Appendix I

Histocompatibility (HLA)
Specimen Collection & Processing

TESTING

HLA Typing

HLA Class I (ABC) and HLA Class II (DR, DQ)

HLA Single Antigen testing either Class I or Class II (specify antigen)
- HLA B27 screen
- Narcolepsy associated antigen screen (DQB1*0602)
- Celiac Associated HLA-DQ Type

HLA DNA Tests: HLA Class I and/or HLA Class II, either low resolution or high resolution.

Serum antibody screening

HLA-Antibody screens for Panel reactive antibody (Class I and/or Class II) screen.

HLA-Antibody Identification (Class I and/or Class II)

HLA-Platelet antibody screen

HLA Serum crossmatch: donor and recipient, or autologous

SPECIMEN

1. Anticoagulated sterile blood samples are required for HLA-ABC, HLA-DR, and crossmatching. Appropriate anticoagulants include ACD (yellow top vacutainers) and sodium heparin (green top vacutainers).
   a. Samples should be as fresh as possible, and ideally not more than 48 hours old.
   b. ACD (solution A is preferable to B) is the anticoagulant of choice. An acceptable alternative is sodium heparin at 25-50 units of Na heparin **(no preservatives)** per ml of blood
   c. Samples collected in ACD may contain viable lymphocytes up to 96 hours. Testing will be done based on minimum pretest viability greater than 80%.
   d. ACD is the anticoagulant of choice for extended transport of samples.
   e. All samples must remain at room temperature, and be kept from temperature extremes for transport and storage.
   f. DO NOT SPIN TUBES
2. Serum samples for antibody screening and crossmatching are drawn in a dry (red top) tube (no anticoagulant). The sample should remain at room temperature or may be refrigerated for transport. Frozen serum, separated from the clot, should remain frozen during transport.
3. Lymph nodes and spleen are alternative sources for lymphocytes from deceased donors. Samples should be collected aseptically and suspended in RPMI 1640 with antibiotics or other support media, and kept at 4º C. Saline should not be used for tissue collection or as transport media.
4. Requirements for histocompatibility testing
   a. HLA typing by lymphocytoxity
      - HLA-ABC: 10-20 ml of anticoagulated blood (ACD or heparin)
      - HLA-DR: 10-20 ml of anticoagulated blood (ACD or heparin)
      - HLA-ABC & DR: 30 - 50 ml anticoagulated blood (ACD or heparin)

      Minimum requirements:
      1. Pediatric: 3 - 5 ml
      2. Adult: 10 ml
      3. A patient who is lymphopenic or undergoing chemotherapy may require 60 ml peripheral blood drawn to provide an adequate cell number for testing. A minimal WBC of 1.0 required.

   b. Crossmatches
      - **Each donor**: 30 - 40 ml anticoagulated blood (ACD or heparin)
      - Deceased donor: 60 - 80 ml anticoagulated blood (ACD or heparin)
      - If donor has been transfused with >2 units of blood, pre-mortem nodes should be requested.
      - **Recipient:** 5 - 10 ml of clotted blood (serum dry clot tube)

c. HLA Antibody screening (any method)
   - 5 - 10 ml of clotted blood (serum dry clot tube)
   - Minimum: 1 ml of serum

d. HLA typing by DNA
   - 10 - 20 ml of ACD or EDTA (lavender tube) anticoagulated blood
   - Heparinized blood is known to interfere with the PCR process

e. Platelet antibody screening
   - 1 ml of plasma or serum

Specimen should be examined to determine that they were appropriately collected and maintained.

Unacceptable specimens include:

- Unlabeled specimens (no name or name and no date)
- Refrigerated green (heparinized) or yellow (ACD) top tubes
- Cracked or leaking tubes
- Heparinized specimens >72 hours old for crossmatch. (Testing will be based on viability, if it cannot be redrawn)
- Specimens drawn in Lithium heparin
- Grossly hemolyzed tubes
- Unclotted clot tubes
- Mismatched labels, label on tube does not match the form label, either in ID number or name.