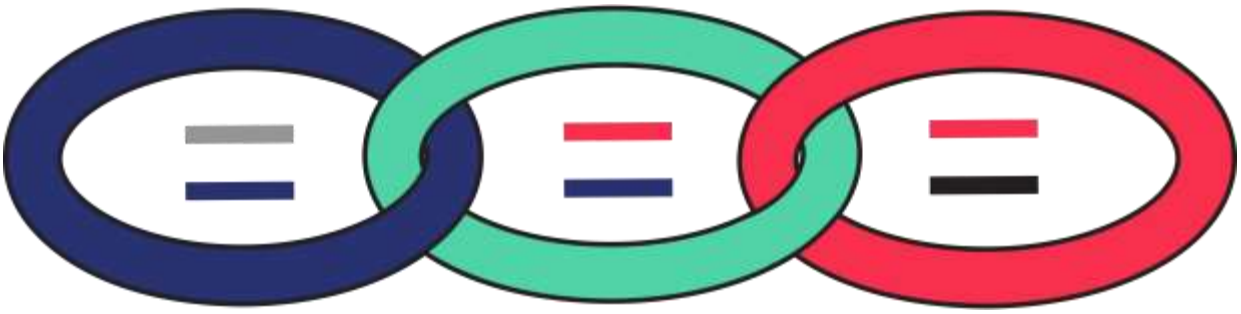
UNIVERSITY OF PUERTO RICO
MEDICAL SCIENCES CAMPUS - SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

HISTOCOMPATIBILITY AND DNA LABORATORY



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

Name	Phone
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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 low resolution testing for Class I HLA by serologic and molecular techniques and low and high resolution of Class II HLA by molecular techniques for purposes such as identification of the best possible donor for a patient in need of a transplant, typing deceased donor, disease association, crossmatch for solid organs and parentage testing assessment.

Parentage Testing: Provides DNA (STR) 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. Deceased 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 (proper 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 5 ml, yellow top Buccal Swab (Whole blood if requested)	HLA-A, B typing HLA-DR, DQ typing (PCR/SSP) DNA Polymorphism (STR)
Over 10 years	10 ml, green top 10 ml, yellow top Buccal Swab (Whole blood if requested)	HLA-A, B typing (serology) HLA-DR, DQ typing (PCR/SSP) DNA Polymorphism (STR)

General Procedure for Venipuncture

1. Check patient identification band against labels and requisition 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 identification. Do not draw any specimen without proper identification of the patient.

2. Address the patient, and inform the patient what is to be done. Reassure the patient to avoid as much tension as possible.

3. Position the patient properly, depending on whether the patient is sitting or prone, for easy, comfortable to the antecubital fossa.

4. Assemble equipment and supplies, including collection tubes, tourniquet, preparations for cleansing the area, syringes if necessary, sterile blood collection needle, and holder used for secure the needle (for evacuated collection tube system). Gloves and laboratory coat must be worn according to established policies.

5. Ask the patient to make a fist so veins are more palpable.

6. Select a suitable vein for puncture. Veins of the antecubital fossa, in particular the median cubital, are preferred. If one arm has an intravenous line, use the other arm to draw a blood specimen.

7. Cleanse the venipuncture site with 70% isopropanol alcohol solution. Begin at the puncture site, and cleanse outward in a circular motion. Allow the area to dry. Do not touch the cleaned area with any unsterile object.

8. Apply a tourniquet several inches above the puncture site. Never leave the tourniquet in place longer than one minute.

9. Anchor the vein firmly, both above and below the puncture site. Use either the thumb and middle finger or thumb and index finger.

10. Perform the venipuncture: **a) enter** the skin with the needle at approximately a 15 degree angle to the arm, with the bevel or the needle up. Follow the geography of the vein with the needle, **b) insert** the needle smoothly and fairly rapidly to minimized patient discomfort. Do not bury the needle, **c) using** a syringe, pull back on a barrel with a slow, even tension as blood flows into the syringe. Do not pull back too quickly to avoid hemolysis or collapsing the vein, **d) if** using an evacuated system, as soon as the needle is in the vein, ease the tube forward in the holder, firmly securing the needle holder in place. When the tube has filled, remove it by grasping the end of the tube and pull gently to withdraw.

11. Release the tourniquet when blood begins to flow. Never withdraw the needle without removing the tourniquet.

12. After all blood has been drawn, have the patient relax his or her fist. Do not allow the patient to pump the hand.

13. Place a clean sterile cotton ball or gauze lightly over the site. Withdraw the needle, and then apply pressure to the site.

14. Apply an adhesive bandage strip over the cotton ball or gauze to adequately stop bleeding and avoid a hematoma.

15. Mix and invert tubes with anticoagulants; do not shake the tube. For syringe-draw specimens, transfer blood to adequate tubes, taking caution not to hemolyze the specimen(s) and observing needle safety. Follow any special handing procedures.

16. Label the tubes.

17. Check condition of patient.

18. Dispose of contaminated material.

Notes:

a. Label each specimen tube with patient's name, phlebotomist's initials, draw date and time.
b. It is important to follow manufacturer and institution specific instructions for proper use of drawing supplies. Pay careful attention that anticoagulated samples are properly mixed.

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 the viability of the sample. Deceased donor typing or crossmatch are available 24 hours a day, seven days a week.

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. 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 and the sample will be noted 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.
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 deceased donor 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 specimen have been completed. Final crossmatch and stat 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)

Crossmatch, Deceased Donor (heart) Complement Dependent Cytotoxicity AHG

Special instructions The test must be notified to the MT on call.
Specimen Whole blood, serum.
Volume/Container 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.
Storage instructions Maintain specimens at room temperature.
Causes for rejection Clotted specimen, hemolysis, specimen refrigerated, old samples.
Use Evaluate compatibility between donor and recipient.
Schedule Around the clock
Methodology Cytotoxicity, T lymph (AHG) B lymphocytes
TAT 5 hours

Deceased Donor Evaluation

Special instructions Contact laboratory before drawing specimen.
Specimen Whole blood.
Volume/Container 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, and specimen refrigerated.
Use HLA typing.
Schedule Around the clock.
Methodology Cytotoxicity, PCR-SSP Molecular
TAT 4 hours

PRA Screening Class I/Class II (HLA Antibody Detection)

Specimen serum
Volume / Container 10 ml specimen (red top tube)
Storage instructions Maintain specimens at room temperature
Causes for rejection Improperly labeled specimen, specimen received more than 48 hours of venipuncture, lypemic serum.
Use Luminex Screening assay for the detection of IgG Antibodies to HLA Class I and/or Class II
Methodology Microarray (Luminex)
TAT 4 hours (stat), 3 days (routine)

Antibody Specificity Class I

Specimen serum
Volume / Container 10 ml specimen (red top tube)
Storage instructions Maintain specimens at room temperature
Causes for rejection Improperly labeled specimen, specimen received more than 48 hours of venipuncture and has not been centrifuged, separated the serum and freezed, lypomic serum.
Use: Assay for the qualitative detection and assignment of IgG Antibodies to HLA Class I molecules

Methodology Microarray (Luminex)
TAT 4 hours (stat), 1-2 weeks (routine)

Antibody Specificity Class II

Specimen serum
Volume / Container 10 ml specimen (red top tube)
Storage instructions Maintain specimens at room temperature
Causes for rejection Improperly labeled specimen, specimen received more than 48 hours of venipuncture and has not been centrifuged, separated the serum and freezed, lypomic serum.
Use: Assay for the qualitative detection and assignment of IgG Antibodies to HLA Class II molecules
Methodology Microarray (Luminex)
TAT 4 hours (stat), 1-2 weeks (routine)

HLA-B 27

Test includes human leukocytes antigen testing for detection of B27 antigen.
Special instructions The test must be scheduled with the laboratory one day in advance, since test required viable lymphocytes.
Specimen Whole blood
Volume/Container 7 ml specimen (green top tube / heparin)
Storage instructions Maintain specimens at room temperature
Causes for rejection Clotted specimen, hemolysis, specimen refrigerated, old samples
Use Evaluate spondyloarthritis and other disorders associated with this antigen
Schedule Monday to Thursday
Methodology Cytotoxicity
TAT 2 days

HLA Typing - A and B Locus

Special instructions The test must be scheduled with the laboratory one day in advance, since test required viable lymphocytes.
Specimen Whole blood
Volume / Container 10 ml specimen (5 ml minimum / green top tube [heparin] or yellow top tube [ACD])
Storage instructions Maintain specimens at room temperature
Causes for rejection Clotted specimen, hemolysis, specimen refrigerated, old specimen
Use Epidemiological marker, correlation with disease syndromes, transplantation candidate matching, transfusion of specifically compatible blood components, among others. See also paternity studies listing.
Methodology Cytotoxicity
TAT 2 days

HLA Typing A, B, C LOCUS (Low Resolution)

Special instructions The specimen should arrive in the laboratory Monday to Thursday and within 48 hours of venipuncture.
Specimen Whole blood
Volume / Container 10 ml specimen (5 ml minimum / yellow top tube [ACD])
Storage instructions Maintain specimens at room temperature
Causes for rejection Clotted specimen, improperly labeled specimen, specimen received more than 48 hours of venipuncture.
Use Epidemiological marker, correlation with disease syndromes, transplantation candidate matching, among others.
Methodology PCR-SSP Molecular
TAT 4 days

HLA Typing DRB1 and DQB1 (Low Resolution)

Special instructions The specimen should arrive in the laboratory Monday to Thursday and within 48 hours of venipuncture.
Specimen Whole blood
Volume / Container 10 ml specimen (5 ml minimum / yellow top tube [ACD])
Storage instructions Maintain specimens at room temperature
Causes for rejection Clotted specimen, improperly labeled specimen, specimen received more than 48 hours of venipuncture.
Use Epidemiological marker, correlation with disease syndromes, transplantation candidate matching, among others.
Methodology PCR-SSP Molecular
TAT 4 days

HLA Typing DRB1 (High Resolution)

Special instructions The specimen should arrive in the laboratory Monday to Thursday and within 48 hours of venipuncture.
Specimen Whole blood
Volume / Container 10 ml specimen (5 ml minimum / yellow top tube [ACD])
Storage instructions Maintain specimens at room temperature
Causes for rejection Clotted specimen, improperly labeled specimen, specimen received more than 48 hours of venipuncture.
Use Epidemiological marker, correlation with disease syndromes, transplantation candidate matching, among others.
Methodology PCR-SSP Molecular
TAT 4 days

Parentage Testing Assessment

Test includes DNA-STR
Specimen Buccal swab (whole blood if requested)
Quantity 4 color coded buccal swabs for each individual (Pink: Mother; Yellow: Child and Blue: Alleged Father).
Volume for Whole Blood Adults: 5 ml EDTA whole blood; Pediatrics: 3 ml EDTA whole blood

Collection Witnesses required during collection of color coded buccal swabs or blood samples from mother, child and alleged father. All three samples must be labeled with complete name and date of collection and phlebotomist's initials. Photographs and thumb prints are to be placed on the "Client 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 Specimens will be not being obtained until all parties are present at the same time.
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 Short Tandem Repeats
Schedule Monday to Friday
TAT 2 - 3 weeks