

**UNIVERSITY OF PUERTO RICO  
MEDICAL SCIENCES CAMPUS - SCHOOL OF MEDICINE  
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE  
HISTOCOMPATIBILITY AND DNA LABORATORY**

***HLA AND DNA  
LABORATORY CATALOG***

# ***HLA AND DNA LABORATORY CATALOG***

## **Introduction**

The purpose of this manual is to assist you regarding the tests offered by the Histocompatibility and DNA Laboratory. The catalog of tests procedures includes instructions for obtaining specimens, methodology and use of the tests. If you have questions regarding the laboratory functions, special needs of your patients or laboratory, please contact the responsible persons listed in the manual.

## **Laboratory Staff**

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## **General Description of the Laboratory**

### **Location**

The Histocompatibility and DNA Laboratory is located in the third floor of the Medical Sciences Campus, School of Medicine, Department of Pathology and Laboratory Medicine, Suite B-310, at the Puerto Rico Medical Center.

### **Services Offered**

***Histocompatibility*** : Provides testing for Class I and Class II HLA by molecular serologic and by serologic techniques for purposes such as identification of the best possible donor for a patient in need of a transplant, typing cadaver donor, disease association, crossmatch for solid organs and paternity evaluation.

*Parentage Testing* : Provides DNA (RFLP) testing for child support offices and private parties.

## Working Hours

The regular working hours are from 8:00 am to 4:00 pm, Monday through Friday. Cadaver workup (typing and crossmatch) is available 24 hours a day, seven days a week.

## Laboratory Request form Documentation

All laboratory request forms submitted must be properly filled out for acceptance of the specimen being tested. The request form must have the patient's name, date of birth, physician's name and hospital or laboratory name.

## Specimen Collection and Preparation

Proper specimen collection and preparation is essential in order for us to provide a timely accurate test result and interpretation. Prior to each specimen collection, please review the specimen requirements (correct specimen, amount, specimen container, collection material, storage and handling).

## Specimen Containers

Red Top Tube : contains no anticoagulant/preservative

Lavender Top Tube : contains liquid EDTA as anticoagulant

Yellow top Tube : contains acid citrate dextrose (ACD) as anticoagulant/preservative

Green Top Tube : contains heparin as anticoagulant

## Sample Requirement for Pediatric Patients

Age	Tube Type	Typing
0-10 years	5 ml, green top	HLA-A,B typing (serology)
	5 ml, yellow top/lavander	HLA-DR, DQ typing
	5 ml, yellow top/lavander	HLA-DR,DQ typing (PCR/SSP)
	5 ml, lavender top	DNA Polymorphism (RFLP)
over 10 years	10 ml, green top	HLA-A,B typing (serology)
	10 ml, yellow top/lavander	HLA-DR, DQ typing

10 ml, yellow top/lavander  
5 ml, lavander top

HLA-DR, DQ typing (PCR/SSP)  
DNA Polymorphism (RFLP)

## **General Procedure for Venipuncture**

1. Check patient identification band against labels and requisitions forms (hospitalized patients). Ask the patient for her or his full name. If the patient identity is unknown or questionable, give the patient a temporary identificacion. Do not draw any specimen without properly identification of the patient.

## **Delivery Requirements**

Blood samples must be delivered by courier service. All samples should be considered as biohazard. Samples for serologic HLA typing should not be delivered from Friday 4:00 pm to Monday 8:00 am or the day before a holiday, to ensure viability of the sample. For cadaver typing or crossmatch, contact (787) 402-2337 Unit 230-2719.

## **Unacceptable Specimens**

Proper identification of specimens is essential to provide accurate laboratory results for the correct patient. The laboratory will not accept unlabeled specimens, even when accompanied by paperwork or request form bearing the patient=s name. Incomplete or inaccurately labeled specimens will be evaluated by the general supervisor and director in order to determine whether acceptable identification can be made and a report issued. If a sample is rejected for this reason, the physician will be notified by phone and the sample will be noted in the log book, including the cause for rejection.

Sample integrity is crucial to accurate test results. Samples can be compromised due to conditions during collection, storage or transportation. The most frequent causes of unacceptable sample are hemolysis (for HLA serology), inappropriate transport temperature, incorrect anticoagulant or samples more than 48 hours old. If a sample is rejected for this reason, the physician will be notified by phone immediately and the sample will be registered in the log book, including the cause for rejection.

## **General Rejection Criteria Include:**

1. Containers contaminated on the outside, since they are dangerous and unacceptable
2. Improper storage of specimen before reaching the laboratory

3. Unlabeled specimen or incorrectly labeled specimens
4. (Date and time of collection indicates that the specimen is inadequate) Samples more than 48 hours old.
5. Incorrect specimen (serum versus plasma)
6. Incorrect anticoagulant
7. Insufficient quantity of specimen
8. Discrepancy between request form and specimen
9. Hemolyzed or clotted specimen, depending on the test

### **Turn Around Time (TAT)**

The estimated Turn Around Time (TAT) is listed in the catalog.

### **STAT Testing**

Stat testing is available for cadaver workup (typing and crossmatch procedures).

### **Results and Reports**

Final test results are usually sent by courier service to the referring institution when all tests on a specimens have been completed. Final crossmatch results are reported immediately by phone, in addition to the written report.

### **Consultation**

The medical faculty, supervisor and technologists of the laboratory are available for consultation. Our laboratory staff is committed to helping you to provide the best patient=s care.

### **Laboratory Accreditation**

Health Care Financing Administration (CLIA certificate number 40D0658324)  
Puerto Rico Department of Health (Lic. 596)  
American Society of Histocompatibility and Immunogenetics (ASHI), 11-3-PR-02-1  
UNOS #03PRtl

## List of Tests (Alphabetical Order)

### Cadaveric Donor Evaluation

**Special instructions** Contact laboratory before drawing specimen  
**Specimen** Whole blood  
**Volume** 10 ml heparin (1 green top tube) 20 ml ACD (2 yellow top tubes)  
**Storage instructions** Maintain specimens at room temperature  
**Causes for rejection** Clotted specimen, hemolysis, refrigerated specimen  
**Use** HLA typing  
**Schedule** around the clock  
**Methodology** cytotoxicity  
**TAT** 4 hours

### Crossmatch, cadaveric donor (heart) Complement Dependent Cytotoxicity AHG

**Special instructions** The test must be notified to the MT on call  
**Specimen** Whole blood, serum  
**Volume** 10 ml heparin (green top) tube and 10 ml clotted (red top) tube from the recipient and 10 ml heparin (green top) tube and 20 ml ACD (yellow top) from the donor  
**Container** Green top tube (heparin), red top tube (no anticoagulant), yellow top tube (ACD)  
**Storage instructions** Maintain specimens at room temperature  
**Causes for rejection** Clotted specimen, hemolysis, refrigerated specimen, old samples  
**Use** Evaluate compatibility between donor and recipient  
**Schedule** Around the clock  
**Methodology** Cytotoxicity, T lymph (AHG) + B lymphocytes  
**TAT** 4 hours

### HLA-B 27

**Test includes** human leukocytes antigen testing to the detection of B27 antigen  
**Special instructions** The test must be scheduled with the laboratory one day in advance, since test requires viable lymphocytes.

**Specimen** Whole blood  
**Volume** 7 ml specimen  
**Container** Green top tube (heparin)  
**Storage instructions** Maintain specimens at room temperature  
**Causes for rejection** Clotted specimen, hemolysis, refrigerated specimen, old samples  
**Use** Evaluate spondyloarthritis and other disorders associated with this antigen  
**Schedule** Monday to Thursday  
**Methodology** Cytotoxicity  
**TAT** 3 days

### HLA Typing (A and B locus)

**Special instructions** The test must be scheduled with the laboratory one day in advance, since test requires viable lymphocytes. Include date and time  
**Specimen** Whole blood  
**Volume** 10 ml specimen  
**Container** Green top tube (heparin) or yellow top tube (ACD)  
**Storage instructions** Maintain specimens at room temperature  
**Causes for rejection** Clotted specimen, hemolysis, refrigerated specimen, old specimen  
**Use** Epidemiological marker, correlation with disease syndromes, transplantation candidate matching, transfusion of specifically compatible blood components, among others.  
**Methodology** Cytotoxicity, SSP  
**TAT** 3 days

### HLA Typing (DR and DQ Locus)

**Special instructions** The test must be scheduled with the laboratory one day in advance, since test requires viable lymphocytes. Include date and time  
**Specimen** Whole blood  
**Volume** 20 ml specimen  
**Container** Yellow top tube (ACD) or lavender top tube (FDTA)  
**Storage instructions** Maintain specimens at room temperature  
**Causes for rejection** Clotted specimen, hemolysis, refrigerated specimen, improperly labeled, old specimen  
**Use** Epidemiological marker, correlation with disease syndromes, transplantation candidate matching, transfusion of specifically compatible blood components, among others.  
**Methodology** Cytotoxicity  
**TAT** 3 days

### HLA Typing DRB and DQB 1 by SSP (Low Resolution)

**Special instructions** Schedule family studies in advance. The specimen should arrive in the laboratory Monday to Thursday and within 48 hours of venipuncture

**Specimen** Whole blood

**Volume** 5 ml specimen

**Container** Yellow top tube (ACD)

**Storage instructions** Maintain specimens at room temperature

**Cause for rejection** Clotted specimen, improperly labeled specimen, specimen received with more than 48 hours of venipuncture

**Use** Epidemiological marker, correlation with disease syndromes, transplantation candidate matching, among others.

**Methodology** PCR-SSP

**TAT** 2 days

### HLA Antibody Detection

**Specimen** serum

**Volume** 10 ml specimen

**Container** Red top tube (no anticoagulant)

**Storage instructions** Maintain specimens at room temperature

**Causes for rejection** Improperly labeled specimen, specimen more than 48 hours from venipuncture.

**Use** HLA antibodies detection in patients candidates to heart transplant

**Methodology** Cytotoxicity AHG

**TAT** 4 hours (stat), 5 days (routine)

### Parentage Testing Assessment

**Test includes** DNA-RFLP, HLA-A and B loci (if needed)

**Specimen** Whole blood

**Volume** adults: 10 ml heparinized whole blood (for HLA), 5 ml EDTA whole blood;  
pediatrics: 5 ml heparinized whole blood, 3 ml EDTA whole blood

**Container** EDTA (lavander top) tube

**Collection** Witnesses required during collection of blood samples from mother, child and presumptive father. All three samples must be labeled with complete name, date and time of collection and phlebotomist's initials. Photographs and thumb prints are to be placed on

the AClient Authorization@ form. Race and history of transfusion must be stated. Chain of custody will be followed.

**Storage instructions** Maintain specimens at room temperature

**Causes of rejection** All parties must be at the laboratory at the same time, appointments are required. Specimens will be not be drawn until all parties are present.

**Use** Determine the nonpaternity or the chance of paternity in cases of disputed paternity; establish blood relationship of potential immigrants, possible exchange of infants in nursery.

**Methodology** Cytotoxicity. Restriction fragment length polymorphism

**Schedule** Monday to Thursday

**TAT** 3 weeks

**NOTE :**       **Currently we are using DNA-RFLP almost exclusively for paternity testing. The next semester of 2002 this method will changed to DNA-STR which is a faster method.**