Deceased Donor Workup for Solid Organ Transplantation

PROCEDURAL REVISION

Author: Karen Roush, MD

Revised by: Jodee Sedalnick

Revision Accepted by:

HLA Laboratory Medical Director — Date: 7/11/24

Surgical Director of Kidney Transplant Programs — Date: 7.16.24

Medical Director of Kidney Transplant Programs — Date: 7/16/24

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

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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 will be performed only 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 be performed only 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.

      - ABO B recipients will have additional orders for an Anti-A IgG titer/ DTT treated. Titer results of ≤4 allow for preliminary eligibility in the ABO Intended Incompatible Transplant protocol.
      - After initial qualifying titer, two additional titers are required. Additionally, a titer is performed at the time of final crossmatch. Anti-A IgG titer/ DTT treated titers will also 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.

   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 assessed for Anti-A IgG titer/ DTT treated. 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.
      - After initial qualifying titer, two additional titers are required. Additionally, a titer performed at the time of final crossmatch. Anti-A IgG titer/ DTT treated titers will also be performed per the schedule required by the OPTN.

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

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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.
    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.

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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.
    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 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 sample 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, Thymoglobulin, 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. For recipients transplanted under the non-A1 protocol
    i. Blood Bank will be given the name of the recipient and administer only AB plasma/platelets to type B patients, or A plasma/platelets 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.
f. Donor specific antibody (DSA): Upon initial evaluation for DSA, the patient serum is screened for the presence of Class I and Class II Luminex Single Antigen DSA.
g. Results are provided to ordering clinician, giving each donor directed antibody and the highest MFI (mean fluorescent intensity) corresponding to each DSA.
h. 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.

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.

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Deceased Donor Workup for Solid Organ Transplantation

I. PRINCIPLE:

The HLA Laboratory of Methodist Dallas Medical Center provides compatibility testing for deceased donor kidney, kidney/pancreas and kidney/liver transplantation for The Transplant Institute at Methodist Dallas and in association with Southwest Transplant Alliance. The series of events involved in the compatibility testing between recipients and a deceased donor is described herein.

II SPECIMEN:

Any properly labeled specimen submitted to the MDMC HLA Laboratory for transplant or cellular immunologic testing.

III. REAGENTS AND SUPPLIES:

Reagents and supplies are outlined in the specific procedures referred to throughout this document.

IV. PROCEDURE:

A. Request for Preliminary Compatibility Assessment

The laboratory or technologist on call will be notified by MDMC transplant coordinator of a potential deceased donor organ offer for a kidney or kidney pancreas recipient. The coordinator will communicate the donor's UNOS ID and Match run ID number, the recipient name, DOB/ Medical Record number and request a Preliminary Compatibility Assessment. This is performed to determine if the potential recipient has any donor directed HLA antibodies, or if they can be considered for transplant with this donor.

1. To complete this assessment, you will need to access the donor's molecular typing in DonorNet:
   a. Https://Portal.Unos.org
   b. UNET requires Multi-factor authentication (MFA) to access the system, thus, you will need to open your Authy authenticator app and approve the request to complete the log in process.
   c. Upon log in, choose DonorNet.
   d. If the donor of interest is listed on the DonorNet home page, click on the Match ID to bring up the donor information.
   If the donor is not listed, choose Donors and Search from the top tool bar. Enter the required information to access the donor of interest.
   e. Choose the *Donor Summary* tab and click on the small *Print* icon on the right side of the page. Select *Crossmatch & HLA* and click on the *Print Summary* at bottom of the page, click again to print. Use this official copy to enter the donor's type in the LIS; see B, 5 below.
   f. To view a more detailed version of the HLA type, return to the Donor Summary tab and click on the sub-tab, *Attachments*. Scroll down to find the attachment labeled HLA (ex ADFY435-HLA) and print.

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2. Looking at the donor HLA typing, determine if the intended recipient has any donor directed antibodies. The recipient's HLA antibodies are listed on their LIS HLA Hist report. Additionally, check the front of the folder to confirm if there are any DPA antibodies not yet listed on the Hist. Additionally, check the patient folder and LIS for any pending PRA results and evaluate for the presence of donor directed antibodies.

3. If no donor directed HLA antibodies are present, let the coordinator know the Preliminary Assessment is negative.

4. If any HLA- A, B, C, DR, DRB3,4,5 or DQB donor directed antibodies are present, or if any DQA, DPA or DPB donor directed antibodies are present and any one bead of the DSA has an MFI of ≥ **10,000**, let the coordinator know the Assessment is Positive, and thus a contraindication for transplant.

   If any HLA- A, B, C, DR, DRB3,4,5 or DQB allele specific donor directed antibody is present, consult the medical director prior to reporting the assessment.

   If there are any DQA, DPA and DPB donor directed antibodies with **MFI** (of any 1 bead) **between 2,000 and 9,999**, notify the transplant coordinator of the possibility of a positive crossmatch, however, we will proceed to determine clinical relevance.

5. If the donor/ recipient is being considered for the ABO Intended Incompatible Transplant protocol, verify recipient has a total of 3 Anti-A IgG titer/DTT treated titers performed prior to presentation for transplant, all titer results must be ≤4. The most recent titer must be dated within the prior 90 days, +/- 20 days from the day of planned transplant. If this is not the case, a titer must be performed when patient presents for transplant, notify the transplant coordinator.
   Verify the donor ABO in UNET to be A, non-A1. Either result is discrepant, immediately notify the transplant coordinator of contraindication for transplant.

6. Upon completion of the Preliminary Compatibility Assessment, the coordinator will provide ETA for donor harvest and recipient arrival.

7. Note all information about the donor and recipient, any communication with the transplant coordinator and/or transplant clinicians in Final Crossmatch Logbook.

8. Kidney/ liver recipients do not have HLA antibodies listed in UNET. Rather upon organ offer, a Final Virtual Crossmatch is performed as outlined below in Section C, 4.

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B. **Deceased Donor and Recipient Samples and LIS orders**
Note, steps 1-6 apply to Kidney and Kidney/Pancreas deceased donor and potential recipients.

Receipt of deceased donor material for ABO typing and final crossmatch

Verify each specimen is properly labeled with the UNOS number, date and time of collection and collector's identification

1. Prior to organ harvest, the lab often receives donor blood (ACD-A tubes and red top tube) that can be used for both final crossmatch and preliminary ABO verification.
2. Use of the ACD-A tubes for Final Crossmatch testing should occur within 48 hours of blood draw, but may exceed 48 hours if cell viability is ≥ 80%.

   Preliminary ABO typing/ subtyping should occur within 3 days of draw time. If this is not the case, Blood Bank may call asking if we want the ABO, as they will have to override a flag in their LIS. Confirm yes, we do want ABO.

   Upon harvest of donor organs, HLA will receive "Final" donor material which arrives with the organ and can be used for final crossmatching and must be used for Final ABO verification. Donor material may include lymph node, spleen, ACD-A tubes and red top tube. Lymph node, spleen or blood can be used for final crossmatching; ACD-A, red top tube or spleen can be used for Final ABO verification. Exception: if the donor is for transplant using the ABO Intended Incompatible Transplant protocol, a red top tube must be used for ABO and subtyping.

   Prior to the release of the final crossmatch report for kidney, kidney/pancreas transplant, the donor ABO must be verified using a sample drawn at the time of organ harvest and/or received with the organ.

3. Complete the registration of the deceased donor in the LIS via Requisition Entry. Register the donor with **SWTA HLA as the Submitter regardless of the OPTN procuring the organ**. Order an HLA TYPE CLASS I AND II NO CHARGE [LAB3429] and [LAB6965] Transplant Evaluation ABO/RH No Charge; request ABO subtyping when applicable, using TRANSPLANT A SUBTYPE [LAB4100].
4. Any donor ABO discrepancy from that reported in UNET is brought to the immediate attention of the laboratory director. In cases where the donor has been transfused, the ABO type may not be clear. The HLA director will advise on how to proceed/ who to notify.
5. If the donor/ recipient is being considered for the ABO Intended Incompatible Transplant protocol, verify the donor ABO A subtype is non-A1. Any discrepancy is immediately brought to the attention of the HLA medical director.
6. Using the HLA typing report printed from DonorNet; answer and verify out the HLA TYPE CLASS I AND II NO CHARGE [LAB3429] in the LIS leaving the ABO field blank as it is not applicable to deceased donors. Add a comment to the report stating typing information obtained from UNET.

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Our laboratory will retype the donor only when HLA typing information is not available to laboratory personnel, the donor has a serologic typing, rather than molecular or at the discretion of the HLA laboratory director.

NOTE: Donors may receive numerous transfusions in order to maintain their stability. In the event we retype the deceased donor, HLA typing results are acceptable only if the HLA antigens are unequivocally defined with no more than 2 antigens per locus. Equivocal typing results require repeat using lymph node or spleen cells, both of which should provide accurate results unaffected by transfusion history.

7. Make a folder for the donor that includes the HLA typing and Transplant Candidate Evaluation ABO/RH from the LIS and the typing information from DonorNet report(s). Make certain to redact any donor identifying information. Submit these results to the director or designee for review.

Receipt of recipient blood for final crossmatch for kidney and kidney/pancreas:

1. When the recipient presents to the hospital, blood is drawn for ABO typing and sent directly to the Blood Bank.
2. Blood for HLA crossmatch is sent to our laboratory
    a. Kidney and Kidney/Pancreas recipients presenting to the hospital for Final Physical Crossmatch or qualifying for Final Virtual Crossmatch will have orders in the LIS for an HLA Crossmatch, Flow Allo and Flow Auto [LAB3451]. This blood draw includes yellow top ACD-A tubes, a pink top and a red top tube.
    b. For recipients receiving the 3 Month Remote Sera Final Physical Crossmatch, the remote final crossmatch is performed prior to the recipient presenting to the hospital. Therefore, HLA staff will submit an order in the LIS for the HLA Crossmatch, Flow Allo and Flow Auto [LAB3451], as discussed in C, 3 below. Upon admission, blood will be drawn for HLA Crossmatch, Flow Allo and Flow Auto [LAB3451]. Store or use as discussed in E, 3 below.
    c. The primary recipient for the organ, (recipient listed first on the match run), will be charged for the donor ABO retyping, both initial and final ABO. Drop the charge(s) as follows:
        - Double click on the current admission for the recipient; click on the charges button on the right side of the screen; click on HLA button.
        - On next screen click on BOTH the HC ABO TYPE and HC RH TYPE button.
        - Edit service date to date ABO collected AND change Service Provider to the treating nephrologist.
        - When/if a second ABO is performed on donor, repeat process.

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3. Receipt and processing of Kidney/Liver recipient samples is discussed in section C, 4 below. Blood Bank personnel are responsible for ordering and reporting ABO typing on Kidney/ Liver and Liver donors. No donor material is required for our testing purposes (unless Final Physical Crossmatch is requested) and HLA personnel do not input donor HLA typing in the LIS.
4. All communication from MDMC kidney and liver transplant coordinators and physicians must be noted in the Final Crossmatch Logbook including date, time and person communicating information.

C. **Final Crossmatch Protocols for Kidney, Kidney/ Pancreas and Liver/ Kidney Recipients**

Prior to performing a Final Physical or Final Virtual Crossmatch , you will need to determine which protocol to follow as outlined below.

1. **ABO Compatible and ABO Intended Incompatible Final Physical Crossmatch for Kidney, Kidney/ Pancreas Recipients**

   a. A Recipient must present to the hospital for blood draw for a Final Physical Crossmatch if their cPRA >30% and they do not have a PRA within the prior 3 months. Testing will include a Flow Allo and Auto crossmatch and PRA.

   b. Information regarding transfusion or other sensitizing event are discussed with the medical director and recorded in the Final Crossmatch logbook.

   c. **When the recipient is being considered for the ABO Intended Incompatible Final Physical Transplant protocol, verify the recipient meets the titers requirements as outlined in IV**, A 5. In addition, verify the donor ABO subtypes as non-A1.

2. **ABO Compatible and ABO Intended Incompatible Final Virtual Crossmatch for Kidney and Kidney/ Pancreas Recipients**

   a. Kidney and Kidney/Pancreas transplant recipients may qualify for these protocols as follows:
   Both primary and regraft recipients qualify for this protocol if all of the following criteria are met:

      i. **The recipient must have a cPRA history of ≤ 30% AND all PRAs devoid of any partial or allele specific donor directed HLA antibodies.** The ≤ 30% cPRA is based on HLA antibodies identified on the Single Antigen PRA assay. The criteria for this

protocol negates FCPRA results. Check the recipient's HLA chart by viewing each Luminex Single Antigen PRA report for such antibodies. Any partial or allele specific DSA **disqualifies** the recipient from the Final Virtual Crossmatch protocol and the recipient must proceed with a Final Physical Crossmatch. Additionally, check for any PRAs in process to assure no antibodies, partial or otherwise are present.

ii. **The recipient must have a minimum of three Single Antigen. PRAs tested, one of which must have been collected within the previous three months.**

iii. **Recipient cannot have any sensitizing events since the last qualifying PRA**. Sensitizing events include transfusion, pregnancy, and transplant with any human tissue or organ since the last qualifying PRA. Verify this with the Transplant Coordinator.

b. The three required PRAs for this protocol must be tested by Single Antigen beads and do not need to be within a particular time frame, as long as there is a minimum of three and one of these is dated within the previous three months. If no PRA has been completed within the prior three months, or the recipient has only two Single Antigen PRA samples tested thus far, a PRA must be performed on a sample drawn when the recipient presents for transplant or other qualifying sample (a serum sample that has arrived in the laboratory but has not yet been tested). If this third sample result has cPRA ≤30% and is devoid of any HLA donor directed antibodies, partial or allele specific, the patient may proceed to transplant without a Final Physical Crossmatch, assuming all criteria outlined in a-f are met.

c. If the Luminex is inoperable and the patient requires a PRA in order to meet protocol, the recipient must proceed to Final Physical Crossmatch. Notify the coordinator.

d. When the recipient is being considered for the ABO Intended Incompatible Transplant protocol, verify the recipient meets the titers requirements as outlined in IV, A 5. In addition, verify the donor ABO subtypes as non-A1.

e. If the recipient meets all of these criteria, you must call the HLA Laboratory Director, followed by the on call surgeon and nephrologist. All persons must agree to proceed with this protocol. Document this information in the Final Crossmatch logbook, listing all persons called, times and dates.

f. Prior to proceeding to transplant, the **HLA Sensitization History for Transplant Patients** form must be filled out and signed by the recipient and witness as outlined in Section E, 10c and d below.

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3. **ABO Compatible and ABO Intended Incompatible 3 Month Remote Sera Final Physical Crossmatch for Kidney and Kidney/ Pancreas Recipients**

For this protocol, the date of the sample draw is Day 0.

a. To qualify for the 3 Month Remote Sera Final Physical Crossmatch protocol, the kidney or kidney/pancreas recipient, **MUST** have a serum sample dated within the prior 3 months from the day of planned transplant **AND**, have been PRA tested on a **minimum of two** Luminex Single Antigen PRA samples. Fewer than two PRA samples will require recipient to present to the hospital for Final Physical Crossmatch and PRA. The report of a sensitizing event, ie transfusion, pregnancy, or transplant with any human tissue or organ within the prior 4 months, requires discussion with the medical director to determine if we can proceed with this protocol or the recipient will revert to the Final Physical Crossmatch protocol.

b. Using this protocol, the Final Physical Crossmatch will take place prior to the recipient presenting to the hospital. Once the recipient presents to the hospital for transplant, crossmatch blood will be drawn (2-4 yellow top ACD-A tubes and 1 red top tube). If the allogeneic crossmatch is negative, no further testing is needed. If the allogeneic crossmatch is positive, an autologous flow crossmatch is required and is performed using this blood **but**, using same sera used on the allogeneic crossmatch. A PRA may be performed at the discretion of the medical director.

c. When the recipient is being considered for the ABO Intended Incompatible Transplant protocol, verify the recipient meets the titer requirements as outlined in IV, A 5. In addition, verify the donor ABO subtypes as non-A1.

4. **Final Virtual Crossmatch for Kidney/Liver:**
This section discusses virtual crossmatching for Kidney/ Liver recipients; a Final Physical Crossmatch for Kidney/Liver recipients is available upon request.

a. Prior to transplant, a Final Virtual Crossmatch must be performed for Kidney/Liver recipient to assess the presence of any donor directed HLA antibodies. Although donor directed antibodies are not a contraindication for transplant in Kidney/Liver recipients, identifying these antibodies is necessary to manage their care post-transplant. As such, a Luminex Class I and II PRA is performed on the recipient prior to, or upon listing, and again when the patient presents for transplant.

b. A patient may proceed to transplant with the Final Virtual Crossmatch based on a prior PRA result **if,** the sample was collected within the prior 3 months of planned transplant **and** the patient has had no sensitizing

events in the prior 4 months. If neither is the case, a PRA is performed using the sample drawn when the patient presents for transplant.

c. Upon acceptance of an organ offer, the Liver Hold test is ordered by the transplant clinician. The Liver Hold is not a laboratory test per se, rather serves to let the phlebotomist know what tubes to draw for the final virtual crossmatch and possible post-transplant testing. With this order, we will receive 1 red top tube for PRA and 2 yellow-top ACD-A tubes for buffy coat storage (place one ACD-A tube in the DNA rack).

d. Upon receipt of the sample, pull the liver hold test into Result Entry section in the LIS, answer and finalize. Next, add on an HLA CROSSMATCH - FC ALLO AND FC AUTO [LAB3451] to the Liver Hold order. If a PRA is required for the Final Virtual Crossmatch, add on the HLA PRA CLASS I AND II BY LUMINEX SINGLE ANTIGEN [LAB3417]. The Final Virtual Crossmatch is reported as outlined in section F.

e. When the Final Virtual Crossmatch is performed using the PRA sample drawn within the prior 3 months, no PRA is required. In this case, **the patient must sign the HLA Sensitization History for Transplant Patients form and this form must be received in the HLA lab prior to release of the Final Virtual Crossmatch report.**

f. A Final Physical Crossmatch for Kidney/Liver **is required when the Luminex is inoperable and the recipient had a sensitizing event within the prior 4 months or when the Luminex is inoperable and the most recent PRA sample is greater than 3 months old**. The crossmatch may be performed concurrent with surgery. When a crossmatch is required, notify the surgeon immediately. Results of the crossmatch must be reported to the surgeon and transplant nephrologist as outlined in section E below.

g. ABO B or O Kidney/Liver recipients may receive an ABO Intended Incompatible Transplant but are not required to have anti-A IgG titers. The strength of the Anti-A titer in these recipients is not a contraindication for transplant. Blood Bank personnel are responsible for ordering and reporting ABO typing and subtyping on Kidney/ Liver and Liver donors.

D. **Testing Required for ABO Compatible and ABO Intended Incompatible Final Physical Crossmatch, 3 Month Remote Sera Final Physical Crossmatch and Final Virtual Crossmatch for Kidney and Kidney/ Pancreas Recipients**

When the Flow Cytometer is inoperable, contact the UTSWMC laboratory as outlined in in FLF 1.19.A Memorandum for Instrument Downtime in the Flow Cytometry procedure manual.
All recipients chosen for Final Physical Crossmatch will receive both Flow Cytometric allogeneic and autologous T cell and B cell crossmatches, with the

exception of the 3 Month Remote Sera Final Physical Crossmatch as outlined below.

1. Each Final Physical Crossmatch will include 2 serum samples:
   Final crossmatch sample –a serum sample drawn at the time the recipient presents to the hospital for transplant with a current donor **OR** a sample that has been collected within 3 months of planned transplant, provided the recipient qualifies as described above and by transplant/pregnancy/transfusion history.

   Peak serum sample - recipient serum sample showing the highest sensitization/ most number of identified antibodies for Class II followed by Class I. If multiple serum samples show equal number of antibodies, choose the one with the higher MFIs. If partial donor directed antibodies appear on a sera, choose this sample as the peak (again sera with highest MFI of partial DSA should be selected).

   Remote serum sample – in rare circumstances a third serum sample will be used for crossmatch. For example, a recipient has allelic donor directed antibody for both Class I and II seen on separate serum samples. One sample may be used as the peak and the other as a remote/ third sample. Consult with laboratory director/ design when other circumstances occur that may require a remote sample

   Serum sample locations are found in the LIS, or written on the requisition attached to the PRA report. For samples moved to remote R boxes, the locations are found in the LIS or the H: drive in the R box location folder.

2. For Final Physical Crossmatch perform a Flow Allogenic and Autologous crossmatch using the final crossmatch sample and peak serum samples; remote sample where applicable.

3. For 3 Month Remote Sera Final Physical Crossmatch perform a Flow Allogenic crossmatch using a sample that has been collected within 3 Months of planned transplant and a peak serum sample; remote sample where applicable.

   **Recipients qualifying for the 3 Month Remote Sera Final Physical crossmatch do not require an autologous Flow crossmatch unless the allogeneic crossmatch is positive. Notify the transplant coordinator on call if the auto crossmatch is required.

4. For Final Virtual Crossmatch, no physical crossmatch is performed, although blood for crossmatch is drawn and processed as outlined in Section E, 13 below. A Final Crossmatch report is released as outlined in E, 10c and d below.

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E. **Reporting Final Crossmatches**

1. Unusual crossmatch results must be discussed with the HLA Medical Director who will advise how to proceed and any comments to add to the crossmatch report. These can include positive allogeneic crossmatch with positive autologous crossmatch or positive crossmatch with no known donor directed antibody, etc.

2. For recipients receiving a Final Physical Crossmatch, orders for HLA CROSSMATCH FC ALLO AND FC AUTO [LAB3451], are placed in the LIS by the treating physician.

3. For recipients crossmatched using the 3 Month Remote Sera Final Physical Crossmatch protocol, HLA staff will place the order in the LIS for final crossmatch, as the crossmatch is performed and reported prior to the recipient presenting to the hospital. Using Requisition Entry, order HLA CROSSMATCH FC ALLO AND FC AUTO [LAB3451]. Additionally, notify the Transplant Charge Auditor, who will move the final crossmatch charges to the correct encounter.
   When the recipient is admitted for transplant, the treating physician will order the HLA CROSSMATCH FC ALLO AND FC AUTO [LAB3451]. The blood drawn in association with the physician ordered final can be used for autologous flow crossmatch and/or PRA, as needed. Regardless, the final crossmatch report is submitted using the crossmatch ordered by the HLA staff. Ultimately, the crossmatch ordered by the physician is cancelled; however, the sera drawn with this crossmatch is stored in the TXP box as described in step 13. Serum can be stored even if crossmatch is cancelled.

4. Prior to the release of the final crossmatch report for kidney, kidney/pancreas transplant, the donor ABO, and ABO subtyping where appropriate, must be verified using a sample drawn at the time of organ harvest and/or received with the organ. The recipient must have an ABO result from blood drawn when they present to the hospital for transplant.

5. Upon completion of the Final Physical and 3 Month Remote Sera Final Physical Crossmatches, results must be called to the on call MDMC transplant coordinator, Nephrologist and Surgeon, with read back. When calling the results of a final crossmatch using 3 Month remote sera, report the sample date used for crossmatch.
   Calls to each person are required as is documentation in the Final crossmatch Logbook.

6. Kidney/ Liver Final Virtual Crossmatch results **must** be called to the on call nephrologist, with read back.

7. Record all communication, test results, date, time and names of personnel notified in the Final Crossmatch logbook.

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8. For crossmatch compatible kidney and kidney/pancreas recipients, a final crossmatch report must be present on the recipient's hospital chart prior to surgery. And, any recipient proceeding to transplant with the 3 Month Remote Sera Final Physical Crossmatch or Final Virtual Crossmatch **must sign the HLA Sensitization History for Transplant Patients form verifying they have had no sensitizing events. This form must be received in the HLA lab prior to release of the final crossmatch report.** A copy of this form remains in the recipient's HLA chart and the original form is sent to the transplant floor or the OR, along with the final crossmatch and ABO reports.
This form is also required when a transplant will take place greater than three days from the time of final crossmatch blood draw.

9. Kidney/ Liver Final Virtual reports are typically available prior to transplant; however, transplant may proceed prior to completion of the testing and reporting. When the patient proceeds to transplant with a Final Virtual Crossmatch based on PRA drawn within the prior 3 Months, the recipient **must sign the HLA Sensitization History for Transplant Patients form and this form must be received in the HLA lab prior to release of the final crossmatch report.**

10. Each final crossmatch is reported using the LIS Pre-Transplant-Final Crossmatch report, assessing compatibility between the recipient and donor. How the final is reported is dependent on the crossmatch protocol employed. **When Epic is unavailable for crossmatch reporting, use the downtime HLA crossmatch report as discussed in FL1.19 Flow Cytometry Cross Match for Solid Organ Transplantation.**

    a. Reporting **ABO compatible Final Physical Crossmatch or 3 Month Remote Final Physical Crossmatch for Kidney, Kidney/Pancreas Recipients:**
    Using the LIS Pre-Transplant-Final Crossmatch report, fill out as follows:

        i. Use the Current Serum date and associated Allo and Auto T and B cell result fields to report the final crossmatch serum sample or 3 Month remote sample. For the 3 Month serum sample, it is possible this sample might be the patient's peak serum; however, the crossmatch result of this sample is always considered the "current" serum. In this instance, make a note in the Comments section of the report, that the 3 Month remote sample is also the patient's peak sample.
        Fill out the result fields for Peak Serum Date and when applicable, Remote Serum Date and associated Allo and Auto T and B cell result(s).
        Answer No to the result for "Qualifies for Final Virtual Crossmatch".

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ii. Once crossmatch results are entered, at the HLA XM Report result line, pull in and complete the HLAFINALXM crossmatch template. Final verify the crossmatch report and send a copy of this report along with copies of the recipient and donor ABO and HLA typings to the transplant floor or the OR. In addition, for 3 Month Remote Final Physical protocol patients, the **HLA Sensitization History for Transplant Patients** form must be received in lab prior to release of the final report, and is also sent to the transplant floor or the OR. A copy of the form remains in the recipient's HLA chart.

b. Reporting **ABO Intended Incompatible Final Physical or ABO Intended Incompatible 3 Month Remote Final Physical Crossmatch for Kidney, Kidney/Pancreas:**

i. Using the LIS Pre-Transplant-Final Crossmatch report, follow step a.,i above.

ii. Once crossmatch results are entered, at the HLA XM Report result line, pull in and complete the HLAFINSUBA crossmatch template The HLAFINSUBA report contains an ABO compatibility chart for this protocol, including questions verifying ABO titer and subtyping.
Final verify the crossmatch report and send a copy of this report along with copies of the recipient HLA typing, ABO and most recent Anti-A IgG titer, donor HLA typing and ABO/ ABO Subtype results to the transplant floor or the OR. In addition, for 3 Month Remote Final Physical protocol patients, the **HLA Sensitization History for Transplant Patients** form must be received in lab prior to release of the final report and, is also sent to the transplant floor or the OR. A copy of the form remains in the recipient's HLA chart.

iii. For recipients receiving an ABO Intended Incompatible transplant, **notify Blood Bank with the recipient's name** so they can place an alert on the patient's record to give the correct type of plasma/platelets

c. Reporting **ABO Compatible Final Virtual Crossmatch for Kidney and Kidney/ Pancreas Recipients**

i. Using the LIS Pre-Transplant-Final Crossmatch report, answer Yes to the result for "Qualifies for Final Virtual Crossmatch". Leave the Current, Peak and Remote serum dates and crossmatch fields blank (when the report is finalized, those answer spots will not appear on the report). At the HLA XM Report result line, pull in and complete the HLAFNLNOXM template. This template has pre-printed

comments discussing the Final Virtual Crossmatch requirements.

ii. Final verify the crossmatch report and send a copy of this report along with copies of the recipient and donor ABO, HLA typings and the completed **HLA Sensitization History for Transplant Patients form** to the transplant floor or the OR. A copy of this form remains in the recipient's HLA chart

d. Reporting the **ABO Intended Incompatible Transplant Final Virtual Crossmatch for Kidney and Kidney/ Pancreas Recipients**

i. Using the LIS Pre-Transplant-Final Crossmatch report, answer Yes to the result for "Qualifies for Final Virtual Crossmatch". Leave the Current, Peak and Remote serum dates and crossmatch fields blank (when the report is finalized, those answer spots will not appear on the report). At the HLA XM Report result line, pull in and complete the HLAFINSUBANOXM template. This template has pre-printed comments discussing the Final Virtual Crossmatch requirement and ABO compatibility chart for this protocol, including questions verifying ABO titer and subtyping.

ii. Final verify the crossmatch report and send a copy of this report along with copies of the recipient HLA typing, ABO and most recent Anti-A IgG titer/DTT treated, and donor HLA typing, ABO/ ABO Subtype along with the completed **HLA Sensitization History for Transplant Patients form** to the transplant floor or the OR. A copy of this form remains in the recipient's HLA chart.

iii. **Notify the Blood Bank with the recipient's name** so they can place an alert on the patient's record to give the correct type of plasma/platelets.

e. Reporting **Crossmatch Incompatible Kidney, Kidney/ Pancreas** recipient:

i. Using the LIS Pre-Transplant-Final Crossmatch report, fill out the Current and Peak Sera Date fields along with the associated Allo and Auto T and B cell result fields. Answer No to the result for "Qualifies for Final Virtual Crossmatch".

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ii. At the HLA XM Report result line, pull in and complete the appropriate crossmatch template for your donor/ recipient pair. Do not verify out the report.

iii. Attach copies of the recipient and donor ABO and HLA typings to the folder. The laboratory director or designee will review and final out the crossmatch report.

f. Reporting the ***Kidney/Liver Final Virtual Crossmatch protocol:***

i. Using the LIS Pre-Transplant-Final Crossmatch report, answer Yes to the result for "Qualifies for Final Virtual Crossmatch". Leave the Current, Peak and Remote serum dates and crossmatch fields blank (when the report is finalized, those answer spots will not appear on the report). At the HLA XM Report result line pull in and complete the LAB HLA FINAL KL REPORT – NO XM template.

ii. If no donor directed antibodies are identified, answer as follows:
'The virtual final crossmatch using sera dated xx/xx/xx was performed and found to be Negative.'

iv. If donor directed antibodies are present:
'The final virtual crossmatch using sera dated xx/xx/xx, was performed and found to be Positive'.
List each donor directed antibody, along with the highest MFI for each antibody. When there are multiple beads for a particular antigen, report the highest MFI within that series of beads.
Lastly, add the comment 'Recipient HLA type is unknown'. (This is added because we are making the assumption that the antibodies are donor directed as we do not know recipient's HLA type.)

v. The final report does not include HLA typing information for the recipient, as we routinely do not type kidney/ liver recipients. The ABO donor typing is reported by Blood Bank and is not included on this final report.

vi. Once completed, final verify the crossmatch report. For Kidney/ Liver, the crossmatch report is not sent to the transplant floor or the OR. However, the results must be called to the nephrologist on call for transplant. Report the sera date, all DSA and the MFI information.

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REMEMBER, when the Final Virtual Crossmatch is performed using a PRA drawn within the prior 3 Months, **the patient must sign the HLA Sensitization History for Transplant Patients form and this form must be received in the HLA lab prior to release of the Final Virtual Crossmatch report.**

vii. When a **Final Physical Crossmatch for Liver/ Kidney** is **requested or required** (i.e. when the Luminex is inoperable):

Unless otherwise requested, crossmatch may take place concurrent with transplant surgery.
In all cases below, it is not necessary to notify the laboratory director of unusual crossmatch results.

a) Using Requisition Entry, order HLA CROSSMATCH FC ALLO AND FC AUTO [LAB3451]. Using the LIS Pre-Transplant-Final Crossmatch report, use the Current Serum date and associated Allo and Auto T and B cell result fields to report the final crossmatch serum sample and at the HLA XM Report result line, pull in and complete the HLAFINALXM crossmatch template according to step b-e below.

b) If the crossmatch is negative, answer crossmatch template per protocol noting in the comments the recipient is receiving a liver/kidney transplant. Results of the Final Physical Crossmatch will be reported to the liver transplant coordinator, surgeon and nephrologist.
Final verify the crossmatch report and send a copy of this report to transplant floor or the OR.

c) If the crossmatch is positive and donor directed antibodies from are present, answer the comments section of the report as follows, 'A PRA dated xx/xx/xx shows a donor directed antibody xxx, with an MFI of xxx, as such, the positive crossmatch is not unexpected. These results do not preclude kidney/ liver transplant from this donor to this patient.' And add statement 'Recipient HLA type is unknown'.
Results of the final crossmatch are reported to the liver transplant coordinator, surgeon and nephrologist.

Final verify the crossmatch report and send a copy of this report to transplant floor or OR.

d) If the crossmatch is *positive* and there are ***no*** donor directed antibodies to explain positivity, answer the comments section of the report as follows:
'A PRA dated xx/xx/xx on this patient shows no donor directed antibodies to explain the positive crossmatch; however, this does not preclude kidney/ liver transplant from this donor to this recipient. A specificity PRA will be performed on the sample used for crossmatch to determine if novel donor directed antibodies are present.'

Results of the final crossmatch are reported to the liver transplant coordinator, surgeon and nephrologist.
Final verify the crossmatch report and send a copy of this report to transplant floor or the OR.
If PRA is preformed to explain unexpected crossmatch result, report findings to the on call transplant nephrologist.

11. For all organ types, once final crossmatch reports are sent to the transplant floor or OR, call and verify their receipt. Note all communications in the Final Crossmatch Logbook.

12. A copy of the final crossmatch reports and related documents are attached to the recipient's HLA folder and must be reviewed by the laboratory director or designee during the next business day of regular laboratory operation.

13. **All** sera used in the final crossmatch must be frozen and stored **in the TXP** (transplanted) sera box for a minimum of 5 years. Note the location of the serum sample in the LIS.

a. Recipients receiving a Final Physical Crossmatch will have <u>both</u> the current and the peak sera stored the TXP box. If a remote sample was tested, store this as well.

b. Recipients receiving a 3 Month Remote Sera Final Physical Crossmatch will have <u>three</u> sera stored in the TXP box; the 3 Month remote sample, the peak sample and the serum sample drawn when the recipient presented to the hospital for transplant. The latter sample may be needed for future testing. If a remote sample was tested, store this as well.

c. For Kidney/ Liver recipients proceeding to transplant based on a Final Virtual Crossmatch from a PRA drawn within the prior 3 Months: pull this

serum sample from its T location and store in a TXP location along with the serum sample collected when the recipient presented for transplant.

For Kidney/ Liver recipients transplanted with a Final Virtual Crossmatch based on the PRA drawn when the patient presents for transplant, file this sample in a TXP location.

14. Place a sample from the donor (ACD-A tube or isolated lymph/spleen cells) in the DNA rack for buffy coat storage. This donor sample is saved for a minimum of 5 years. Note the location of the sample in the LIS.

G. **PROCEDURE NOTES:**

If there is a typing discrepancy between laboratories, a variance report describing the discrepancy is signed by laboratory director.

H. **REFERENCES:**

N/A

I. **RELATED PROCEDURES:**

FL 1.19 Flow Cytometry Cross match for Solid Organ Transplantation
FLF 1.19.G HLA Downtime Crossmatch Forms
HLA 4.11 LABScreen (One Lambda) Class I and Class II Single Antigen Assay