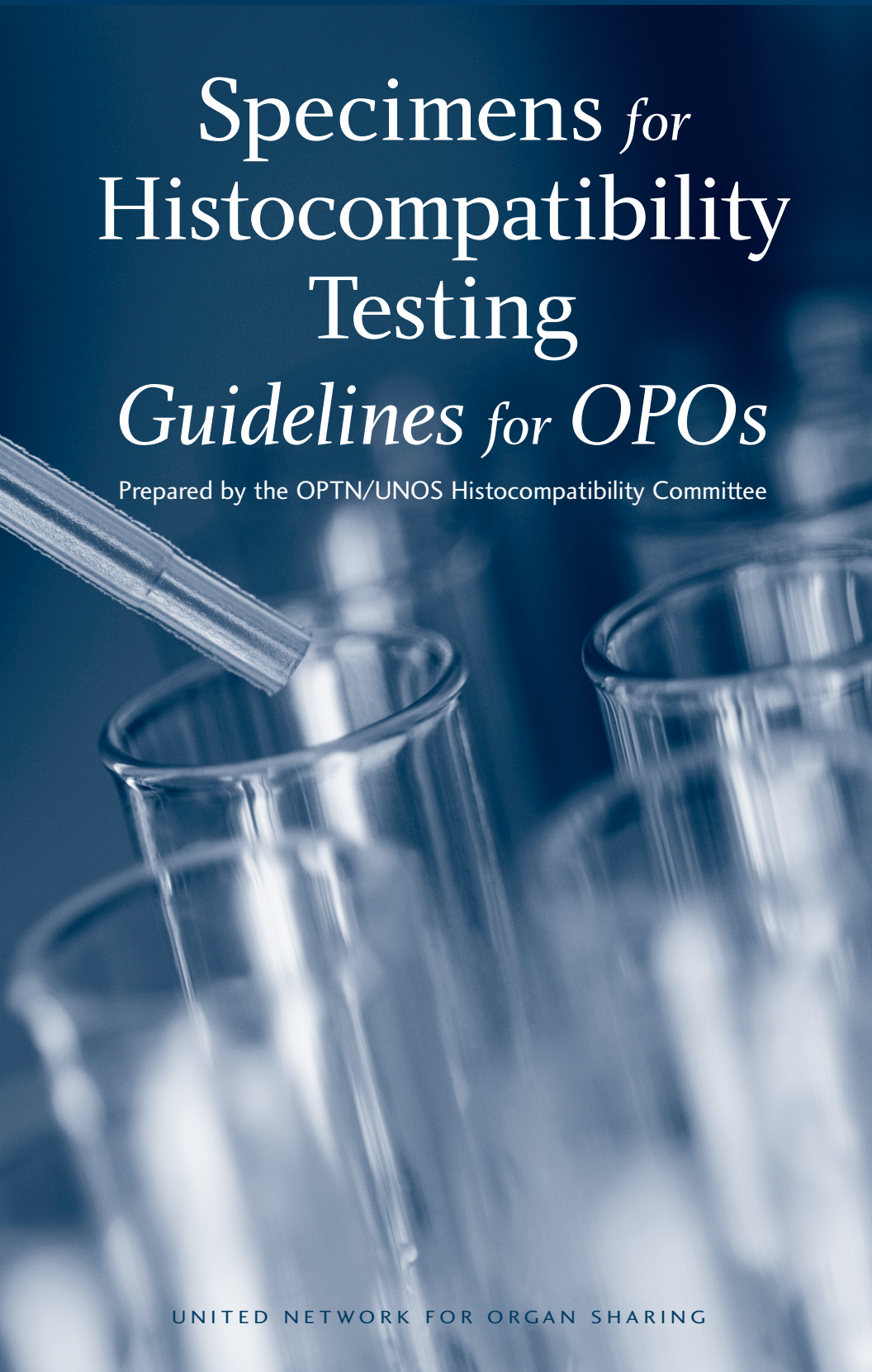
TALKING ABOUT TRANSPLANTATION

# Specimens *for* Histocompatibility Testing *Guidelines for OPOs*

Prepared by the OPTN/UNOS Histocompatibility Committee



UNITED NETWORK FOR ORGAN SHARING

# Specimens *for* Histocompatibility Testing
## *Guidelines for OPOs*

**The guidelines in this booklet will help you determine the appropriate number and type of specimens you need to collect from deceased organ donors for histocompatibility testing.**

**We'll also give you tips on how to store and ship your specimens to the Histocompatibility Laboratory for the highest-quality test results.**

**OPTN policy 2.5.5 lists the minimum tissue-typing materials you must collect for each donor. These minimum requirements, however, may not meet the needs of all OPOs and their Histocompatibility Laboratories. This document will help you establish a policy that will maximize the use of each donor organ.**

**The following table summarizes the types of tests that Histocompatibility Laboratories perform and the types of specimen required.**

| Procedure | Specimen Type | Anticoagulant(s) or Preservatives | Storage Temperature |
|---|---|---|---|
| Pre-recovery donor HLA typing *and* Pre-recovery preliminary crossmatches | Peripheral blood | 1. ACD<br>2. Na Heparin<br>3. EDTA | Room temp (RT) |
| | Lymph nodes | Tissue culture media | On ice (4°C) |
| ABO typing | "red-top" tube clotted blood | None | RT or on ice (4°C) |
| Organ recovery specimens | Lymph nodes | Tissue culture media | On ice (4°C) |
| | Spleen | Tissue culture media | On ice (4°C) |

# Histocompatibility Tests and Specimens Required

OPTN policy 2.5.5 requires that "Each OPO, with their respective histocompatibility laboratories, establish *minimum* written requirements for tissue typing material required to generate match runs for local or regional placement of all organs. Organ procurement organizations will establish minimum requirements for tissue typing material required for local disposition of livers, hearts and lungs. In view of the frequent need for regional shipment of pancreas and kidney allografts, however, sufficient specimens for several crossmatches are required."

At a minimum, you must obtain the following typing materials for each kidney and pancreas:

- One 7 to 10 ml. clot (red topped) tubes
- 2 ACD (yellow top) tubes
- 3 to 5 lymph nodes
- One 2x4 cm. wedge of spleen in culture medium, if available.

## Need for Additional Specimens

The specimen amounts listed in the policy may not always be sufficient for the lab's needs. Regional sharing agreements, the size of the service area, or the number of candidates on the waiting list can affect the number of crossmatches the Histocompatibility Laboratory needs to perform. For example, laboratories that serve an OPO with a large waiting list will require a greater volume of specimen (number of tubes of peripheral blood and/or number of lymph nodes) to perform HLA typings and preliminary crossmatches. Also, keep in mind that specimens often need to be sent with any organs shipped to another center, where additional histocompatibility testing must be performed.

It is critical that your OPO and your Histocompatibility Laboratory develop a mutually agreeable protocol for the number, amount, and types of specimens you need to collect for testing.

Specimens are needed to perform the HLA typing and crossmatching. These tests are critical in determining recipient selection. The HLA type of the donor is necessary to determine if there are any "0-antigen mismatched candidates", nationally. The kidney allocation system in UNet[SM]/DonorNet[SM] also uses the HLA type to determine the order of potential recipients on the match run for kidneys and pancreata. Cells obtained from these specimens are also used to perform pretransplant crossmatch tests for recipients of almost all types of solid organ transplants; including kidneys, pancreata, hearts, livers, and lungs.

The accuracy of the results and the time taken by the Histocompatibility Laboratory to do the multiple tests on deceased donors depend partly on the amount and quality of the tissue typing materials. If the specimens have been handled or stored incorrectly, or if there simply aren't enough of them, the lab may need more time to perform the tests and the results may not be completely accurate. In some cases the lab will not be able to obtain satisfactory results, and they will need new specimens to repeat the testing.

The viability of the cells used is critical. The longer a specimen is stored, even under ideal conditions, the fewer the number of viable cells. The lack of viable cells can make it more difficult for the lab to perform the test and could delay the time to transplantation. If a specimen has been incorrectly handled (e.g., frozen), the lab will be unable to isolate viable cells. By collecting the correct tissue

typing specimens and handling them correctly, you will ensure that the Histocompatibility Laboratory has the materials it needs to perform pretransplant testing.

Specimen types used for histocompatibility testing include:

- anticoagulated peripheral blood
- lymph nodes
- spleen

OPTN policy states that whenever possible, you should test deceased organ donors prior to organ recovery. This testing speeds the allocation process and reduces cold ischemia time. For this reason, most labs perform HLA typing and preliminary crossmatching of deceased donor organs using anticoagulated peripheral blood that they collected before organ recovery. If final crossmatches are necessary, the lab will generally use lymph node or spleen specimens collected at organ recovery.

## Anticoagulated Peripheral Blood Specimens

You can collect peripheral blood specimens in a variety of anticoagulants and thus tube types. Because of the volume of blood required for pre-procurement testing, coordinators often obtain venous blood from a central line from deceased donors. Be careful to collect the specimen in an aseptic manner and do not contaminate it with IV fluids. If the specimen is sterile, the viability of the cells is assured for a much longer time. If the Histocompatibility Laboratory needs to perform a preliminary crossmatch, be sure to collect an adequate number of tubes. Specimens should be transported at room temperature to the lab as quickly as possible. If you place the samples on ice or refrigerate them, you will significantly reduce the viability of the cells in peripheral blood specimens. You should also inform

the lab about any blood transfusions the donor received 72 hours before the specimens were collected. Blood transfusions administered to the donor in the immediate precollection period may have an impact on the accuracy of HLA typing. Each OPO and its lab should establish a policy regarding the need for confirmatory HLA typing of donors who have been transfused.

## Types of Anticoagulants

**Acid Citrate Dextrose (ACD) (yellow top tubes).** Most labs prefer this anticoagulant. It preserves cell viability very well, often for several days. Be careful not to confuse ACD vacutainer tubes with serum separator vacutainer tubes, which often have a yellow top as well. The serum separator vacutainer tubes are intended for clotted blood. An ACD tube has 2-3 mls of liquid anticoagulant in it while a serum separator tube has a gel-like substance in the bottom. Many smaller donor hospitals may not have ACD tubes available, so you may find it advantageous to carry a supply with you.

**Sodium Heparin (green top tubes).** This anticoagulant is suitable for most HLA typing and crossmatching assays. It preserves cell viability for between 48 to 72 hours. Take care to not use lithium heparin tubes (usually they have a lighter green top) as the lithium interferes with cell viability and are unacceptable for most HLA assays. Sodium heparin tubes are more commonly available in smaller donor hospitals and may be used as a substitute for ACD tubes if necessary.

**Ethylenediaminetetraacetic Acid (EDTA) (purple top tubes).** Some labs may request EDTA tubes for DNA-based testing, especially if ACD tubes are not available. Technicians can easily isolate DNA from EDTA anticoagulated specimens. These types of anticoagulants,

however, interfere with complement-dependent assays, (e.g., cytotoxicity crossmatches). EDTA tubes should not be used unless your Histocompatibility Laboratory specifically requests them. Plus, you should not collect all of the specimens in EDTA as specimens often have to be shared with other laboratories.

### Clotted Specimens

OPTN policies now require OPOs to collect a "red-top" tube of clotted blood so that receiving OPOs and/or transplant centers can confirm donor ABO blood type. A "red-top" vacutainer tube contains no anticoagulant, thus allowing the specimen to clot. Be careful not to substitute "serum separator" tubes, which also allow the blood to clot. The gel barrier in these tubes doesn't allow the lab access to the red cell part of the specimen, which is necessary for ABO typing. OPO coordinators must collect enough "red-top" tubes so that one specimen can accompany each transported organ and the receiving transplant centers can perform the required ABO verification. If an unforeseen event occurs and there is no "red-top" tube available, labs and blood banks may be able to perform an ABO type on almost any anticoagulated tube of blood (e.g., one of the ACD tubes left over from the HLA typing).

## Pediatric Donors

It may difficult or impossible to collect the same volume of peripheral blood samples from a pediatric donor that you would from an adult donor. Even with the use of a central line, it may not be possible to obtain 60–100 mls of peripheral blood from an approximately 15 kg donor with an estimated total blood volume of 1200 mls. In these situations, OPO personnel should communicate closely with the Histocompatibility Laboratory personnel performing the testing.

Knowledge of the donor's ABO type and the potential organs to be recovered will allow the Histocompatibility Laboratory staff to better estimate the volume of blood specimen needed for pre-recovery testing. For example, with very small donors, you might not expect kidney recovery. This expectation would mean fewer pre-recovery crossmatches performed and a smaller volume of specimen needed by the lab. Additionally, a smaller volume of transfused blood products may have an impact on the accuracy of HLA typing in a donor with a reduced blood volume. As a general rule, greater than one transfusion administered in the previous 24 hours for a pediatric donor may necessitate repeat HLA typing of the donor from lymph node or spleen specimens obtained with the organ recovery. In the pediatric donor setting, close communication between the OPO and the Histocompatibility Laboratory is essential from referral to organ recovery.

## Tissue Specimens

Tissue specimens collected at organ recovery for histocompatibility testing purposes include lymph nodes and spleen. Collecting adequate numbers and carefully preserving them is essential. Many labs isolate B lymphocytes for crossmatching and/or HLA typing. B lymphocytes represent only about 5% of the white cells in a peripheral blood specimen but account for up to 25–50% of the white cells obtained from a lymph node or spleen sample. The use of these tissue samples greatly enhances the ability of the lab to provide crossmatching results in as short a time as possible. To best preserve the tissue specimen, place it in tissue culture media and keep it on ice (4°C).

Some OPOs may recover lymph nodes prior to organ recovery. Handle these lymph nodes the same way you handle tissue specimens obtained at organ recovery.

## Lymph Nodes

The advantage of lymph node specimens is that a large number of lymphocytes, uncontaminated by other cell types, can be quickly isolated. It takes less time to isolate usable cells from lymph nodes than from any other type of sample. The greater numbers and quality of lymphocytes from lymph nodes, including B lymphocytes, also reduces the possibility of error due to poor cell quality in interpretation of both HLA typing and crossmatching results. While collection of lymph node specimens during organ recovery may seem like a less-important task than organ recovery, obtaining sufficient numbers of lymph nodes and correctly preserving them can significantly assist the Histocompatibility Laboratory in performing the pretransplant crossmatch tests, and also improve the quality of the results of those tests. If multiple organs are recovered, you will need 3 to 5 well-defined lymph nodes per organ recovered so that you can ship adequate lymph node specimens with each organ. Histocompatibility Laboratories greatly appreciate nodes that have been carefully dissected from the mesenteric fat. Surgeons or surgical assistants are clearly better qualified to do this and ensure that lymph nodes (and not fat) are actually present in the specimen.

## Spleen

You also can collect spleen samples or the entire spleen during organ recovery. If inadequate numbers of lymph nodes are obtained, a small (3x5cm) specimen of spleen can easily provide adequate numbers of lymphocytes for most labs to complete all of the necessary assays. The time taken by the laboratory to prepare lymphocytes from the spleen is longer than it is for lymph nodes, but adequate numbers and quality of cells, especially B lymphocytes, can be obtained from spleen. If you obtain the entire spleen, you should divide it into 5 to 6 equal sections for shipment and/or use by multiple labs, if necessary.

## Preservation of Tissue Specimens

To ensure adequate viability of the cells obtained from lymph node and spleen samples, you need to preserve them carefully and maintain them at temperatures colder than peripheral blood samples. As soon as the lymph node and spleen samples are recovered in the operating room, you should place them in some form of liquid tissue culture media. This will assure viability and stability. Histocompatibility Labs use many different types of tissue culture media. You need to consult with your laboratory beforehand and determine which type of tissue culture media and containers the lab prefers. Sterility of these samples is also critical. Make sure you use sterile containers and avoid using outdated bottles of tissue culture media. If you use a bottle of tissue culture media more than once, you should add an antibiotic solution. The samples must be correctly labeled and placed on ice for transport to the Histocompatibility Laboratory or stored at 4°C for later shipment to a recipient's transplant center. Allowing these specimens to "dry out" is the worst situation that can occur and will result in the laboratory being unable to isolate sufficient viable cells. If tissue culture media isn't available, place the specimens in sterile isotonic saline and transplant to the lab as quickly as possible.

## Specimen Labeling

Labeling the specimens correctly is as critical as obtaining the right samples. You must label each individual tube. OPTN policies require that all specimens obtained for histocompatibility testing and shipped with organs for transplant must be labeled with the UNOS Donor ID number, the date and time the specimen was collected, the donor's ABO type, and the type of specimen (e.g., lymph nodes, spleen). As the host OPO, you are responsible for labeling the specimens correctly. Either the tube or the accompanying requisition should indicate the individual who collected the specimen so that information

regarding source of the collection, possible contamination, or other fluids and drugs administered can be obtained quickly, if necessary. In some instances, labs may perform pre-recovery HLA typing before a UNOS donor number is available. In these situations, you and your Histocompatibility Laboratory should have a prearranged system for identifying the samples. The donor name alone should not be used as a unique identifier. You should add either the donor's date of birth or medical record number as well.

It is never acceptable to submit unlabeled specimens to the Histocompatibility Laboratory.

Both the Histocompatibility Laboratory and the OPO have the same goal of ensuring the highest quality of service in the fastest manner possible to maximize the use of each donor organ. Working together and developing mutually agreeable protocols for collecting specimens will help ensure that histocompatibility assays can be performed in the shortest time possible with the highest degree of accuracy.

**References:**

1. OPTN policy 2.5.5, Specimens for Tissue Typing Purposes, Minimum Procurement Standards for Organ Procurement Organizations.
2. OPTN policy 5, Standardized Packaging and Transporting of Organs and Tissue Typing Materials:
3. Guidelines for Specimen Collection, Storage and Transportation, ASHI Laboratory Manual, 4th edition, 2000.

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