HLA TESTING FOR LIVING DONOR TRANSPLANTATION

PROCEDURAL REVISION

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I. PRINCIPLE:

Candidates for transplantation and their Living Donor, Altruistic, Non Domino Therapeutic or Kidney Paired Donor are referred to the MDMC HLA Laboratory through The Transplant Institute at Methodist Dallas. Each potential recipient and donor receive a specified number of tests including ABO, HLA tissue typing and crossmatching. The purpose of the following procedure is to delineate the laboratory testing protocol for the Living Donor / Recipient work-up.

II. SPECIMEN:

Recipient, for both preliminary and final crossmatch:
2-4 10ml yellow top ACD tubes
7mL red top tube (either no anticoagulant or serum separator only)
1 EDTA (at least 7 mL)

Donor:
Pre-Transplant:
3-4 10mL yellow top ACD tubes
1 EDTA (at least 7 mL)

Upon admission for donation:
1 EDTA (at least 7 mL)
7mL red top tube (either no anticoagulant or no serum separator only)

III. REAGENTS, EQUIPMENT, AND SUPPLIES:

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

IV. PROCEDURE:

A preliminary workup for donor and recipient is outlined below. The types of testing depend on a number of factors as discussed herein.

A. **LIS Orders for recipient and donor preliminary workup:**
Recipient:

i. If ABO has not been established on the recipient, order an ABO, Rh type [LAB895]. If a second confirmatory ABO is requested, order [LAB6965] Transplant Evaluation ABO/RH No Charge.

ii. For blood type B and O recipients: if the potential donor's ABO is A or AB we will order subtyping as described below. If donor subtypes as A, non-A1 or AB, A non-A1B, we need to assess the recipient's Anti-A titer to determine if they qualify for the ABO Intended Incompatible Transplant protocol. If an Anti-A DTT Treated titer has not been established previously, HLA will order TRANSPLANT ANTI-A DTT TITER BY GEL [LAB5789].

iii. HLA Crossmatch – Flow Allo and Flow Auto [LAB3451]

iii. If HLA Typing has not been established on the recipient, order HLA TYPE

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FOR ABC AND DRDQDP BY SSO [LAB3438].

iv. PRA as needed, order HLA PRA CLASS I AND II BY LUMINEX SINGLE ANTIGEN (AKA HLA PRA LUSAG) [LAB3417].

Donor:

i. ABO, Rh type, ABO/RH [LAB895].

ii. HLA will request ABO subtyping for ABO A or AB donors, when recipient is ABO B or O. Order TRANSPLANT A SUBTYPE [LAB4100].

ii. HLA TYPE FOR ABC AND DRDQDP BY SSO [LAB3438] as outlined in step B below.

iv. Living Donor Crossmatch [LAB4374] (ordered only when donor is drawn in the MDMC Outpatient lab). LAB4374 is not a laboratory test per se, rather serves to let the phlebotomist know what tubes to draw on the living donor. The living donor crossmatch will appear on the HLA Outstanding list. To result and finalize this test, click on the test and then click on the pencil icon next to the test name on the right side of the screen. Choose Yes in the Value box and final verify the result.

B. **HLA typing for recipient and donor**:

i. ABO and crossmatch compatible donors will be HLA typed. Typing is performed by molecular SSO low or intermediate resolution typing for HLA A, B, C, Bw4, Bw6, DRB1, DRB3, 4, 5, DQA1, DQB1, DPA1 and DPB1.

ii. HLA typing will not be performed on crossmatch incompatible donors unless:

- Immunologic intervention is considered through the Desensitization protocol
- The donor/ recipient pair preliminarily qualifies for the ABO Intended Incompatible Transplant protocol.
- Upon request for donor consideration in Altruistic, Non Domino Therapeutic or the Kidney Paired Donor exchange.

Regardless, a buffy coat is collected and stored on all ABO incompatible donors for possible testing as stated above.

iii. When HLA typing of the recipient or donor is known from previous work-up in the MDMC HLA laboratory or a referring laboratory, consult HLA 2.17 Receiving Samples and Ordering Tests, Section B, for retyping protocol.

iv. When HLA typing and/or PRA is performed by another laboratory, the results are accepted upon director approval. These results should be faxed to the MDMC HLA laboratory or identified through Epic Care Everywhere or Epic chart notes. To document this information in the patient's LIS chart, use Requisition

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Entry, to order and answer HLA PRA Class I And II by Luminex Single Antigen No Charge [LAB5748], and/or HLA TYPE CLASS I AND II NO CHARGE [LAB3429] (Do not answer ABO field in this instance). Submit the results to the laboratory director for review and approval.

C. **Testing for the ABO Intended Incompatible Protocol:**
Living donors who are ABO incompatible with the intended recipient, as noted below, may be eligible for the ABO Intended Incompatible Transplant protocol, also known as the A_{sub} or $A_{sub}B$ protocol. Request Blood Bank perform ABO A subtyping (TRANSPLANT A SUBTYPE [LAB4100]) on the following potential living donors:

Donor is A and recipient is B
Donor is AB and recipient is B
Donor is A and recipient is O

i. If the donor types as ABO A, non-A1 or AB, A non-A1B, the recipient will be assessed for Anti-A titer by placing an order in the LIS for TRANSPLANT ANTI-A DTT TITER BY GEL [LAB5789]. Titer results of ≤4 allow for preliminary eligibility in the protocol and the recipient/ donor pair will proceed to preliminary crossmatch. If the recipient's Transplant Anti-A DTT titer is >4, the pair cannot be considered for this protocol.

Please NOTE Any titer results preformed with the test INDIRECT ANTIHUMAN GLOBULIN TITER [LAB275] were NOT performed using DTT and the results should NOT be assessed when determining eligibility for this protocol.

Acceptable titer results include Ref Transplant ABO Antibody DTT Titer and/ or TRANSPLANT ANTI-A DTT TITER BY GEL.

ii. For crossmatch compatible pairs initially qualified for the ABO Intended Incompatible protocol, the following applies:

a. Recipient - After initial qualifying titer, two additional titers must be performed (preferably monthly). The HLA Lab will request blood for titer from the recipient's dialysis center or notify the Transplant Coordinator to place the order. To remain in consideration for this protocol, all titers must be ≤4. Any result >4 will disqualify, or require immunologic intervention to continue in this protocol. The transplant coordinator should be notified of these results. Subsequent to the qualifying 3 titers, the Transplant Anti-A DTT Titers must be performed per the schedule required by UNET (every 90 days +/- 20 days).

b. Donor - A second ABO with subtype must be performed on a separate sample, preferably prior to presentation for final crossmatch. Notify the Transplant Coordinator to order the test and cc HLA manager. Any discrepancy in ABO subtype should be brought to the immediate attention of the laboratory director/designee for investigation. Notify transplant coordinator, as needed.

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c. Any recipient and living donor pair listed for Kidney Paired Donation, who meet the above criteria, may be considered for KPD non-A1 program.

D. **Preliminary and Final Crossmatch Protocols**:

i. The following tests are performed:
Flow Cytometric allogeneic and autologous T cell and B cell crossmatches,
LIS order: Flow Allo and Flow Auto [LAB3451]
The workup may proceed in the absence of the autologous crossmatch; however, if the allogeneic crossmatch is positive, an autologous flow crossmatch must be performed (using the same serum sample(s) as on allo crossmatch) to determine compatibility and the need for perform donor HLA typing.
Note, Preliminary Crossmatch testing should occur within 48 hours of blood draw, but may exceed 48 hours if cell viability is ≥ 80%.

Recipient PRA where applicable.

NOTE: When Epic is unavailable for crossmatch reporting, use the downtime HLA crossmatch report as discussed in FL 1.19 Flow Cytometry Cross Match for Solid Organ Transplantation.

ii. Serum sample selection and crossmatch scenarios are as follows:
Two samples should be used for the crossmatch.
Current crossmatch sample –a serum sample drawn at the time the recipient presents for testing with a current donor. If recipient current blood is not available when donor blood arrives, select a remote sample. Consult director or designee.

Peak serum sample - recipient serum sample showing the highest sensitization/ most number of reactive beads for Class II followed by Class I. If multiple serum samples show equal number of reactive beads, choose the one with the higher MFIs. When recipient and donor HLA typing are available, and partial/ allele specific donor directed antibodies are noted, the peak serum sample showing the highest MFI for this partial/ allele specific antibody should be tested.

If the patient is non-sensitize, choose a serum sample dated with in the proceeding few months (i.e. a remote sera).

Additional Peak 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 will be used as the peak and the other as an additional peak. Consult with laboratory director/ design when other circumstances occur that may require this type of 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 can be found in the LIS or the H: drive in the R box location folder.

iii. As discussed in section E below, Altruistic, Non-Domino and KPD donor/

recipient pairs have unique identifiers to maintain confidentiality. As such, when performing the preliminary crossmatch, follow instructions on how to identify the donor **on all flow histograms, Flow Crossmatch and LIS reports.**

E. **Reporting Preliminary Crossmatches**

Complete a crossmatch report in the LIS for all recipient/living donor pairs, regardless of ABO or crossmatch compatibility. For the preliminary crossmatch, the technologist performing the crossmatch is responsible for filling out the Preliminary Flow Crossmatch Result portion of the report. The technologist performing the HLA typing will complete the Preliminary Living Donor Crossmatch Summary section of the report. This involves assessing any aberrant crossmatch result or any donor directed antibodies. When there is a positive allogenic crossmatch result with a positive autologous crossmatch, verify on the Lyric report if the auto result accounts for the allo result and make note of this to the medical director.
When there is a negative preliminary crossmatch in the presence of a donor directed antibody, let the medical director know if there are any donor director antibodies or any partial/allele specific antibodies. If the antibodies are DQA, DPA, or DPB include the highest MFI with the note to the medical director.

i. **For all scenarios listed below**, order, the **HLA PRELIMINARY Flow Crossmatch Report** by clicking on the Prelim Flow XM Result button in the HLA Crossmatch – Flow Allo and Flow Auto report. Complete and final verify this report by the next business day following crossmatch. The report acts as a reference for the transplant coordinator in managing the donor/ recipient workup. This report is completed on all workups regardless of ABO or crossmatch compatibility. Instructions for completing this report are outlined in steps iii-xi below, according to recipient/ donor type.

In all scenarios below, when completing the Preliminary Living Donor Crossmatch Summary section of the report and answering the question 'Has the donor/recipient pair been evaluated for the presence of donor directed antibodies', the answer is always Yes. In instances where donor directed antibodies are present, list the DSAs, and attach a note to the crossmatch to make the medical director aware of the antibodies. The medical director will assess significance of the antibodies and comment accordingly.

ii. For those crossmatches that require the HLA Crossmatch – Flow Allo and Flow Auto report, there is a question 'List known antigens that this donor possesses to which this recipient has been previously exposed through transplant:'. To determine if the recipient is a regraft (has had any prior transplant, solid organ or otherwise), and access prior donor typing, follow the steps below.
Log into UNET https://Portal.Unos.org
- 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
- Choose Waitlist
- Click in Transplant hospital field and choose TXMC Methodist Dallas Medical Center – Transplant Hospital
- Click on the Search waiting list icon

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- Enter recipient's SS# or name and click on Search.
- Click on the SSN number to bring up recipient information.
- Refer to the section entitled Kidney Organ Information. If the recipient has been previously transplanted, there will be information as to the Organ, Transplant Date and Graft Fail Date.
- To determine previous transplant antigens, return to the main UNET log in screen, Choose Waitlist and choose Transplant hospital followed by Run a Report
- Scroll to the bottom and choose Prior donor HLA report and enter the candidates SSN, click search.
- The donor HLA type will come up and it is possible there will be multiple entries, i.e. one entry for each organ and/or one entry for each prior donor type.

For recipients not yet Waitlisted, prior transplants may be listed in the 'Transplant Episode History' section of the recipient's Phoenix chart or, contact their Transplant Coordinator for this information and prior donor's HLA type.

iii. For **ABO Incompatible pairs** for which *no* crossmatch is performed:

a. Complete and final verify the HLA *Preliminary Flow Crossmatch Report*, in addition, note, when applicable, if pair was assessed for the ABO Intended Incompatible Protocol, and this result.

b. Next, using the Preliminary Living Donor Crossmatch Summary report, leave all Current/Remote/Peak crossmatch results **blank,** (when the report is finalized, these answer spots will not appear on the report). Under the comments section of the report, answer 'the recipient/donor pair is ABO incompatible', and note, when applicable, the pair was assessed for the ABO Intended Incompatible Protocol.

c. **Altruistic/ Non-Domino/ Kidney Paired Donor/ Recipient pairs** should be ABO compatible, as ABO is considered as part of the match run. In the unlikely event the pair is ABO incompatible, refer to the scenarios below for reporting and proper donor identification.

iv. For **crossmatch incompatible pairs**:

a. Complete and final verify the HLA *Preliminary Flow Crossmatch* Report, and note, when applicable, the pair was assessed for the ABO Intended Incompatible Protocol, and this result.

b. Next, using the Preliminary Living Donor Crossmatch Summary report, fill out the Current/ Peak Sera and if applicable, additional Peak Sera results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the appropriate crossmatch report depending on the type of recipient/donor pair, taking care to use the appropriate donor identification outlined below. Do not verify out the report. The report will be reviewed and final verified by the HLA medical director.

v. For **ABO/ Crossmatch compatible pairs**:

a. Complete and final verify the HLA *Preliminary Flow Crossmatch* Report.

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b. Next, using the Preliminary Living Donor Crossmatch Summary report, fill out the Current/ Peak Sera results and if applicable, Additional Peak Sera results for the auto and allo crossmatches and at the HLA XM Report result line pull in and complete the *HLAPRELIM* crossmatch report.

c. Order and HLA type HLA TYPE FOR ABC AND DRDQDP BY SSO [LAB3438] on the donor (and recipient if not previously typed).

vi. For **ABO Intended Incompatible Protocol pairs**:

a. Complete and final verify the HLA *Preliminary Flow Crossmatch* Report noting that the pair preliminarily qualifies for the ABO Intended Incompatible Transplant protocol, and noting the recipient's Anti-A titer and donor's ABO subtype result. As stated previously, the recipient will need two additional titers of ≤4 to officially qualify for the protocol.

b. Next, using the Preliminary Living Donor Crossmatch Summary report, fill out the Current/ Peak Sera and if applicable, Additional Peak Sera results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the *HLAPRESUBA* crossmatch report. This report contains an ABO compatibility chart for this type of transplant and additional questions verifying ABO titer and subtyping. In the comments section of the report, note that the pair preliminarily qualifies for the ABO Intended Incompatible Transplant protocol.

c. Order and HLA type HLA TYPE FOR ABC AND DRDQDP BY SSO [LAB3438] on the donor (and recipient if not previously typed).

vii. For all **Altruistic/ Non Domino Therapeutic/ Kidney Paired Donors, donor information (name, social security number, any other combination of information felt to lead to donor identification) will not be released to the recipient or placed in the recipient's medical record.**
To ensure donor confidentiality, each type of recipient and donor has unique identifiers used in lieu of name and medical record number.
Follow instructions very carefully.

viii. For the **Altruistic** or **Non Domino Therapeutic Donor/ Recipient pairs**:
This type of donor/ recipient pair will be ABO compatible. The unique identifier used when reporting results on this type of donor is their **UNOS number** assigned upon the match run and emailed to the HLA lab by transplant office personnel.

a. Print the donor HLA type from UNET using the UNOS number/ Match ID supplied by the transplant coordinator. Order and answer the donor HLA TYPE CLASS I AND II NO CHARGE [LAB3429] in their LIS chart. The donor's LIS chart has their name (later we will create a chart with the UNOS

number and link the charts through aliases). At this point, notify the living donor transplant coordinator to add the donor's UNOS Number as an alias to their LIS chart.

b. When performing the preliminary crossmatch on this type of workup, use the **donor's UNOS Number** on **all flow histograms** and **the flow reports**. Donor **DOB** will act as the second unique identifier.

c. When completing the HLA *Preliminary Flow Crossmatch* Reports, in lieu of donor name, use the **donor's UNOS Number. Donor DOB** will act as the second unique identifier on this report. Additionally we will include the recipient's Waitlist ID on the report. This is done in order to have a link between this preliminary crossmatch report and the final crossmatch and HLA Living Donor ABO reports.
To locate the recipient's Waitlist ID:
   - Log into UNET, choose Waitlist, click on the Transplant hospital, top right corner and select TXMC as the institution; then choose Search waiting list
   - Enter recipient SS# or name, click search
   - To view the recipient's Kidney Candidate screen, click on SSN. The Waitlist ID is found next to Demographic Information in the second section.

d. Next, using the Preliminary Living Donor Crossmatch Summary report, fill out the Current/ Peak Sera and if applicable, Additional Peak Sera results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the *HLAPRELIM* crossmatch report. When completing this portion of the report:
   - The recipient's Waitlist ID (not including the numbers in parenthesis), will need to be added to the report; add this as a line above the Recipient ABO
   - Answer the donor Name: as N/A
   - Answer 'ID:' using **the donor's UNOS Number**
   - Donor **DOB** serves as the second unique identifier.
   - For Relationship, enter Altruistic or Non Domino Therapeutic, accordingly.
   - When filling out the Comments section**, use donor's UNOS Number in lieu of name.**

ix. For **ABO Compatible Kidney Paired Donor / Recipient pair,**

a. When performing the preliminary crossmatch on this type of transplant pair, use the **donor's KPD ID** on all **flow histograms** and **the flow reports. Donor DOB** will act as the second unique identifier.

To locate the KPD ID and HLA typing of the donor:
   - Contact the Living Donor Transplant Coordinator and ask for the Exchange Number for the donor. Log into UNET, choose KPD
   - Click on Matches and enter the Exchange number

- Open the donor record and the KPD Candidate ID will appear at the top of the screen next to the donor name.
- Click on the HLA tab to view and print the donor HLA typing.

b. Order and answer the donor HLA TYPE CLASS I AND II NO CHARGE [LAB3429] in their LIS chart. The donor's LIS chart is created using their name. Because we identify the donor by their KPD ID in the crossmatch report, this KPD ID should be added to their LIS chart as an alias. Notify the living donor transplant coordinator to add the KPD ID.

c. When completing the HLA *Preliminary Flow Crossmatch* Reports, use the **donor's KPD ID** in lieu of donor name. **Donor DOB** will act as the second unique identifier on this report. Additionally we will include the **recipient's KPD ID** on the reports. This is done in order to have a link between this preliminary crossmatch report and the final crossmatch and HLA Living Donor ABO reports, when the pair proceed transplant. Locating the Recipients KPD ID differs slightly from the donor's:
   - Log into UNET and choose KPD
   - In the Candidate and Donor Eligibility section click on Eligible Candidate(s)
   - On the following screen locate your recipient name and to the left of the name you will find the KPD candidate ID

d. Next, using the Preliminary Living Donor Crossmatch Summary report, fill out the Current/ Peak Sera and if applicable, additional Peak Sera results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the *HLAPRELIM* crossmatch report. When completing this portion of the report:
   - The recipient's KPD Candidate ID, will need to be added to the report; add this as a line above the Recipient ABO
   - Answer the donor Name: as N/A
   - Answer "ID:' using **donor's KPD ID**
   - **DOB** is used as the second unique identifier.
   - Answer the 'If Living Related' line as N/A
   - For Relationship, enter Kidney Paired Donor.
   - When filling out the Comments section, **donor's KPD ID will be used in lieu of name.**

x. For **ABO Intended Incompatible Kidney Paired Donor/ Recipient pairs**

a. In order for the recipient to qualify for this type of KPD transplant, the recipient will have already had three Transplant Anti-A DTT titers and have been listed in the KPD as qualifying for the protocol. Verify that the most recent Transplant Anti-A DTT titer date is within the prior 90 days (+ / - 20 days). If not, a titer must be ordered.

b. Order and answer the donor HLA TYPE CLASS I AND II NO CHARGE [LAB3429] in their LIS chart. The donor's LIS chart is created using their

name. Because we identify the donor by their KPD ID in the crossmatch report, this KPD ID should be added to their LIS chart as an alias. Notify the living donor transplant coordinator to add the KPD ID.
See section ix a. above to locate KPD ID and donor HLA typing.

c. When performing the preliminary crossmatch on this type of transplant pair, use the **donor's KPD ID** on all **flow histograms** and **the flow reports**. **Donor DOB** will act as the second unique identifier.

d. When completing the HLA *Preliminary Flow Crossmatch* Report, in lieu of donor name, use the **donor's KPD ID**. **Donor DOB** will act as the second unique identifier on this report. Note on the report that the pair qualifies for the ABO Intended Incompatible Transplant protocol and list recipient Transplant Anti-A DTT titer and donor ABO subtype result.
Additionally we will include the **recipient's KPD ID** on the report. This is done in order to have a link between this preliminary crossmatch report and the final crossmatch and HLA Living Donor ABO reports, should the pair proceed transplant. See ix c. above for instruction.

e. Next, using the Preliminary Living Donor Crossmatch Summary report, fill out the Current/ Peak and if applicable, Additional Peak results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the *HLAPRESUBA* crossmatch report. This report contains an ABO compatibility chart for this type of transplant and additional questions verifying ABO titer and subtyping.
When completing this portion of the report:
   - The recipient's KPD Candidate ID will need to be added to the report; add this as a line above the Recipient ABO.
   - Answer the Donor Name: as N/A
   - Answer 'ID:' using **donor's KPD ID**
   - **DOB** is used as the second unique identifier.
   - Answer the 'If Living Related' line as N/A.
   - For Relationship, enter Kidney Paired Donor.
   - When filling out the Comments section, **donor's KPD ID will be used in lieu of name.** Make certain to check if the recipient has any donor directed antibodies and notify HLA director/ designee immediately.

xi. To **maintain donor confidentiality, Altruistic, Non-Domino and KPD donors** will have a separate HLA chart. At the point of preliminary crossmatch, the donor is identified by their name in their LIS chart; therefore their name will appear on the ABO and HLA reports. Thus the need for a separate chart.

xii. The Pre-Transplant-Preliminary Crossmatch report, ABO and HLA typing(s) are reviewed and final verified by the HLA laboratory director or designee. For the ABO Intended Incompatible recipient/ donor pairs, include the Transplant Anti-A DTT treated titer and ABO A subtyping results for

director review.
The finalized report is distributed via Epic to the appropriate ordering physician.

F. **Final Physical Crossmatch Procedure and Protocols**:

When the Flow Cytometer is inoperable, **contact the UTSWMC laboratory** as outlined in FLF 1.19.A Memorandum for Instrument Downtime in the Flow Procedure Manuel.

NOTE: **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.**

i When all living donor/ recipient pairs are scheduled for final crossmatch, personnel from the Transplant office will email HLA lab the Living Donor Transplant **UNOS ID**. This identifier will be used in the Final Crossmatch and Living Donor Crossmatch reports as outlined below.

ii. At the time any living donor pair is scheduled for final crossmatch and again when they present for crossmatch, review the recipient's HLA Hist report and the front of the patient folder and paperless yellow sheet to verify that none of the antibodies listed is donor directed. Additionally, review any unverified/ in process antibody testing that may reveal donor directed antibodies.

Consult with the medical director when any of the following are true:

- 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 with MFIs of ≥ **10,000**. **This will be a contraindication to transplant.**
- If any DQA, DPA or DPB partial or allele specific directed antibodies are present regardless of MFI.
  Note: When reviewing partial DQA, DPA or DPB reactivity **if** there is **no** allele specific reactivity within the partial antibody, there is no need to consult the director. For example, donor is DQA1*03:01 and recipient only has reactivity to DQA1*03:02 and 03:03, then no donor directed allele specific or partial antibody is present.
- If any HLA- A, B, C, DR, DRB3,4,5 or DQB allele specific donor directed antibodies are present.
  Note: As DQ and DP antibodies are present on Luminex Single Antigen PRAs as pairs of alpha and beta antibodies, both alpha and beta forms will be considered as partial or allele specific donor directed HLA antibodies if the pattern of reactivity cannot be attributed specifically to the alpha or beta form. However, if all beads for a particular alpha form are positive and not all beads of a particular beta form are positive, the reactivity can be attributed to the specific alpha form and thus the beta form will not be considered positive in regards to partial or allele specific donor directed HLA antibodies.

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- And as is protocol, if any DQA, DPA or DPB donor directed antibodies are present, with MFI <10,000, give the medical director and living donor coordinator a heads up but continue with final crossmatch.

Exception: If the pair presenting for final crossmatch are enrolled in the desensitization protocol, follow the criteria outlined in the appropriate MDMC Desensitization procedure.

iii. If the pair presenting for transplant are enrolled in the ABO Intended Incompatible Transplant protocol, verify all Transplant Anti-A DTT treated titer results must be **≤4**. If This is not the case, immediately notify the HLA medical director/ designee.

NOTE: Any titer results preformed with the test INDIRECTANTIHUMAN GLOBULIN TITER [LAB275] were NOT performed using DTT and the results should NOT be assessed when determining eligibility for this protocol.
Acceptable titer results include Ref Transplant ABO Antibody DTT Titer and/ or TRANSPLANT ANTI-A DTT TITER BY GEL.

Additionally, verify that the donor has been subtyped as ABO A, non A-1 on two separate blood draws. If this is not the case, the testing must be completed prior to transplant to confirm non A-1 result.

iv. Recipient/ donor pair will have the following tests ordered upon final crossmatch:

Recipient

a. ABO Type and Screen

*For a recipient pursuing and ABO Intended Incompatible transplant, the most recent REF Transplant ABO Antibody DTT titer [LAB5500] or TRANSPLANT ANTI-A DTT TITER BY GEL [LAB5789] must be dated with in the prior 90 days +/- 20 days of intended transplant. If this is not the case, add on this test to their ABO. **Verify the result remains ≤ 4** before the patient proceeds to transplant. If the intended recipient's current titer is >4, notify the HLA Medical Director/Designee and the Transplant Coordinator immediately that the candidate will not be suitable for transplant under this protocol. The coordinator is to convey this information the transplant surgeon and nephrologist.

b. Flow Cytometric allogeneic and autologous T cell and B cell crossmatches. LIS order: HLA Crossmatch – Flow Allo and Flow Auto [LAB3451]. Final Crossmatch testing should occur within 48 hours of blood draw, but may exceed 48 hours if cell viability is ≥ 80%.

Note: Surgery may proceed in the absence of autologous flow crossmatch per HLA medical director/ designee approval.

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c. PRA where applicable.

Donor

a. Type and Screen and where applicable, ABO subtyping
*For ABO intended incompatible donors, verify the donor's ABO: A subtyping result(s) as ABO A, non-A1 or AB, non-A1B, and that the subtype was confirmed on a second sample. If the subtyping was only performed once, order TRANSPLANT A SUBTYPE [LAB4100]. Verify this result before recipient/ donor proceeds to transplant.
Notify the laboratory director/designee immediately of an aberrant result.
b. Living Donor Crossmatch [LAB4374]
c. HLA Living Donor ABO Report for OR [LAB3408]. HLA will order this test.

In all cases above, **any ABO discrepancy** is immediately brought to the attention of the laboratory director/ designee, transplant coordinator, surgeon and nephrologist.

v. All recipients receiving a final physical crossmatch should have a PRA within the previous three months. If this is not the case, or if the patient has been PRA tested fewer than three times, a LUSAG PRA [LAB3417] must be performed on the same serum sample used for the final physical crossmatch or other qualifying sample (A qualifying sample is a serum sample that has arrived in the laboratory but has not yet been tested). Upon completion verify there are no donor directed antibodies.

vii. At the time of final crossmatch, fill out the HLAF 1.12.C Final Crossmatch log with recipient and donor information. Any information pertaining to steps i - vi above must be noted in the logbook including all calls, names, dates and times. Additionally, the person(s) performing the final crossmatch must identify themselves in the logbook.

viii. **Serum selection for final crossmatching and crossmatch scenarios include:**
Each Final Physical Crossmatch will include 2 serum samples, and possibly a Third sample.
Final crossmatch sample – a serum sample drawn at the time the recipient presents to the hospital for blood draw in preparation for transplant with the current donor. (Typically 24-48 hours prior to schedule transplant.)
Peak serum sample - recipient serum sample showing the highest sensitization/ most number of reactive beads for Class II followed by Class I. If multiple serum samples show equal number of reactive beads, choose the one with the higher MFIs.

If the patient is non-sensitize, choose a serum sample dated with in the proceeding few months (i.e. a remote sera).

Additional Peak Sample where applicable – when there are Class I and II partial/ allele specific antibodies, two peak samples may need to be tested.

Discuss serum selection with the director/ designee.

ix. As discussed in section G below, **Altruistic, Non-Domino and KPD donor/ recipient pairs** have unique identifiers to maintain confidentiality. As such, when performing the final crossmatch, use instructions below to identify the donor **on all flow histograms**, the **Flow Crossmatch and LIS reports**.

G. **Reporting Final Crossmatches**

i. All final crossmatch results must be reported to the on call Transplant Coordinator, Surgeon and Transplant Nephrologist. Negative crossmatches may be communicated via the LIS Secure Chat. In this circumstance, no call is necessary unless the secure chat is not timely acknowledged.
Unusual crossmatch results such as positive allogeneic crossmatch, or positive allo crossmatch with positive autologous crossmatch, must be discussed with the HLA Medical Director prior to communicating results to the clinicians. Communicate these results to the coordinator, nephrologist and surgeon through a phone call, and advise them of the medical director's assessment of the result.

ii. All communications will be recorded in the Final Crossmatch logbook including name, date and time of communication.

iii. In most cases, transplant will take place within 2-3 days of final crossmatch/ final serum draw date. If this is not the case, repeat crossmatch is not required as long as the transplant takes place within 60 days of the **final serum draw date**, **and** sensitization history is assessed via any subsequent PRAs and the **HLA Sensitization History for Transplant Patients form.** Instructions for how to proceed in reporting this exception see step xi below:

iv. As with preliminary crossmatches, there are separate instructions for each type of recipient/donor pair to properly report final crossmatch results. Follow instructions carefully to ensure proper donor identification.

v. Resulting **ABO and Crossmatch Compatible Living Donor/ Recipient pair**:

a. <u>For the recipient,</u> using the LIS Pre-Transplant-Final Crossmatch report, answer the Current/ Peak Sera and where applicable, Additional peak results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the *HLAFINALXM* crossmatch template. When prompted for the **Donor ID**, use the donor's **UNOS ID** number.

b. Final verify the Final Crossmatch report. This report, along with the recipient <u>and</u> donor's ABO and HLA typing reports are sent to the transplant floor.

c. <u>For the donor</u>, order and complete the HLA Living Donor ABO Report for OR [LAB3408]; in the smart text box, enter HLALDABO to pull in and answer the report. When prompted for the Living Donor ID Number, **use the donor's UNOS Number.**

d. Final verify this report. The report will be printed by OR staff the day of surgery.

vi. Resulting **ABO Intended Incompatible Living Donor/Recipient pair**:

a. For the recipient, using the LIS Pre-Transplant-Final Crossmatch report, answer the Current/ Peak Sera where applicable, Additional peak results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the *HLAFINSUBA* crossmatch report. The *HLAFINSUBA* report contains an ABO compatibility chart for this protocol and additional questions verifying ABO titer and subtyping.
When prompted for the **Donor ID**, use the donor's **UNOS ID** number.

b. **For recipients qualifying for the ABO Intended Incompatible transplant, notify the Blood Bank with the recipient's name and date of birth,** so they can place an alert on the patient's record to give the correct type of plasma.

c. Final verify the Final Crossmatch report. This report, along with the recipient and donor's HLA typing reports, recipient ABO report including Anti-A titer result and donor ABO report including ABO A subtyping result, are sent to the transplant floor.

d. For the donor, order the HLA Living Donor ABO Report for OR [LAB3408]; in the smart text box, enter *HLALDSUBA* to pull in and answer the report. The *HLALDSUBA* report contains an ABO compatibility chart for this protocol and additional questions verifying ABO titer and subtyping.
When prompted for the Living Donor ID Number, **use the donor's UNOS Number.**

e. Final verify this report. The report will be printed by OR staff the day of surgery.

vii. Resulting **Altruistic or Non Domino Therapeutic Donor/ Recipient pairs**:

a. When performing the final crossmatch for this type of transplant, enter the **donor's UNOS ID on all flow histograms** and flow reports. Donor **DOB** will act as the second unique identifier.

b. For the recipient using the LIS Pre-Transplant-Final Crossmatch report, fill out the Current/ Peak Sera where applicable, additional peak serum results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the *HLAFINALXM* crossmatch report. When completing this portion of the report:
   - The recipient's UNOS Waitlist ID (not including the numbers in parenthesis) will need to be added to the report; add this as a line above the Recipient ABO. To locate the Waitlist ID, refer to Section E viii c above. This Waitlist ID will serve to link the preliminary and final crossmatch reports and HLA Living Donor ABO Report for OR.

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- Enter the **donor's UNOS Number in the Donor ID field**.
- **Donor DOB** will be the second unique identifier.
- Answer the 'If Living Related' line as N/A
- For Relationship, enter Altruistic or Non Domino Therapeutic, accordingly.
- When filling out the Comments section, **donor's UNOS Number will be used in lieu of name.**

Final verify the report.

c. For the donor, at the time of final crossmatch, **we will register the donor in Epic using their UNOS Number, just as we do with deceased donors**. Order the HLA TYPE CLASS I AND II NO CHARGE [LAB3429] and answer the report, including the ABO field, which appears as 'Transcribed ABO Grouping' on the printed report.
Ask the transplant living donor coordinator to add the donor's name as an alias to this Epic chart.

Final verify this report.

Registering the donor in the LIS using the UNOS ID allows a way for the donor HLA type to be reported *without their name appearing on the report, and for the report to be placed in the recipient's hospital chart, thus maintaining donor* confidentiality.

Ask the transplant living donor coordinator to add the donor's name as an alias to this Epic chart.

d. Using this newly created **donor LIS chart**, order and complete the HLA Living Donor ABO Report for OR [LAB3408]; in the smart text box, enter HLALDABO to pull in and answer the report. When answering this report:
   - **In lieu of Recipient Name, use the recipient's UNOS Waitlist ID** (not including the numbers in parenthesis).
   - **Edit Recipient Medical Record Number to read Recipient DOB;** answer accordingly.
   - The **Donor's UNOS ID** is used in the 'Living Donor ID Number' field.

Final verify this report. The report will be printed by OR staff the day of surgery.

e. The final crossmatch report, recipient's ABO and HLA typing reports and the donor's HLA typing with transcribed ABO report are sent to the transplant floor. **Remember, do not send the Donor LIS ABO report to the floor as it contains the donor name and medical record number.**

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viii. Resulting **ABO Compatible Kidney Paired Donor / Recipient pair**:

a. When performing the final crossmatch for this type of transplant, use the **donor's UNOS ID on all flow histograms** and **the flow reports** Donor **DOB** will be second unique identifier.

b. For the recipient, using the LIS Pre-Transplant-Final Crossmatch report, answer the Current/ Peak Sera where applicable, additional peak results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the *HLAFINALXM* crossmatch report.

When completing this portion of the report:

- The **recipient's KPD Candidate ID**, **will need to be added to the report; add this as a line above the Recipient ABO**. Again, the KPD ID will serve to link the preliminary and final crossmatch reports with the HLA Living Donor ABO Report for OR. See Section E ix c. to locate recipient KPD Candidate ID.
- When prompted for the **Donor ID** enter the **donor's UNOS Number and the donor's KPD ID used on the preliminary crossmatch report**. Refer to Section E ix a. to locate.
- **Donor DOB** will be the second unique identifier.
- Answer the 'If Living Related' line as N/A.
- For Relationship, enter Kidney Paired Donor.
- When filling out the Comments section, **donor's UNOS Number will be used in lieu of name.**

Final verify the report

c. For the donor, at the time of final crossmatch, **we will register the donor in Epic using their UNOS Number, just as we do with deceased donors**. Order the HLA TYPE CLASS I AND II NO CHARGE [LAB3429] and answer the report, including the ABO field, which appears as 'Transcribed ABO Grouping' on the printed report.
Final verify the report.

Ask the transplant living donor coordinator to add the donor's name as an alias to this Epic chart.
Registering the donor in the LIS using the UNOS ID allows a way for the donor's HLA type to be reported *without their name appearing on the report, and for the report to be placed in the recipient's hospital chart, thus maintaining donor confidentiality.*

d. Using the newly created **donor LIS chart**, order and complete the HLA Living Donor ABO Report for OR [LAB3408]**;** in the smart text box, enter HLALDABO to pull in and answer the report. When answering this report:

- **Use the recipient's KPD candidate ID in lieu of Recipient Name**.
- **Edit Recipient Medical Record Number to read Recipient DOB;** answer accordingly.
- The **donor's UNOS ID** is used in the 'Living Donor ID Number' field.

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Final verify this report. The report will be printed by OR staff the day of surgery.

e. The final crossmatch report, recipient's ABO and HLA typing reports and the donor's HLA typing with transcribed ABO report are sent to the transplant floor. **Remember; do not send the Donor LIS ABO report to the floor as it contains the donor name and medical record number.**

ix. **ABO Intended Incompatible Kidney Paired Donor/ Recipient pairs**

a. When performing the final crossmatch for this type of transplant, use the **donor's UNOS ID on all flow histograms** and **flow reports.** Donor **DOB** will be second unique identifier.

b. For the recipient, using the LIS Pre-Transplant-Final Crossmatch report, fill out the Current/ Peak Sera and where applicable additional Peak sera results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete *HLAFINSUBA* crossmatch report. The *HLAFINSUBA* report contains an ABO compatibility chart for this protocol and additional questions verifying ABO titer and subtyping.
When completing this portion of the report:
- The **recipient's KPD Candidate ID**, **will need to be added to the report; add this as a line above the Recipient ABO**. Again, the KPD ID will serve to link the preliminary and final crossmatch reports with the HLA Living Donor ABO Report for OR. See Section E ix c. to locate recipient KPD Candidate ID.
- When prompted for the **Donor ID** enter the **donor's UNOS Number and the donor's KPD ID used on the preliminary crossmatch report**. Refer to Section E ix a. to locate.
- **Donor DOB** will be the second unique identifier.
- Answer the 'If Living Related' line as N/A
- For Relationship, enter Kidney Paired Donor.
- When filling out the Comments section, **donor's UNOS Number will be used in lieu of name.** Again, these unique identifiers ensure donor confidentiality.

Final verify the report

c. **For recipients qualifying for the ABO Intended Incompatible transplant**, **notify the Blood Bank** with the recipient's name and date of birth, so they can place an alert on the patient record to give the correct type of plasma.

d. For the donor, at the time of final crossmatch, **we will register the donor in Epic using their UNOS Number, just as we do with deceased donors**. Order the HLA TYPE CLASS I AND II NO CHARGE [LAB3429], and answer the report including the ABO field and note the **ABO subtype of the donor**. The ABO and subtype will appear as 'Transcribed ABO Grouping' on the printed report.

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Final verify the report

Ask the transplant living donor coordinator to add the donor's name as an alias to this Epic.
Registering the donor in the LIS using the UNOS ID allows a way for the donor HLA type and ABO to be reported *without their name appearing on the report, and for the report to be placed in the recipient's hospital chart, thus maintaining donor confidentiality.*

e. Order and complete the HLA Living Donor ABO Report for OR [LAB3408]**;** in the smart text box, enter *HLALDSUBA* to pull in and answer the report. The HLALDSUBA report contains an ABO compatibility chart for this protocol and additional questions verifying ABO titer and subtyping.

When answering this report:
- **Use the recipient's KPD candidate ID in lieu of Recipient Name**.
- Edit **Recipient Medical Record Number to read Recipient DOB;** answer accordingly.
- The **donor's UNOS ID** is used in the 'Living Donor ID Number' field.
- Ask the transplant living donor coordinator to add the UNOS ID as an alias in the donor's LIS chart.

Final verify this report. The report will be printed by OR staff the day of surgery.

f. The final crossmatch report, recipient's ABO and HLA typing reports and the donor's HLA typing with transcribed ABO report are sent to the transplant floor.
Remember; do not send the Donor LIS ABO report to the floor as it contains the donor name and medical record number.

x. Resulting **crossmatch incompatible pairs**
For the recipient, using the LIS Pre-Transplant-Final Crossmatch report, fill out the Current/ Peak Sera and where applicable additional Peak sera results for the auto and allo crossmatches and at the HLA XM Report result line, pull in and complete the appropriate crossmatch template for the particular type of donor (as outlined above). Do not verify out the report. The report will be reviewed and final verified by the HLA medical director.

xi. **Resulting crossmatch for transplant delayed more than 3 days**
In the event a transplant is cancelled/ temporarily delayed, repeat crossmatch is not required as long as transplant takes place within 60 days of the **final serum draw date**, **and** sensitization history is assessed via any subsequent PRAs and the **HLA Sensitization History for Transplant Patients form.** Sensitizing events include transfusion, pregnancy, and transplant with any human tissue or organ since the last qualifying PRA. Verify this with the Transplant Coordinator. Additionally, ask the coordinator if there have been any hospitalizations since the last qualifying PRA. If there has been hospitalization,

consult with the HLA Medical Director, who will advise if the patient can still qualify for the protocol.

a. For this circumstance, the initial final crossmatch report will be verified out and submitted for director review.

b. When the transplant is rescheduled, confirm if latest PRA serum draw date is within 3 months of planned transplant. If this is not the case, have the transplant coordinator add HLA PRA CLASS I AND II BY LUMINEX SINGLE ANTIGEN (AKA HLA PRA LUSAG) [LAB3417] to blood draw.

c. When the pair again presents for transplant, blood must be collected for **ABO Type and Screen** and HLA Crossmatch – Flow Allo and Flow Auto, and if needed, PRA.

d. Fill out the Final Crossmatch Log Book as if this was a new event.

e. Using the newly order HLA Crossmatch – Flow Allo and Flow Auto, complete and verify out the report using crossmatch results from the previous final, taking care to evaluate any interim PRA results for donor directed antibodies. In the comments section add the following:
Final Physical crossmatch between this donor/recipient pair was performed within the prior 60 days and the patient has no evidence of sensitizing history and/or donor directed specificity during the interim. As such, the prior final crossmatch results prevail, and have been added to this this report.

f. Upon completion of the report call the Transplant Coordinator and the on call nephrologist and surgeon with crossmatch result and letting them know crossmatch result was based on original result, date of xxx.

d. Final verify and print the Final Crossmatch report. This report, along with the recipient and donor's ABO **from updated draw date** and HLA typing reports are sent to the transplant floor. If the recipient / donor pair happens to be Altruistic / Non-Domino / Kidney Paired Donor / Recipient pairs, follow appropriate instructions for Final Crossmatch report.

e. For the donor, order and complete the HLA Living Donor ABO Report for OR [LAB3408]; in the smart text box, enter HLALDABO to pull in and answer the report. When prompted for the Living Donor ID Number, **use the donor's UNOS Number.** Final verify this report. The report will be printed by OR staff the day of surgery.

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For Altruistic/ Non-Domino/ Kidney Paired Donor/ Recipient pairs follow appropriate instructions for HLALDABO report.

f. The **HLA Sensitization History for Transplant Patients form must be received in our laboratory before the Final Crossmatch and HLA Living Donor ABO Report for OR reports can be released.** Record this information in the Final Crossmatch logbook. A copy of the form will remain on the patient's HLA chart and a copy sent to the transplant floor with final reports.

g. Serum from the final physical crossmatch as well as serum collected when the recipient presents for transplant will be stored as outlined in step xiii below.

h. As filing a new crossmatch report will drop a charge for the test, **this charge needs to be nullified**. Contact the Point of Care Manager and request the crossmatch charge be credited.

xii. Results of all final crossmatches, including copies of the final crossmatch, ABO and HLA typing reports are submitted for laboratory director/ designee review. Review will take place by the next business day of regular laboratory operation.

xiii. **All** sera used for 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. Recipients receiving a Final Physical Crossmatch will have the current and the peak/ additional peak sera stored the TXP box. Note: for a **transplant delayed more than 3 days,** in addition to freezing all sera used on the final crossmatch, also freeze the serum sample drawn when the patient is redrawn for transplant.

xiv. **Donor samples**:
As of June 1, 2021, the recovery hospital will obtain, and the HLA laboratory will store, donor specimens appropriate for serological and NAT testing. An EDTA tube and a 7mL red top tube (either no anticoagulant or serum separator only) will be drawn when the donor presents to the hospital for donation. The buffy coat from the EDTA tube and serum from the red top tube will be stored in the HLA *Living Donor* box, and locations will be noted in the LIS. Samples will be saved for a minimum of 10 years after the date of transplant and available for retrospective testing.

xv. If the donor did not have a buffy coat saved from their initial workup, place a sample (ACD-A tube or cells from crossmatch) in the appropriate rack so DNA can be saved. This donor sample will be saved for a minimum of 5 years. Note the location of the sample in the LIS.

V. REFERENCES:

N/A

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VI. RELATED PROCEDURES:

FL 1.19 Flow Cytometry Cross Match for Solid Organ Transplantation
FLF 1.19.G.01 HLA Downtime Crossmatch Forms
HLA 4.11 LABScreen (One Lambda) Class I and Class II Single Antigen Assay
HLAF 1.12.C Final Crossmatch Logbook