at low-resolution for all required loci (HLA-A, B, C, DRB1, DQB1, and DPB1). The HCT program will be responsible for ordering these samples, as the TIIL does not have the ability to order testing.

Allogeneic Cord Blood Units

- **Initial and verification typing**: Is provided by the cord bank holding the cord unit.
- **Additional verification typing to confirm the correct unit was shipped** must be carried out using a sample from a segment attached to the CBU, and, if possible, should be resulted before infusion. The attached segment must be removed from the unit at the time of the HLA-verification typing request.
  - HLA verification typing is resulted at low resolution, and is performed on the HLA-A, B, C, and DRB1 loci, and any other loci to which the recipient has antibodies.
  - At a minimum, the loci that are used for donor selection must be verified: HLA-A, B, C, and DRB1.
  - For a CBU to be used when there is no attached segment available for HLA-verification typing: verification typing will be ordered by the clinical program before infusion. The clinical program will decide if infusion of the cord unit will occur before reporting of verification typing to avoid delaying infusion of a thawed product; the TIIL will be notified in such cases. Should the TIIL find that the verification typing is not concordant with previous typing; the TIIL will notify the HCT clinical director or their delegate, immediately.

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-TIILLab@uchospitals.edu or by pager #8722.

4. **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 may be 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.
   - For NMDP typing discrepancies, the NMDP HLA Typing Discrepancy Review Process Policy, updated June 2008, is followed.

5. **Turnaround time from receipt of sample to reporting of results to the transplant program**:
   - HLA typing (low resolution): 4 business days (BD), STAT: 48 hours
   - HLA typing (high resolution): 7 business days (BD), STAT: the TIIL will expedite review and reporting
   - Cord unit verification typing: 4 business day (BD), ASAP: 2 BD, STAT: same day
   - Antibody testing: 7 BD, STAT: 48 hours

   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.

6. **Process to obtain sensitization history for each patient when ordering antibody testing**: The sensitization history for each patient is to be provided by the nurse coordinator, physician, or other member of the
transplant team 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

7. **Assay format for recipient antibody screening**: HLA class I and class II antibody testing is performed by solid-phase immunoassays at the request of the program. Based on antibody profile, antibody titers may be performed. Upon request, MFIs can be provided to the HCT program.

   - The HCT program is encouraged to request HLA antibody testing prior to final donor selection, preferably prior to formal donor search.
   - The HCT program is encouraged to request HLA antibody testing after any known sensitizing event (e.g. blood transfusion, transplant), or if the last tested sample is 3 months old or older.

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

   - If possible, DNA specimens collected from recipient and donors are stored frozen in the TIIL for a minimum of 5 years.
   - Serum specimens collected from recipients are stored frozen in the TIIL for a minimum of 6 months.

9. **Protocol for monitoring antibody levels pre and post-desensitization treatment**: Samples for antibody monitoring are tested pre- plasma exchange (PE) and post-PE. A minimum of pre and post the initial PE and consequent third PE is required to be able to monitor patient response to. To facilitate interpretation of test results, it is recommended that the program indicates type of desensitization treatment and timing with each sample sent to the laboratory for testing. To ensure immediate pre- and post-pheresis sample draws, it is preferred that samples are drawn by the Blood Bank team members.

10. **Additional Testing may be performed in the following cases**:

   - To distinguish Common and Well Documented null alleles listed on NMDP Policies P00079 and P00080
   - Homozygous typing: homozygosity will be confirmed at the time of verification typing, which will include typing by a different method. If a recipient’s initial typing will be used to start a formal search, then the program can request (by email at #HLA-TILLab@uchospitals.edu or by pager #8722) verification of homozygous loci on the initial sample.
   - When four family haplotypes are needed and requested by the HCT program and have not been unambiguously determined.
   - When genotype cannot be clearly defined.
   - When an allele-group-level ambiguity is obtained.

**NOTES**: Flow cytometric crossmatch (FCXM) testing is available upon request by the program.