

**Maine Medical Center
Transplant Program
Policies and Procedures
Joint Operating Policy on Histocompatibility Strategies for the
Maine Transplant Program & NorDx Immunogenetics (HLA) Laboratory**

This policy addresses the following:

Organ Procurement and Transplantation Network (OPTN) Bylaws. Histocompatibility laboratories must have written agreements with every transplant program the laboratory serves, unless clinical urgency prevents such an agreement.

I. Sample requirements for histocompatibility testing (HLA typing, ABO typing, and crossmatching)

Generally, ACD anti-coagulated whole blood (yellow-top) tubes and serum (red top) is used for all testing.

Panel	Description	TEST CODE	TEST	SPECIMEN
RECIP	HLA RENAL RECIP PRE-TXP EVAL	ABC	HLA ABC TYPING CLASS I	60 ml ACD (Yellow Top) 10 ml Clotted Blood (Red Top)
		DR	HLA DR TYPING CLASS II	
		AUTXM	HLA AUTO CROSSMATCH	
		FCXM	HLA PRE-TXP CROSSMATHC FLOW CYTO	
		ABO	HLA ABO TYPING	
DONEV	HLA LIVING DONOR EVALUATION	ABC	HLA ABC TYPING CLASS I	90 ml ACD (Yellow Top) 6 ml Clotted Blood (Red Top)
		DR	HLA DR TYPING CLASS II	
		ABO	HLA ABO TYPING	
HLABS	HLA MONTHLY ANTIBODY SCREEN	WBC I	HLA PRE-TXP AB SCREEN CLASS I	20 ml Clotted Blood (Red Top)
		WBC II	HLA PRE-TXP AB SCREEN CLASS II	
FINLD	HLA LIVING DONOR FINAL PRE-TXP XM	ABO	HLA ABO TYPING	10 ml Clotted Blood (Red Top) 60 ml ACD (Yellow Top)
		JICLD	HLA EXTRA ACD TUBES DRAW	
FINPT	HLA RENAL RECIP FINAL PRE-TXP XM	FCXM	HLA PRE-TXP CROSSMATHC FLOW CYTO	20ml Clotted Blood (Red Top)
		ABO	HLA ABO TYPING	
DSA	HLA DONOR-SPECIFIC ANTIBODY	DSAB I	HLA POST-TXP AB ID CLASS I	6ml Clotted Blood (Red Top)
		DSAB II	HLA POST-TXP AB ID CLASS II	
		DSAFL	HLA DSA FLOW CROSSMATCH	
		JICLD	HLA EXTRA ACD TUBES DRAW	60 ml ACD (Yellow Top)
		RDHLA	HLA RED TOP TUBE	6 ml Clotted Blood (Red Top)

II. HLA loci tested and level of resolution typed.

Testing for HLA- A, -B, -C, -DRB1*, DRB3*/4*/5*, -DQA1*, DQB1*, and DPB1* can be performed by the HLA laboratory. All loci are typed at low resolution (e.g. DRB1*15) except DPB1* which is typed at high resolution (e.g. DPB1* 04:02).

Recipients: The basic core loci tested are HLA-A, -B, -C, -DRB1*, DRB3*/4*/5*, and DQB1* with DQA1* and DPB1* typing as needed.

Donors: The basic core loci tested are HLA-A, -B, -C, -DRB1*, DRB3*/4*/5*, and DQB1* with DQA1* and DPB1* as required (i.e. for all deceased donors and living donors in kidney exchange programs).

III. Extended HLA typing.

Recipient or donor specimens may be automatically reflexed for additional HLA testing (i.e. DQA1*, DRB3* subgroups, and/or DPB1*) when appropriate. For example, if a donor is to be listed in a kidney exchange program additional HLA typing for DQA1* and DPB1* loci alleles is required. A recipient may also be tested for these loci to help in the characterization of unacceptable antibodies.

IV. Reporting HLA typing results to the OPTN Contractor.

HLA typing results no longer reported to NEOB.

V. Resolving HLA typing discrepancies and errors.

If a discrepancy occurs involving HLA typing further investigation is performed by the Laboratory Director and staff to determine the cause of the error and appropriate corrective action (e.g. issuing a corrective report, re-education or training as necessary). If a discrepancy or error occurs between our HLA results and another party, follow-up by the laboratory staff may include calling the other HLA laboratories or OPOs and retesting the sample if necessary.

VI. Maximum turnaround time (TAT) from receipt of sample to reporting of HLA typing results to the transplant program.

The TAT varies for different test requests:

Deceased donors: 2 business days for all reviewed/finalized reports.

Living donors and recipients: Five days for finalized reports.

For deceased donors, either local or import, all testing is automatically performed on a STAT basis.

Expected crossmatch turnaround time upon receipt of adequate specimen for a local donor is expected to be ~4 hours with a maximum of 5 hours. It is understood that occasional exceptions may occur.

TATs will be logged and reported to the Transplant QAPI Committee on a quarterly basis.

VII. History of Allosensitization:

Purpose: Patients whose immune systems have been sensitized to allo-antigens are at high risk of rapidly developing an antibody response on re-exposure. It is also important to determine whether any potential sensitizing events have occurred since the patient's antibody status was last tested.

Allosensitization history: provided at initial patient workup and follow-up visits by the transplant staff.

Event	Data	Notes
Previous graft (includes all solid organs, blood vessel, and bone or tendon allografts)	Date of transplant, organ(s)	
	Date of graft loss	
	Dates of graft removal	
	Cause of graft loss (immunological?)	
	HLA typing of previous donor(s)	
Pregnancy (gravida)	Number, years of occurrence	
Births (para)	Number, years of occurrence	
Transfusions	Number	<i>Caveat: A comprehensive and accurate transfusion history is difficult to obtain and confirm</i>
	Type of product (platelets, whole blood)	
	Month and year of occurrence	
Other events	Immunizations	

Goal:

1. Have current information on all patients to eliminate unnecessary testing, reduce unexpected positive results, and obtain optimal clinical outcomes.
2. A central database, EPIC, with pertinent patient information available to the transplant physicians and their support teams. The transplant program staff enters data about sensitization history when known (pregnancy, prior transplant and blood transfusion).

VIII. Periodic Sample Collection:

Purpose: to develop an alloantibody history, identify trends in reactivity patterns, and to facilitate final crossmatches.

Monthly serum samples for all patients should be collected and then maintained by the HLA laboratory. The HLA lab staff will inform the Transplant coordinators of delinquent specimens. Patients should have specimens collected between the 25th and the 1st of the month whenever possible for arrival in the HLA lab no later than the 1st of the month.

Goal: Have current specimens collected on all listed patients.

IX. Antibody (Ab) screening and identification:

- A. Select assay format for Ab screening:

Purpose: Evaluate the patient's current antibodies in the context of their sensitization history. This should help estimate the risk of producing a positive crossmatch when tested against a potential donor. The absence of detectable antibodies does not necessarily mean the absence of sensitization.

Assays to screen for the presence of alloantibody, %PRA, and Ab specificity

Solid phase assays (HLA specific; IgG)	Description and Use
Antibody screening	
Solid phase- Flow PRA beads (class I & II)	Thirty populations of beads each for class I (HLA-A,B,C) and class II (HLA-DP, DQ, DR). The beads are coated with purified HLA molecules. Used for the detection of anti-HLA class I or class II IgG antibodies and to estimate the % panel reactive antibody. **Not used for determination of cPRA**
Antibody Identification (Specificity)	
Solid phase- Luminex platform Single antigen beads (class I or II)	Used to determine the specificity of anti-HLA class I or class II (IgG) antibody and to determine the cPRA.

Note: To determine the calculated PRA (cPRA) patient sera are analyzed for the presence HLA class I and II specific antibodies. HLA specificities with a normalized strength of approx. 3,000 MFI or greater are entered into the UNet and NKR computer systems as unacceptable specificities. This acts as a preliminary virtual crossmatch between the ABO compatible patients and a specific donor eliminating patients with unacceptable donor-specific HLA antibodies from the match-run. For determining compatibility between a patient and a living donor the donor specific antibodies (DSAs) are used as immunologic markers for risk assessment and not necessarily to eliminate the person as a possible donor.

B. Frequency of Ab screening

Purpose: Test patient specimens at adequate frequencies to detect any deleterious antibodies and estimate the likelihood of transplanting a patient within a given time frame. A patient with broadly reactive HLA specific antibodies (high %cPRA) is at a high risk for a positive crossmatch with a prospective donor. A patient who has no detectable anti-HLA antibodies is unlikely to have a positive crossmatch test, assuming there have been no intervening allosensitizing events.

1. New patients:

Perform HLA specific IgG Ab screening test (e.g. Flow PRA beads) and Ab identification by a solid phase method (e.g. Luminex single antigen beads) with the initial specimen received by the HLA laboratory. Updating of HLA specific Ab ID will occur approximately every 3-5 months as needed.

2. Current Patients:

All patients are screened monthly for HLA class I and class II antibody.

- i. **Non-sensitized Patients:** Patients that have been negative for HLA class I and class II Ab by both the flow PRA screening assay and by the single antigen bead assay (luminex) as well as no new sensitization events since testing.
- ii. **Sensitized Patients: Regrafts and/or patients with class I and/or class II Ab or an HLA specific antibody identified.**

As part of our Transplant program's quality assurance plan the Maine transplant program along with the HLA lab have been monitoring patient compliance with monthly specimen draws needed in the HLA lab.

Goals:

- 1. Have current HLA class I and II cPRA% on all patients
- 2. Have Ab identification (class I and/or II) for all HLA Ab positive patients in a timely fashion.

X. Crossmatching

Purpose: To identify the presence of preformed donor reactive antibodies in order to prevent hyperacute rejection. A positive flow cytometry cross match has been deemed by the MTP to be an absolute contraindication for transplantation. A weak positive flow cytometry cross match is deemed to be a major risk factor for antibody mediated rejection. Transplantation across a weak positive FCXM is discouraged though may be permissible under some circumstances (for example, if the patient is highly sensitized and is unlikely to be otherwise transplanted). If a patient is transplanted across a weak positive FCXM, donor specific antibody surveillance will be performed (see below) and enhanced immunosuppression provided that may include plasmapheresis and IVIG. **In some patients the flow crossmatch results may not be reliable due to immunotherapy (e.g. Rituximab). This will prompt discussion among the clinical staff as to the timing and possible short-term discontinuation of immunotherapy allowing for transplantation to proceed.**

A. Assay method used to identify donor specific alloantibody*

Flow cytometer based assay*	Description and Use
High sensitivity: Flow cytometry	The most sensitive crossmatch test for donor-specific IgG antibody (Pronase treatment is used to lower background reactivity)
T cells (CD3+ cells)	Detect anti-donor IgG T-cell reactivity (more sensitive than CDC methods)
B cells (CD19+ cells)	Detect anti-donor IgG B-cell reactivity (more sensitive than CDC methods)

To help rule out Autoantibody (& IgM activity)	
Heat inactivation of serum	Inactivates mostly IgM; help differentiate IgG from IgM
Autologous cells: Flow method	Detect auto-antibody (IgG)

**The above method may also detect non-HLA specific antibody. e.g. ATG, Rituximab*

Note: As of March 1, 2022 the Complement-dependent cytotoxicity (CDC) CX is being discontinued.

Current scoring of flow crossmatches:

Interpretation scale:	Current serum	Historical sera	Interpretation
Flow CX T cell result	Neg	Neg	Compatible
	Neg	Wk Pos/Pos*	Elevated risk with DSA
	Pos	Pos	Incompatible
	Wk Pos	Wk Pos	Elevated risk with DSA

Interpretation scale:	Current serum	Historical sera	Interpretation
Flow CX B cell result	Neg	Neg	Compatible
	Neg	Wk Pos/Pos*	Elevated risk with DSA
	Pos	Pos	Incompatible
	Wk Pos	Wk Pos	Elevated risk with DSA

** Historical reactivity in the presence of DSA may represent an increased risk of rejection due to a past sensitization event.*

Interpretation scale:	Negative	Weak Positive	Positive
T cell channel shift	<35	35-70	≥71
B cell channel shift	<90	90-150	≥151

Note: Values currently used as of 2017

- B. Determine the timing and specimen requirements for performing a donor work-up including a crossmatch**

Deceased donors:

Regional Donors (NEDS) and Shipped in donors (UNOS):

Donors from our UNOS region 1 that are handled through the New England Donor Services (NEDS) have been typed for ABO and HLA (HLA-A*, -B*, -C*, DRB1*, DRB3/4/5*, DQA1*, DQB1*, and DPB1*). The on-call HLA technologist receives a call from the MTP coordinator regarding a possible donor and requests information concerning donor status, ABO, transfusion Hx, and specimen type. Upon receipt of specimens, the laboratory performs confirmatory ABO typing as well as appropriate final crossmatching with the intended recipient. Additional crossmatching for local backup recipients may also be performed if requested. Once the crossmatching procedures are started additional patients cannot be added to that run, so it is necessary to run everyone that will potentially be a viable recipient for that donor at the same time. Final T & B cell flow crossmatches are currently required by our program. Specimen quality & quantity can be very limiting in some cases, especially for donor B-cells. Shipping conditions at certain times of year can also affect cell viability. Occasionally, due to poor cell quality and/or quantity, make reliable results difficult to obtain. Once all potential recipients are selected it should generally take from 4-5 hours to complete final ABO confirmation and Flow crossmatch.

Shipped in donors from the United Network of Organ Sharing are intended for a specific recipient. This information will be given to the tissue typing technologist-on-call by the transplant coordinator. HLA testing is the same as for NEDS donors.

Virtual Crossmatch: A UNOS generated match-run performs a preliminary “virtual” crossmatch for deceased donor work-ups. For all patients registered in UNet all unacceptable HLA specificities (≥ 3000 MFI) are entered and routinely updated when required. Currently, this includes: HLA- A, B, and C class I loci as well as DRB1*, DRB3*/4*/5*, DQB1*, DQA1*, and DPB1* class II loci. In generating the match-run on a specific deceased donor UNOS is essentially running a virtual preliminary crossmatch for each ABO compatible patient as the match run will exclude any patients with unacceptable antibody.

Crossmatch (Final):

Flow crossmatch: For all patients a flow crossmatch using the recipient’s current specimen (<30 days old) and 1-2 additional historical sera, when available, on tested on pronase-treated donor lymphocytes prospectively. Any additional recipients, used as backups, should be set up at the same time since cell number is limited and turn-around-time delayed. Any repeats due to poor donor cell quality/quantity may cause a delay since the crossmatch procedure would need to be started from unprocessed new specimen. This may take approx. 4 hrs after new specimen is received by the lab.

Information about the presence of donor-specific antibody is to be communicated to the Transplant Doctor/Coordinator on-call and will be included in the comments section of the HLA crossmatch report.

Recipient’s current Specimen: If it is documented by the transplant doctors/staff that no sensitization events have taken place since the last specimen was drawn, then a specimen of

up to 30 days old may be used as the current specimen and a more recent specimen may not be necessary before transplant. A current specimen <7 days old is preferred when available.

Several recipient (patient) sera should be evaluated when possible:

- i. Current serum: recommended <30d for non-sensitized; < 7 d for sensitized patient
- ii. Plus 1-2 other sera (peak and/or historical)

Donor Specimens: For deceased donors, lymphocytes isolated from lymph nodes or spleen are preferred but a peripheral blood is generally used. If the donor has been extensively transfused and/or medicated aberrant results may be obtained with blood. On occasion, inadequate T and/or B cell quality or quantity may be obtained from the donor, making interpretation difficult or impossible (e.g. B cell results may be QNS).

Living donors:

Preliminary crossmatch: Flow crossmatches are performed using the recipient's current serum on donor T and B cells. When multiple donors are available a virtual crossmatch may be used to screen out incompatible donors based on unacceptable HLA specificities present in the recipient. If the DSA totals are below 10,000 MFI they will also be flow crossmatched to determine the actual level of donor reactivity. The presence of current or historical DSA are used for risk assessment post-transplant and will be noted in the comments of the HLA crossmatch reports along with the approximate strength of the DSA.

Final Cross-match: Generally within two weeks prior to transplantation additional serum samples are drawn and crossmatching repeated. Flow crossmatches are repeated using the recipient's new serum and 1-2 additional historical sera, if available, on donor T and B cells.

Recipient's current Specimen: A specimen less than 30 days old is considered current if it is documented by the transplant doctors/staff that no sensitization events have taken place since the last specimen was drawn. The most current specimen available is tested before transplant.

Several recipient (patient) sera should be evaluated when possible:

- i. Current serum: recommended <30d for non-sensitized; < 7 d for sensitized patient
- ii. Plus 1-2 other sera (peak and/or historical)

Donor Specimens; Lymphocytes isolated from peripheral blood are used.

Goal: Be able to predict final crossmatch compatibility from the preliminary results and differentiate true anti-donor HLA specific Abs that are clinically relevant from other non-deleterious Abs (e.g. Auto-Abs).

In renal transplantation, there may be *exceptional* cases when it would be advantageous to proceed with transplantation before a pre-transplant final crossmatch can be completed. In all cases where a pre-transplant crossmatch is waived, a peri-transplant or retrospective crossmatch is highly recommended to guide post-transplant management. Waiving the final crossmatch should only be considered in a primary deceased donor allograft if an excellent sensitization history has been

maintained, the patient is non-sensitized (no documented HLA class I or II antibodies or <10% PRA), and to minimize cold ischemic time.

XI. Criteria for establishing a risk category for each patient and crossmatching strategy for each category

For deceased donors:

Sensitized patient (PRA >10% or with an identified HLA Ab):	
	Risk category
1 ^o graft:	Moderate
Re-grafts	High
Non-sensitized patient (PRA <10%):	
1 ^o graft:	Low
Re-grafts	Moderate

Living donors:

Sensitized patient (PRA >10% or with an identified HLA Ab):	
	Risk category
1 ^o graft:	Moderate
Re-grafts	High
Non-sensitized patient (PRA <10%):	
1 ^o graft:	Low
Re-grafts	Moderate

For both living and deceased donors, patients are crossmatched using flow cytometry methods on T and B lymphocytes.

XII. Criteria and a process for use of unacceptable antigens or acceptable antigens for organ allocation

Transplantation of recipients with currently or historically defined anti-HLA antibodies with specificity to a specific donor will be avoided whenever possible but may be considered in some circumstances.

After activation of the candidate in UNet by the RN Coordinator the HLA Director or designee will be able to enter unacceptable HLA antigens for the candidate. For candidates receiving allocation priority based on a cPRA score of 99% or higher the HLA Director or designee will generate the UNet approval form and forward it to the transplant physician for completion.

The HLA Director and the transplant Physician/Surgeon will sign a written approval of the candidate's unacceptable antigens. Written approval by the transplant Physician/Surgeon and HLA Director must occur PRIOR to entry of the approval names entered into UNet. The names entered into UNet must be the same as those documented on the approval form. The approval form will be forwarded to the RN Coordinator for scanning into the patient EMR.

XIII. Specimens storage for repeat or future testing.

Cells: When possible cells from living and deceased donors are kept short term at -70°C. Viability should be adequate for approx. 3 months.

Serum/plasma: Living donor specimens (serum & EDTA plasma) will be sent to the HLA lab for long term storage (>10yrs) at -70C per UNOS Policy 14 and Maine Transplant Policy, Living Donor Specimen and Storage. Patient serum is generally stored frozen for five years or until specimen is exhausted.

XIV. If desensitization is performed, then a protocol for monitoring antibody levels.

Not applicable.

XV. If the laboratory registers candidates for the transplant program, then a process for bloodtype verification according to *Policy 3.3: Candidate Blood Type Determination before Waiting List Registration*.

Not applicable.

XVI. Process for donor reactive antibody monitoring post-transplant

The development of donor specific antibody (DSA) is a risk factor for rejection and premature graft failure. Assessing DSA may be useful for various purposes:

- Pre-transplant assessment of transplant candidates to determine their rejection risk
- Ongoing assessment of increased immunologic risk transplant recipients
- As a precursor to assessing risk for potential reduction in immunotherapy
- As a risk assessment tool for patients who are on dual immunotherapy or less

Immunologic Risk Assessment

The following are categorized as criteria that define increased immunologic risk

1. Regrafts
2. Elevated cPRA >10%
3. Pre-identified anti-donor HLA antibody
4. Patients with FCXM that is not negative (greater than 30 channel shift T-cell /50 channel shift B-cell)

These patients will have protocol DSA checked:

Weeks 2, 4, 8, 12

Months 6, 9, 12, 18 & 24

At the discretion of transplant team

Donor Specific Antibody may be requested for cause:

1. Evaluation of acute kidney injury after transplantation
2. Evaluation of post-transplant proteinuria
3. At the time of "for cause" allograft biopsy

Methodology:


Serum samples will be screened using the single HLA antigen bead Luminex™ platform.

VXII. Non A1 Kidneys for Transplantation in Blood Type “O” or “B”

Maine Transplant Program has discussed the logistics of using Non-A1 Kidneys for transplantation in blood type “O” or “B” recipients according to changes in UNOS allocation rules effective December 2014. Due to logistic concerns, low anticipated volume and concerns about development and maintenance of laboratory protocols, the leadership of the program decided NOT to implement such a program at this time.

Reviewed and agreed to by:

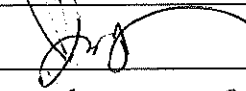
J. Vella, M.D. (Nephrology)



Date

3/1/2022

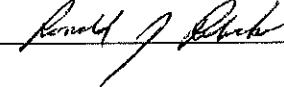
J. Whiting, M.D. (Surgery)



Date

3/2/2022

R. Rubocki, Ph.D. (HLA Lab)



Date

3/1/22

Original Date: June 15, 2011

Revised Date:

August 11, 2011
November 26, 2012
September 30, 2013
October 10, 2014
February 11, 2015
March 23, 2015
January 4, 2016
August 3, 2016
October 12, 2017
November 30, 2018
October 23, 2020
August 30, 2021
February 18, 2022

Appendix: Rationale for Development & Maintenance of Policy

Re: Update to Maine Medical Center **Joint written agreement between the histocompatibility laboratory and the transplant program** (OPTN policy H 3.100)

As required by the policy notice from Dr. Alan Ting (UNOS) sent to HLA Directors, Kidney and Pancreas Transplant Program Directors, Transplant Administrators for Kidney and Pancreas Transplant Programs on Sept 17, 2004. Policy Changes Approved by the OPTN/UNOS Board of Directors

The OPTN/UNOS Board approved the following new Policies, and Appendix D to Policy 3. Policy 3.5.17 refers to **deceased donor kidney** transplants, and 3.8.8 refers to **deceased donor pancreas** transplants. *These Policies shall be implemented by January 1, 2005.*

3.5.17 Prospective Crossmatching. *A prospective crossmatch is mandatory for all patients, except where clinical circumstances support its omission. The transplant program and its histocompatibility laboratory must have a joint written policy that states when the prospective crossmatch may be omitted. Guidelines for policy development, including assigning risk and timing of crossmatch testing, are set out in Appendix D to Policy 3.*

3.8.8 Prospective Crossmatching. *A prospective crossmatch is mandatory for all patients, except where clinical circumstances support its omission. The transplant program and its histocompatibility laboratory must have a joint written policy that states when the prospective crossmatch may be omitted. Guidelines for policy development, including assigning risk and timing of crossmatch testing, are set out in Appendix D to Policy 3.*

According to Appendix D to Policy 3

Histocompatibility testing provides clinicians with data to evaluate the immunological risk of proceeding to transplant. The timing and number of tests may vary depending upon specific needs of the program, waiting times, sensitizing events in individual patients or other considerations. These should be established to best suit the needs and concerns of each transplant program.

This policy addresses the following:

Organ Procurement and Transplantation Network (OPTN) Bylaws (effective date 02/01/15). Histocompatibility laboratories must have written agreements with every transplant program the laboratory serves, unless clinical urgency prevents such an agreement.