Immunogenetics / HLA Laboratory

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I. LABORATORY SERVICES

The UMMC-IMGL is a full-service histocompatibility and immunogenetics testing facility accredited by CLIA, ASHI, OPTN / UNOS and the CAP. Specific testing assays are as follows: HLA Class I and Class II typing using molecular based methods; HLA Class I and Class II antibody analysis using single antigen microbead assays; HLA Class I C1q binding / complement fixation assay; lymphocyte crossmatching using flow cytometry and virtual crossmatch.

II. SPECIMEN INFORMATION REQUIREMENTS FOR TEST REQUESTS.

All testing is ordered electronically by the provider via the Epic / HistoTrac HL7 Interface.
Copies of all orders, specific order set questions/requirements are electronically passed into the HistoTrac LIS from Epic, and posted in HistoTrac per specimen accordingly.

Minimal specimen information captured is:

For PATIENTS / RECIPIENTS:
Date of specimen
Full name of person to be tested
Date of Birth, Gender and MRN
SSN for Abdominal Organ Transplants
Physician requesting test(s)
Test(s) requested

For LIVING DONORS:
Date of specimen
Full name of donor, and relationship to patient (if applicable)
Date of Birth, Gender and MRN
Physician requesting test(s)
Test(s) requested

For DECEASED DONORS:
UNOS ID of donor
Date of Birth, Gender and MRN
Physician requesting test(s)
Test(s) requested

For NMDP DONORS:
Date of specimen
Donor NMDP ID
Patient NMDP ID
Physician requesting test(s)
Test(s) requested

III. SPECIMEN CONTAINER LABELING REQUIREMENTS

Full name of person or UNOS / NMDP Number
Date of Birth / MRN
Specimen collection date

IV. SPECIMEN HANDLING

a. All specimens must be kept at room temperature prior to delivery to the laboratory.

b. Alternative specimens for DNA based testing includes buccal swabs, paraffin embedded tissue, or other nucleated tissue types. Call the laboratory for collection information.

c. All specimens containers submitted for testing must be properly labeled, otherwise the UMMC-IMGL will issue a specimen rejection notification to the sender and cancel the testing in Epic.

V. TEST REQUISITIONS

a. All testing must be ordered in Epic.

b. If Epic is not available, downtime test requisitions are located on the intranet: https://www.testmenu.com/ummc

c. All downtime test requisitions must be completed with required information listed above in section II and match the included specimens.

VI. SPECIAL ARRANGEMENTS FOR TESTING

a. Notify the laboratory of all testing requests outside of those indicated in this document.

VII. SPECIMEN SHIPPING AND LABORATORY HOURS OF OPERATION

Outside Clients - Send all specimens to:

University of Maryland Medical Center Immunogenetics Laboratory
22 S. Greene St.
Room P2G01
Baltimore, Maryland 21201 - 1595

Inside Clients - Send all specimens through the tube system to STATION 6, or hand deliver to the HLA Laboratory in Room P2G01.

Contact Information –

Normal Hours of Laboratory Operation: Monday through Friday, 8 am – 6 pm

Client Services:	410-328-3271
Email:	IMGL@umm.edu

VIII. Test Turn-Around Times (TAT)

See Laboratory Service Guidelines below for specific test TAT.

Note: the time posted will be affected by the complexity of the case, requirements for additional specimen processing and / or specimen arrival time to the HLA laboratory.

Contact laboratory for specimen arrival time cut off to ensure expedited test processing.

IX. Clinical Consultation

Assistance with donor selection, search analysis or interpretive advice on histocompatibility testing is available from the Laboratory Clinical Director upon request.

Immunogenetics (HLA) Laboratory

OVERVIEW OF TESTS PERFORMED BY THE UMMC IMMUNOGENETICS LABORATORY

Test	Methodology	Specimen Requirement	Turn Around Time	Comment
HLA- A, B, C, DRB1, DRB3/4/5, DQB1, DQA1, DPB1, DPA1 Typing, High Resolution	NGS	Blood: 7 ml ACD whole blood	Routine: 14 calendar days	
HLA- A, B, C, DRB1, DRB3/4/5, DQB1, DQA1, DPB1, DPA1 Typing, Low Resolution	PCR-SSOP	Blood: 7 ml ACD whole blood	Routine: 7 calendar days TAT1: up to 72 hours	
HLA- A, B, C, DRB1, DRB3/4/5, DQB1, DQA1, DPB1, DPA1 Typing, Low Resolution	Real-time PCR	Blood: 7 ml ACD whole blood	Routine: 7 calendar days TAT1: up to 24 hours	
HLA Class I and Class II Antibody Identification by Single Antigen Antibody Analysis	Flow Cytometry - Microbead Array Assay	Serum: 7 ml Red top	Routine: 7 calendar days TAT1: up to 48 hours	Solid Organ transplant recipients MUST be drawn pre-dialysis and pre-IVIg
HLA Virtual Crossmatch (VXM)– T and B Cell, Allogeneic	Interpretation	Patient: Antibody Analysis by Single Antigen Bead test results and sensitization history Donor: HLA typing / High resolution preferred	Routine / Preliminary: 7 calendar days Final: 24 hours	Refer to program Joint Agreement for specific guidelines. Refer to individual specimen requirements for each test
HLA Lymphocyte Crossmatch – T and B Cell, Allogeneic or Autologous	Flow Cytometry	Recipient: Serum: 7 ml Red top - and Donor: 4 x 7 ml ACD whole blood	Routine / Preliminary: 7 calendar days Final: 24 hours	Whole blood should not be older than 48 hrs
C1q Binding / Complement Fixation Assay for HLA Class I Antibody ONLY	Flow Cytometry - microbead array assay	Serum: 7 ml Red top	Routine: 7 calendar days TAT1: up to 48 hours	This assay is for Platelet Transfusion Support ONLY

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HLA –A, B, C, DRB1, DRB3/4/5, DQB1/DQA1, DPB1/DPA1 Typing, High Resolution Molecular

Method	Next Generation Sequencing (NGS)
Synonyms	HLA Typing; Tissue Typing; Class I and Class II Antigen Typing; Direct sequencing; Sequencing; High Resolution Typing
Test Includes	Molecular definition of Class I A, B, C loci and Class II DRB1, DRB3/4/5, DQB1/DQA1, DPB1/DPA1 alleles currently recognized by W.H.O.
Availability	Monday through Friday for Routine Typing
Turnaround Time	Routine analysis within 14 calendar days
Specimen	Routine specimen is peripheral whole blood, although any nucleated tissue may be used. Contact the laboratory for instructions on collecting alternative tissue types (eg. buccal mucosa swabs)
Volume	7 ml ACD anticoagulated peripheral whole blood
Minimum Volume	1 ml ACD anticoagulated peripheral whole blood
Container	Acid Citrate Dextrose, Solution A or B
Collection	None
Storage Instructions	Store at room temperature
Causes for Rejection	Specimen improperly labeled, inadequate specimen and/or test requisition incomplete.
Use	Donor and recipient stem cell pre-transplant evaluation; Post solid organ transplant for donor allele specific antibody analysis.
Methodology	Molecular PCR based direct sequencing assay.
Limitations	Additional testing may be required to verify alleles which will lengthen test turnaround time.
Contraindications	None

Immunogenetics (HLA) Laboratory

HLA –A, B, C, DRB1, DRB3/4/5, DQB1/DQA1, DPB1/DPA1 Typing, Low Resolution Molecular

Method	PCR-Sequence Specific Oligonucleotide Probe (PCR-SSOP)
Synonyms	HLA Typing; Tissue Typing; Class I and Class II Antigen Typing
Test Includes	Molecular definition of all Class I HLA-A, B, C and Class II HLA-DRB1, DRB3/4/5, DQB1/DQA1, DPB1/DPA1 allele groups currently recognized by W.H.O.
Availability	Monday through Friday for Routine Typing; 24/7 for Deceased Donor Typing ONLY
Turnaround Time	Routine analysis within 7 calendar days; TAT1: up to 72 hours
Special Instructions	None
Specimen	Routine specimen is peripheral whole blood, although any nucleated tissue may be used. Contact the laboratory for instructions on collecting alternative tissue types (eg. buccal mucosa swabs). Typing for deceased donors may be performed using cells from whole blood, lymph node or spleen tissue.
Volume	7 ml ACD anticoagulated peripheral whole blood. For deceased donors, contact the HLA laboratory.
Minimum Volume	1 ml ACD anticoagulated peripheral whole blood. For deceased donors, contact the HLA laboratory.
Container	Acid Citrate Dextrose, Solution A or B. Specimens for deceased donors must follow UNOS Specimen Packaging guidelines.
Collection	None
Storage Instructions	Store at room temperature
Causes for Rejection	Specimen improperly labeled, inadequate specimen and/or test requisition incomplete.
Use	Tissue matching of donors and recipients for organ transplant; transfusion of HLA compatible blood products.
Methodology	PCR-SSO Typing: Molecular PCR based assay which uses oligonucleotide probes to identify the nucleotide polymorphisms present for the HLA locus being tested
Limitations	Additional testing may be required to resolve ambiguous allele combinations which will lengthen test turnaround time. RT-PCR method is the preferred low resolution HLA typing method. PCR-SSOP HLA typing method is only used when RT-PCR is not available.
Contraindications	None

Immunogenetics (HLA) Laboratory

HLA –A, B, C, DRB1, DRB3/4/5, DQB1/DQA1, DPB1/DPA1 Typing, Low Resolution Molecular

Method	Real-time PCR (RT-PCR)
Synonyms	HLA Typing; Tissue Typing; Class I and Class II Antigen Typing
Test Includes	Molecular definition of all Class I HLA-A, B, C and Class II HLA-DRB1, DRB3/4/5, DQB1/DQA1, DPB1/DPA1 allele groups currently recognized by W.H.O.
Availability	Monday through Friday for Routine Typing; 24/7 for Deceased Donor Typing ONLY
Turnaround Time	Routine analysis within 7 calendar days; TAT1: up to 24 hours
Special Instructions	None
Specimen	Routine specimen is peripheral whole blood, although any nucleated tissue may be used. Contact the laboratory for instructions on collecting alternative tissue types (eg. buccal mucosa swabs). Typing for deceased donors may be performed using cells from whole blood, lymph node or spleen tissue.
Volume	7 ml ACD anticoagulated peripheral whole blood. For deceased donors, contact the HLA laboratory.
Minimum Volume	1 ml ACD anticoagulated peripheral whole blood. For deceased donors, contact the HLA laboratory.
Container	Acid Citrate Dextrose, Solution A or B. Specimens for deceased donors must follow UNOS Specimen Packaging guidelines.
Collection	None
Storage Instructions	Store at room temperature
Causes for Rejection	Specimen improperly labeled, inadequate specimen and/or test requisition incomplete.
Use	Tissue matching of donors and recipients for organ transplant; transfusion of HLA compatible blood products.
Methodology	RT-PCR Typing: RT-PCR is a combination of traditional PCR for amplification along with probe technology for allele identification / detection.
Limitations	Additional testing may be required to resolve ambiguous allele combinations which will lengthen test turnaround time.
Contraindications	None

Immunogenetics (HLA) Laboratory

HLA Class I and Class II Antibody Identification by Single Antigen Antibody Analysis

Method:	Luminex (flow cytometry) microbead array assay; Single Antigen Immunoassay
Synonyms	HLA Class I or Class II Antibody Analysis; HLA Class I or Class II Single Antigen (SA) Analysis;
Test Includes	Tests for the presence of preformed HLA antibodies in patient serum using a panel of purified single HLA antigens conjugated to beads.
Availability	Monday through Friday for Routine Testing
Turnaround Time	Routine analysis within 7 calendar days; TAT1: up to 48 hours
Special Instructions	Draw blood prior to dialysis or any intravenous treatment for organ transplant recipients. Do not draw recipient while on dialysis machine or immediately following dialysis.
Specimen	Serum
Volume	7 ml serum
Minimum Volume	2 ml serum
Container	Serum: Red top or clot tube or serum separator (Tiger top / SST)
Collection	Must be delivered to the laboratory within 10 days following collection
Storage Instructions	Store specimen at room temperature. Serum separator (Tiger Top / SST) tubes should be inverted 5 times to mix activator.
Causes for Rejection	The specimen is grossly hemolyzed; Specimen improperly labeled, inadequate specimen and/or test requisition incomplete; Specimen over 10 days old upon receipt by the laboratory.
Use	Determine the presence of preformed HLA antibodies following sensitizing events (e.g. transfusions, pregnancies, transplant, immunizations) and / or evaluation of recipient donor specific antibodies for pre and post-transplant evaluation.
Methodology	Highly sensitive and specific assay using purified HLA Class I or Class II antigen bound to the surface of micro beads.
Limitations	Luminex microbead array antibody analysis is extremely sensitive and may detect antibody not detectable using the standard complement dependent cytotoxicity or ELISA. It is also well known that individuals may have antibody to the bead matrix (anti-latex antibody). This may result in high background fluorescence and the test results will be indeterminate. NOTE: Vendor reagent lot numbers may impact / limit HLA antibody detection for particular alleles.
Contraindications	Results are evaluated on an individual basis for potential donor specific reactivity. Antibody specificities in a broader sense are also used to determine unacceptable antigens to the general donor population.

HLA Virtual Crossmatch (VXM), T and B Cell, Allogeneic

Method	Interpretation
Synonyms	VXM, Paper Crossmatch
Test Includes	The comparison of patient antibody specificities against donor HLA typing with consideration of the recipient's sensitization history provided by the transplant program
Availability	Monday through Friday for Living Donors; 24/7 for Deceased Donors
Turnaround Time	Living Donors: Routine / Preliminary analysis within 7 calendar days; Final Crossmatch results within 24 hours; Refer to "Special Instructions" below. Deceased Donors: per Joint Agreement
Special Instructions	This interpretative report requires completed tests for both donor and recipient. Required recipient HLA Antibody results: HLA Class I and Class Antibody Identification by Single Antigen Antibody Analysis Required both donor and recipient HLA Typing results, with NGS typing preferred: HLA-A, B, C, DRB1, DRB3/4/5, DQB1/DQA1, DPB1/DPA1
Specimen	N/A
Use	Determine the presence of donor specific HLA antibodies to donor cells; Predict the results of the physical flow crossmatch
Methodology	Comparison of identified donor specific antibodies (DSA) by HLA Class I and Class II Antibody Identification by Single Antigen Antibody Analysis, and their reactivity against donor HLA typing for HLA-A, B, C, DRB1, DRB3/4/5, DQB1/DQA1, DPB1/DPA1 by low or high resolution. Serum date selected for DSA is usually last tested. The selection must be indicated by the client on the test requisition.
Limitations	Titer of antibody varies and may not be high enough to be detected using current reagents or it may be masked by a phenomenon known as Prozone. It is recommended that flow cytometric crossmatching be used to confirm virtual crossmatch results.
Contraindications	Crossmatch results are evaluated on an individual basis.

Immunogenetics (HLA) Laboratory

HLA Lymphocyte Crossmatch, T cell and B Cell, Allogeneic or Autologous

Method	Flow Cytometry
Synonyms	HLA Crossmatch; Flow Crossmatch; Allo crossmatch; Auto crossmatch
Test Includes	Flow cytometric test for the presence of preformed antibodies present in prospective recipient serum specific to donor HLA antigens. Both T and B lymphocyte crossmatching is simultaneously performed by identifying the cell types in the assay using monoclonal antibodies.
Availability	Monday through Friday for Living Donors; 24/7 for Deceased Donors
Turnaround Time	Living Donors: Routine / Preliminary analysis within 7 calendar days; Final Crossmatch results within 24 hours; Deceased Donor per Joint Agreement
Special Instructions	Serum must be delivered to the laboratory within 10 days following collection. Draw recipient specimen prior to dialysis. Do not draw recipient while on dialysis machine or immediately following dialysis. Do not draw blood from a PICC line. Donor blood specimens should not be older than 24 hours prior to receipt by the laboratory. Specimens over 24 hours old may result in crossmatch failures due primarily to loss of HLA antigen expression on the cells.
Specimen	Allogeneic crossmatch: Serum from recipient and lymphocyte cells from donor; Autologous crossmatch: Serum and lymphocyte cells from recipient
Volume	7 ml serum, (4)-7 ml ACD Solution A or B anticoagulated peripheral whole blood
Minimum Volume	5 ml serum, (3)-7 ml ACD Solution A or B anticoagulated peripheral whole blood
Container	Serum: Red top or clot tube or serum separator (Tiger top / SST) Peripheral Blood: Yellow top or Acid Citrate Dextrose, Solution A or B
Collection	Draw prior to dialysis, if applicable
Storage Instructions	Store all specimens at room temperature
Causes for Rejection	Specimen improperly labeled, inadequate specimen and/or test requisition incomplete
Use	Determine the presence of donor specific HLA antibodies to donor cells which may be identified by HLA Class I and Class II Antibody Identification by Single Antigen Antibody Analysis
Methodology	Four color flow cytometric assay using a serum specimen from the prospective recipient and a lymphocyte preparation from a prospective donor (allogeneic crossmatch), or the recipients own lymphocytes (autologous crossmatch). IgG antibody in the patient serum binding to the lymphocytes is detected using anti-human IgG antibody conjugated to FITC.

Limitations	Flow cytometric analysis may detect non-HLA antibody reactivity, or non-specific binding with B cell Fc receptors, and must therefore be interpreted in conjunction with recipient immunological history.
Contraindications	Crossmatch results are evaluated on an individual basis.

C1q Binding / Complement Fixation Assay for HLA Class I Antibody ONLY

Method:	Luminex (flow cytometry) microbead array assay
Synonyms	C1q Assay
Test Includes	Tests for the presence of preformed HLA Class I IgG subtypes antibodies in patient serum which can bind the C1q complement product, namely IgG1, IgG2 and IgG3. IgG4 does not bind C1q. This lack of C1q binding by the IgG4 subtype is the critical component of this assay.
Availability	Monday through Friday for Routine Testing
Turnaround Time	Routine analysis within 7 calendar days; TAT1: up to 48 hours
Special Instructions	Draw blood prior to dialysis or any intravenous treatment for organ transplant recipients. Do not draw recipient while on dialysis machine or immediately following dialysis HLA Class I Single Antigen Antibody Analysis testing must also be performed on the same specimen requested for C1q binding assay.
Specimen	Serum
Volume	7 ml serum
Minimum Volume	2 ml serum
Container	Serum: Red top or clot tube or serum separator (Tiger top / SST)
Collection	Must be delivered to the laboratory within 10 days following collection
Storage Instructions	Store specimen at room temperature.
Causes for Rejection	Specimen improperly labeled, inadequate specimen and/or test requisition incomplete; Specimen over 10 days old upon receipt by the laboratory.
Use	Determine the presence of preformed HLA antibodies which bind complement following sensitizing events (e.g. transfusions, pregnancies, transplant, immunizations). This assay is used specifically for selection of Platelets for Transfusion at UMMC.
Methodology	Highly sensitive and specific assay using purified HLA Class I antigen bound to the surface of micro beads followed by detection of bound C1q using an anti-C1q antibody.
Limitations	It is also well known that individuals may have antibody to the bead matrix (anti-latex antibody). This may result in high background fluorescence and the test results will be indeterminate. NOTE: Vendor reagent lot numbers may impact / limit HLA antibody detection for particular alleles.
Contraindications	None