

## Specimen Collection Requirements

### PURPOSE/PRINCIPLE:

To outline proper specimen collection for molecular HLA testing, including tube type, minimum specimen amount, proper tube and requisition labeling, specimen storage conditions, appropriate and inappropriate specimen test requests, unacceptable specimens, and recommended HLA testing policy for patients and donors.

### SPECIMEN COLLECTION:

The following table lists the specimen requirements for each test performed in the molecular HLA laboratory.

<b>Test Name</b>	<b>Specimen Type</b>	<b>Storage Time</b>	<b>Storage Conditions</b>
Mid-Resolution RSSO HLA Class I or Class II typing	1 ACD solution A (8.5 mL) or B (6.0 mL) vacutainer tube--whole blood; cord blood	Up to 7 days	RT (20°C to 26°C)
	Buccal Swab	Up to 24 hrs.	RT (20°C to 26°C)
	DNA	Up to 30 days Up to 1 year	2°C-8°C -20°C
High Resolution RSSO HLA Class I or Class II typing	1 ACD solution A (8.5 mL) or B (8.5 mL) vacutainer tube--whole blood; cord blood	Up to 7 days	RT (20°C to 26°C)
	Buccal Swab	Up to 24 hrs.	RT (20°C to 26°C)
	DNA	Up to 30 days Up to 1 year	2°C-8°C -20°C
High Resolution SSP-PCR Typing for HLA-A, -B, -Cw, -DR and/or -DQ alleles	1 ACD solution A (8.5 mL) or B (8.5 mL) vacutainer tube--whole blood; cord blood	Up to 7 days	RT (20°C to 26°C)
	DNA	Up to 30 days Up to 1 year	2°C-8°C -20°C

Flow Crossmatch	1 ACD solution A (8.5 mL) or B (8.5 mL) vacutainer tube--whole blood	Up to 3 days	RT (20°C to 26°C)
	Lymph Nodes in sterile media	Up to 5 days	2°C-8°C
	Spleen in sterile media	Up to 5 days	2°C-8°C

To collect buccal tissue, roll the Catch-All sample collection swab firmly on the inside of each cheek, approximately 20 times on each side, making certain to move the brush over the entire cheek. Air dry the swab for 20 minutes at room temperature. **NOTE: DO NOT ALLOW THE SWAB TO TOUCH ANY OBJECT, AS IT MUST BE FREE OF CONTAMINATION FOR DNA TESTING.** Return swab to the original tube. Label the tube according to instructions below.

Patient specimens must be labeled properly with the following: Name of patient, medical record number, date and time of draw, and phlebotomist's initials or number. Specimens for family members must be labeled properly with the following: Name of patient, patient's medical record number, name of donor, date and time of draw, and phlebotomist's initials.

A completed laboratory requisition must accompany all samples. Outpatient requisitions must contain the following information:

1. Patient's name
2. Full account number
3. Diagnosis, symptom, or ICD-9-CM code associated with ordered tests
4. Ordering physician or qualified health professional and UPIN number if a non-UAMS physician
5. Date of birth
6. Gender
7. Test(s) ordered
8. Draw date
9. Patient's insurance information if a non-UAMS patient

Donor requisitions must contain the following information:

1. Patient's name and medical record number
2. Donor's name

3. Donor's sex and race
4. Donor's date of birth
5. Donor's relationship to patient (helpful but not required)
6. Test(s) ordered
7. Ordering physician or qualified health professional
8. Draw date

### **MAIL-IN SPECIMENS:**

The transplant coordinators are responsible for contacting all patient/donors who need HLA testing. The coordinators are also responsible for acquiring consent forms and sending the correct tubes and test requisitions contained in the blood draw kits to the designated individuals.

The HLA blood draw kits consist of a test requisition, instructions for drawing the specimens, tube labels, three 7 or 10 ml ACD yellow top tubes (solution A or B), and one red top tube. Test requisitions for mail-in specimens must contain the same essential information as listed for outpatient requisitions in Section 4.0, Specimen Collection. If the facility drawing the specimens has any questions, the laboratory telephone number is listed on the requisition. Specimens drawn outside the institution must be sent by overnight mail to the HLA Laboratory by Wednesday of each week in order to insure arrival by Thursday afternoon. Mail-in specimens requesting HLA Class I or Class II molecular typing are rejected if greater than 7 days old on receipt.

### **UNACCEPTABLE SPECIMENS:**

1. Clotted specimens for molecular HLA-ABC or –DR/DQ testing.
2. For molecular testing, specimens from patients who received chemotherapy or other cytotoxic drug therapy before drug half-life clearance has been achieved.
3. Specimens for molecular HLA-A, -B, -Cw, -DR or -DQ testing more than 7 days old.
4. Less than 6 mL of whole blood for HLA-A, -B, -Cw, -DR or -DQ high resolution SSP-PCR testing.
5. Unlabeled or mislabeled specimens.
6. Specimens that yield a low lymphocyte count.
7. Specimens exposed to extreme temperature changes.
8. Specimens not collected in ACD or EDTA.
9. Specimen other than whole blood, buccal swab, cell culture or cord blood.
10. Specimen that has potentially been contaminated. If it is necessary to test this specimen, the technologist should interpret the results cautiously and the patient report should state that the specimen could be potentially contaminated.
11. Specimens that have been aliquoted and returned to the original tube. If a specimen must be aliquoted, the aliquot must never be returned to the original container.

Specimens that are considered unacceptable for testing must be documented. Documentation includes notification of the reason for unacceptability to the transplant coordinator or other appropriate personnel; reason for unacceptability in the patient's Communication Log; the hospital computer result system; and the Daily QA Log.

### **TESTING POLICY:**

1. Allogeneic bone marrow transplant candidates who will potentially be receiving a related donor must initially have mid-resolution HLA-A and –B locus molecular typing and HLA Class I antibody identification performed. If a related donor is found to match in at least 3 of the 4 Class I loci, then mid-resolution HLA-Cw, -DR and –DQ molecular typing will be performed by reflex. Prior to final donor selection, the recipient must have repeat HLA typing using a new specimen. Typing results previously reported by another laboratory are acceptable if documentation is received in the UAMS HLA Laboratory verifying that the results correlate.
2. Related bone marrow donors have mid-resolution HLA-A and –B locus molecular typing. If the initial findings indicate that a family member or members is a 3/4 or 4/4 match, then mid-resolution molecular typing for HLA-Cw, DR and -DQ alleles is performed by reflex. Prior to final donor selection, the donor must have repeat HLA typing using a new specimen. Typing results previously reported by another laboratory are acceptable if documentation is received in the UAMS HLA Laboratory verifying that the results correlate.
3. Allogeneic bone marrow transplant candidates who will receive a matched unrelated donor must have high resolution Class I and Class II molecular typing. Repeat HLA typing of recipient must be performed using a new sample prior to the initiation of a formal registry search and unrelated donor selection.
4. Unrelated bone marrow donors must have high resolution Class I and Class II molecular typing. Repeat HLA typing of an unrelated donor using a new sample is required for confirmation of initial results prior to stem cell collection. However NMDP typing data is acceptable as the first of these two samples. The UAMS transplant coordinator is notified when an unrelated donor is received in the HLA laboratory and the NMDP HLA typing report is faxed to our lab. The NMDP results are reviewed by the technologist and the medical director to determine if the results correlate with those obtained in our laboratory.
5. Bone marrow patients whose protocol is undetermined by the physician should have mid-resolution HLA-A and -B locus molecular typing and HLA Class I antibody identification/PRA performed.
6. Disease study patients for HLA-B27 or –B57 should have a mid-resolution HLA-B locus molecular typing. If the –B locus mid-resolution, is positive for HLA-B\*57:01, but is not resolved to one CWD allele, then high resolution typing will be recommended on the patient's result. Mid-resolution HLA-DR/DQ typing is performed to aid in diagnosis of celiac disease.

7. Kidney & Heart Transplant patients: Please refer to the transplant protocols.

### **CLINICALLY APPROPRIATE TEST REQUESTS:**

**Mid-Resolution HLA-A and -B locus testing:** Appropriate for patients who, due to their clinical diagnosis, may be considered as bone marrow transplant candidates; for parents and full siblings of these patients; for previously untyped patients with a positive Blood Bank platelet crossmatch screen; for previously untyped patients requiring HLA-matched platelet therapy; for HLA Class I disease association; may be indicated at the transplant coordinator's or physician's request for family members other than parents and full siblings.

**High-Resolution Molecular HLA-A, -B, -Cw testing:** Appropriate for unrelated allogeneic bone marrow candidates and donors.

**High-Resolution Molecular HLA-DR, -DQ testing:** Appropriate for unrelated allogeneic bone marrow candidates and donors.

**HLA Antibody Identification:** Appropriate for patients who, due to their clinical diagnosis, may be considered as bone marrow transplant candidates (an initial request is usual; however, another request is acceptable if a considerable amount of time has lapsed between the initial request and the scheduled transplant, or if the patient has had blood products after the initial request); for patients with a positive Blood Bank platelet crossmatch screen; as a diagnostic aid for thrombocytopenic patients; for monitoring of patients with a known HLA serum antibody.

If there is reason to question the appropriateness of any HLA test request, the transplant coordinator, charge nurse, physician, or Blood Bank resident may be consulted to verify the request. If a consultation with authorized personnel determines that a test ordered in SOFT or E-chart must be deleted, the technologist must document the deletion on the test requisition, including the person authorizing the deletion, the date, and time; in the Daily QA log book; and in the patient's Communication Log. If a test must be added, a nurse or physician must submit the test to HLA on a requisition within 30 days of the request.

**Mid-Resolution HLA-B locus testing for disease association:** Appropriate for patients who, are suspected of a particular diagnosis that would be more easily made if the patient expresses HLA antigens/alleles that have been found to be correlated to the diagnosis.

**Mid-Resolution HLA-DR/DQ locus testing for disease association:** Appropriate for patients who, might have celiac disease that would be more easily diagnosed if the patient expresses HLA antigens/alleles that have been found to be correlate to that diagnosis.

### **REPORTING TEST RESULTS:**

Results for mid-resolution HLA-A, -B and DR/DQ typing are usually reported within 72 hours. Results for high resolution molecular testing are usually reported within 5 working days. HLA antibody screens are usually reported within one week. HLA flow crossmatches are reported with 72 hours/ deceased donor workups within 5 hours. Results will be faxed, e-mailed or given verbally to physicians or the transplant team members.