Joint Written Policy between Methodist Dallas Medical Center Transplant Program (MDMCTP) and Methodist Dallas Medical Center Transplant Immunology Laboratory

PROCEDURAL REVISION

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Annual Review

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Joint Written Policy between Methodist Dallas Medical Center Transplant Program (MDMCTP) and Methodist Dallas Medical Center Transplant Immunology Laboratory

Histocompatibility Evaluation

1. **This written agreement details the Histocompatibility lab services provided to Methodist Dallas Medical Center Transplant Program:**
   a. Kidney and combined kidney/pancreas transplant candidates require ABO typing and confirmation, HLA typing, periodic panel reactive antibody testing and crossmatching prior to transplantation.
   b. Combined kidney/liver transplant candidates require ABO typing and panel reactive specificity antibody testing prior to or upon listing for transplant. Subsequent PRA testing will occur at the time of transplant if, the patient has had a sensitizing event within the prior 4 months, and/or the draw date of the most recent PRA sample is greater than 3 months prior. Candidate HLA typing and or final physical crossmatch will only be performed upon physician request.
   c. Liver transplant candidates require ABO typing and confirmation prior to transplantation. Candidate HLA typing, panel reactive antibody testing and crossmatch will only be performed upon physician request.

2. **Candidate sample requirements, timing of sample requirements and initial evaluation sensitization history:**
   a. 2-4 ACD (at least 10 mLs/tube) yellow top tubes, 1 (at least 7 mLs) red top tube (either no anticoagulant or serum separator only) and 1 EDTA (at least 7 mLs) tube should be provided for initial evaluation and/or prior to listing.
   b. Specimens should be delivered or mailed to the lab within 48 hours of procurement.
   c. All samples must be labeled with the patient name and a unique identifier (either date of birth OR social security number, OR medical record number), the date drawn and drawer's initials. Unacceptable samples are discarded, and laboratory will request recollect.
   d. An LIS order or signed request should be sent to the HLA laboratory with the samples. Request should include sensitization history.
   A kidney or combined kidney/pancreas candidate's initial workup includes an ABO typing, HLA Class I and II typing and Class I and II specificity PRA with antibody identification. HLA Class I and II typing inclusive of all required loci, and /or Class I and II PRA results from an outside accredited laboratory are acceptable upon director review.
   e. A recipient pursing living donor transplant found to be ABO incompatible with the donor, whereby the recipient is type O or B and the donor is type A, or recipient is type B and donor is type AB, will be evaluated for the ABO Intended Incompatible Transplant protocol.
      - If the donor subtypes as ABO A, non-A1, the recipient will be evaluated via Transplant Anti-A DTT Titer by Gel. An Anti-A IgG titer is >4 precludes eligibility for the protocol.
      - Titer results of ≤4 allow for preliminary eligibility in the protocol and the recipient/ donor pair will proceed to preliminary crossmatch.
      - For crossmatch compatible pairs, two additional titers are required on the recipient and an additional ABO subtyping is required on the donor. If the pair meet the protocol requirements, they will be consented for the ABO Intended Incompatible Transplant Protocol, and the recipient will be listed in UNET for this type of transplant. Subsequently, the recipient's Anti-A DTT Titers must be performed per the schedule required by the OPTN.
      - Any subsequent titer >4 will disqualify the recipient from the non-A1 protocol or require immunologic intervention to continue in this protocol. The treating physician will be notified immediately.
   f. A recipient pursing deceased donor transplant found to be ABO B will be evaluated for the ABO Intended Incompatible Transplant protocol, through testing for Transplant Anti-A DTT Titer by Gel. Titer results of ≤4 allow for preliminary eligibility in the protocol.
      - After initial qualifying titer, two additional Anti-A DTT titers must be performed, preferably at one month intervals.

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- Any subsequent titer >4 will disqualify the recipient from the non-A1 protocol or require immunologic intervention to continue in this protocol. The treating physician will be notified as needed.
- Once the three qualifying titers are complete and the patient is consented for the ABO Intended Incompatible Transplant Protocol, they will be listed in UNET for this type of transplant. Subsequently, Anti-A DTT Titers must be performed per the schedule required by the OPTN.
- Any subsequent titer >4 will disqualify the recipient from the non-A1 protocol or require immunologic intervention to continue in this protocol. The treating physician will be notified immediately.

3. **Living donor sample requirements:**
   a. 4 ACD (at least 10 mLs/tube) yellow top tubes and 1 EDTA (3 mLs) tube should be provided for initial evaluation
   b. Specimens should be delivered or mailed to the lab with 48 hours of procurement
   c. All samples must be labeled with the patient name and a unique identifier (either date of birth OR social security number, OR medical record number), the date drawn and drawer's initials. Unacceptable samples are discarded and the laboratory will request a recollect.
   d. An LIS order or signed request should be sent to the HLA laboratory with the samples.
   e. A potential donor's initial workup includes ABO typing and A subtyping if ABO incompatible as stated in #2 e above. If the potential donor is ABO compatible, intended incompatible or is a candidate for paired exchange, HLA typing is performed unless contraindicated by incompatible HLA crossmatch. ABO typing, HLA Class I and II typing inclusive of all required loci, and/or Flow crossmatch results from an outside accredited laboratory are acceptable upon director review.

4. **The Loci and Level of Resolution when typing performed and process for requesting Extended Typing:**
   a. Transplant recipients and living donors will be typed using molecular SSO low resolution typing for HLA A, B, C, Bw4, Bw6, DRB1, DRB3, DRB4 and DRB5, DQA1, DQB1, DPA1 and DPB1.
   b. In most instances low/intermediate resolution typing is sufficient for solid organ transplant testing. Extended typing will be performed upon physician request or as indicated in complex workup (for example if allele specific antibodies are suspected), In such event, the laboratory will arrange for higher or high resolution typing through an outside reference laboratory accredited in the US for high or higher resolution molecular HLA typing.

5. **Reporting HLA typing results to the OPTN and Transplant Program for transplant candidates and potential living donors:**
   a. HLA typing
      i. HLA typing results for HLA A, B, C, Bw4, Bw6, DRB1, DRB3, DRB4 and DRB5, DQA1, DQB1, DPA1 and DPB1 are reported to the transplant program and its affiliates via the hospital LIS system or secure fax prior to candidate registration on the waitlist.
      ii. HLA typing is entered into the OPTN database by an employee of MDMCTP utilizing the typing provided by the HLA laboratory
      iii. The HLA laboratory will periodically review the typing information. Identified discrepancies are corrected and a report made to MDMCTP regarding error detection and correction.
   b. Unacceptable antigen identification
      i. PRA specificity results are reported to the transplant program and its affiliates via the hospital LIS system or secure fax prior to candidate registration on the waitlist. Subsequent PRA results are reported in the same manner. All antibodies identified are reviewed and verified by the laboratory director prior to release.
      ii. Initial and subsequent unacceptable antigens are reported to the OPTN contractor by the HLA laboratory. Unacceptable antigens reported to the OPTN contractor are reviewed for accuracy by a technologist other than the submitting technologist, on a routine basis.
      iii. Error detection and correction will be documented by intralaboratory variance

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c. The HLA laboratory submits donor and recipient histocompatibility forms to the OPTN contractor after transplant.

6. **Resolution of HLA typing discrepancies**:
   a. All HLA typing discrepancies and errors found will be investigated and resolved if resolution is possible
   b. HLA typing discrepancies and errors will be fully re-evaluated including clerical check, new sample procurement, contacting the laboratory where the discrepant typing results originated and/or retyping the original sample as the investigation dictates
   c. All discrepancies will be resolved/closed within 30 days of notification or identification of discrepant HLA typing results
   d. HLA typing discrepancy and the reason for the discrepancy will be reported to the OPTN Contractor if such listing has been made and identified.

7. **Maximum turnaround time from receipt of sample to report availability:**
   a. Initial and periodic candidate testing:
      i. Initial ABO typing results will be reported via hospital LIS system or secure fax within 3 business days of receipt or prior to OPTN candidate listing.
      ii. Initial HLA typing of recipients will be reported via hospital LIS system or secure fax within 14 business days of sample receipt or prior to OPTN candidate listing.
      iii. PRA results
         1. Initial PRA results will be reported to the transplant program within 45 calendar days or prior to candidate OPTN listing via hospital LIS system or secure fax.
         2. Ongoing periodic PRA results will be batched and tested routinely. The maximum time for reporting in all cases will be 45 calendar days. Reports are provided via hospital LIS or secure fax.
   b. Preliminary living donor crossmatch and Donor ABO typing will be available within 2 days of sample receipt. Compatibility assessment, HLA typing and crossmatch report will be available within 14 business days of sample receipt.
   c. Final living donor crossmatch; Donor/recipient ABO typing and compatibility assessment and crossmatch report will be available within 1 business day of sample receipt.
   d. Deceased donor work-ups: ABO typing with compatibility assessment and verified crossmatch results for deceased donor transplants will be available 8 hours after sample receipt. Crossmatch results may be available as early as 4 hours after sample receipt.
   e. Post-transplant PRA testing: upon completion of the test, results are provided to the ordering physician either verbally or via LIS Secure Chat within one business day of receipt. Final report will be provided by the laboratory director via hospital LIS system within 14 business day of sample receipt. Upon physician request, testing may be performed on a stat basis and results reported to the physician verbally or via LIS Secure Chat, and can be made available within 4 hours of sample receipt.

Waitlist

8. **Activation on the Waitlist and ABO Verification:**
   a. MDMCTP is responsible for candidate waitlisting with the OPTN contractor.
   b. MDMCTP will notify the HLA laboratory of patient listings using secure e-mail.
   c. MDMCTP verifies that each transplant candidate has been ABO typed on two separate occasions prior to addition to the OPTN contractor waitlist. .

9. **The process for obtaining sensitization history:**
   a. Each sample mailed to the laboratory should be accompanied by a laboratory requisition, which requests information regarding transfusion history.

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b. Transplant coordinators should notify the laboratory when they are made aware of transfusions, prior transplantation, immunizations, hospitalizations, and nephrectomy of failed graft(s).
c. In addition, prior transplant history may be identified by the HLA laboratory by checking the OPTN contractor database and/or hospital LIS.
d. MDMCTP should request PRA testing after a known sensitizing event.

10. **The frequency of periodic sample collection and antibody screening:**
    a. After waitlisting in the OPTN database, monthly serum should be drawn for a period of three months to establish an antibody profile for the kidney and combined kidney/pancreas recipients. Samples should be provided for PRA testing quarterly thereafter. The laboratory will send mailers for serum collection. Per physician request, samples may be requested monthly for sensitized patients living a far distance from the transplant center and for those patients listed for a Kidney Paired Donor.
    b. Combined kidney/liver recipients should have serum screened prior to or upon listing. Subsequent PRA testing will occur at the time of transplant if, the patient has had a sensitizing event within the prior 4 months, or, the collection date of the most recent PRA sample is 3 months prior.
    c. Samples will be screened using Luminex Single Antigen Class I and II beads. If further testing is needed that is outside the scope of our laboratory practice, methodologies may be sought from an outside accredited laboratory at the discretion of the HLA laboratory director.

11. **Criteria for determination of unacceptable antigens:**
    a. For HLA A, B, C, DR, DQB, unacceptable antigens are determined by the laboratory using solid phase antibody specificity testing methodology utilizing validated cutoffs (contact laboratory for specific methodology and validation studies) in addition to unacceptable antigens identified by physical crossmatch. These findings are reviewed by the HLA director and identified unacceptable antigens are reported to MDMDTP and reported in the OPTN database. Antibodies that show incomplete reaction patterns (i.e. suspected alleles specific antibodies or those with apparent non-specific reaction patterns) or those that are excluded by physical crossmatch results from a cell with the suspected antigen, are not listed as unacceptable antigens.
    b. DQA, DPA and DPB antibodies will not be listed in UNOS unless/until an MFI of ≥ 10,000 is noted or positive crossmatch is seen that is only explained by said antibody. When a DQA, DPA or DPB antibody shows an MFI of <10,000, the technologist will notify the transplant coordinator and the donor/patient pair will proceed to crossmatch. If the crossmatch is negative, the antibodies will be considered clinically irrelevant. If the crossmatch is positive (in the absence of a corresponding autologous crossmatch result that accounts for positivity) without other explanation, that antibody will be listed in UNOS to avoid future donors with the corresponding antigen. Based on our prior low rate of unexpected positive crossmatch, our expectation is that the impact to the transplant program will be low.

12. **Crossmatch criteria:**
    a. Criteria for crossmatching and determining unacceptable antigens used during organ allocation with deceased donors is outlined in detail in the procedure 'Deceased Donor Workup for Solid Organ Transplantation'; a copy of which is on file in the MDMC Transplant office.
    b. Criteria for crossmatching and determining unacceptable antigens used during organ allocation with living donors is outlined in detail in the procedure 'HLA Testing for Living Donor Transplantation'; a copy of which is on file in the MDMC Transplant office.

13. **Desensitization Protocol for monitoring antibody levels:**
    a. A request is sent to the HLA laboratory with the sample for testing.
    b. Sample requirement - 1 (at least 7 mLs) red top tube (either no anticoagulant or serum separator only)
    c. The patient serum is screened for the presence of Class I and Class II antibodies using Luminex Single Antigen beads.
    d. The PRA results will be evaluated for the presence of donor directed antibodies and any DSA with an

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MFI of 15,000 or greater will disqualify the patient from inclusion in this protocol.
e. Subsequent evaluation for potential DSA will include Luminex Single Antigen testing for the appropriate class of antibody until such time as the HLA crossmatch is negative or weakly positive (3 SDs) by flow cytometric analysis.
f. Verbal results are provided to ordering physician upon completion of the test. Final report will be provided by the laboratory director via hospital LIS system

14. Specimen Storage

Storage requirements
a. Buffy coat and/or isolated DNA on all recipients, donors, deceased and living, are stored for a minimum of 5 years after transplant.
b. Serum samples collected upon recipient initial workup and subsequent PRA samples will be frozen and kept for a minimum of 6 months for nonsensitized recipients and 5 years for sensitized recipients.
c. The serum samples used for final crossmatch on transplanted patients is stored for a minimum of 5 years after transplant.
d. As of June 1, 2021, the recovery hospital will obtain, and the HLA laboratory will store, donor specimens appropriate for serological and NAT testing. The specimen(s) will be stored for at least 10 years after the date of transplant and assure the samples are available for retrospective testing.

Post Transplant Monitoring

15. Protocol for Monitoring Antibody

a. A request is sent to the HLA laboratory with the sample for testing.
b. Sample requirement - 1 (at least 7 mLs) red top tube (either no anticoagulant or serum separator only) drawn prior to infusion of therapeutic drugs.
c. In order to accurately interpret results, treatment protocols or rescue therapy (i.e. IVIG, Rituximab, Thymologlobulin, etc.) history may be requested since therapeutic drugs may give false positive/negative results.
d. Donor specific crossmatch will be performed upon request within 24-48 hours post-transplant, dependant on availability of donor material.
e. Donor specific antibody (DSA) evaluation: the patient serum is screened for the presence of Class I and Class II Luminex Single Antigen DSA.
f. Results are provided to ordering clinician, giving each donor directed antibody and the highest MFI (mean fluorescent intensity) corresponding to each DSA.
g. Results are provided to the ordering physician either verbally or via LIS Secure Chat. Final report will be provided by the laboratory director via hospital LIS system.
h. For recipients transplanted under the ABO Intended Incompatible Transplant protocol
    i. Blood Bank will be given the name of the recipient and administer only AB plasma to type B patients, or A plasma to type O patients.
    ii. if antibody mediated rejection is suspected, anti-A IgG titers should be performed in addition to evaluation of HLA donor directed antibodies.

Histocompatibility Laboratory Compliance with Regulatory Agencies

16. If a regulatory agency takes final adverse action against the HLA laboratory, which affects the lab ability to function, the OPTN contractor must be notified.

    Notification must:
    i. Be in writing
    ii. Be submitted within 10 business days after the Histocompatibility Laboratory receives notification of the final adverse action
    iii. Include all documents relating to the final adverse action.