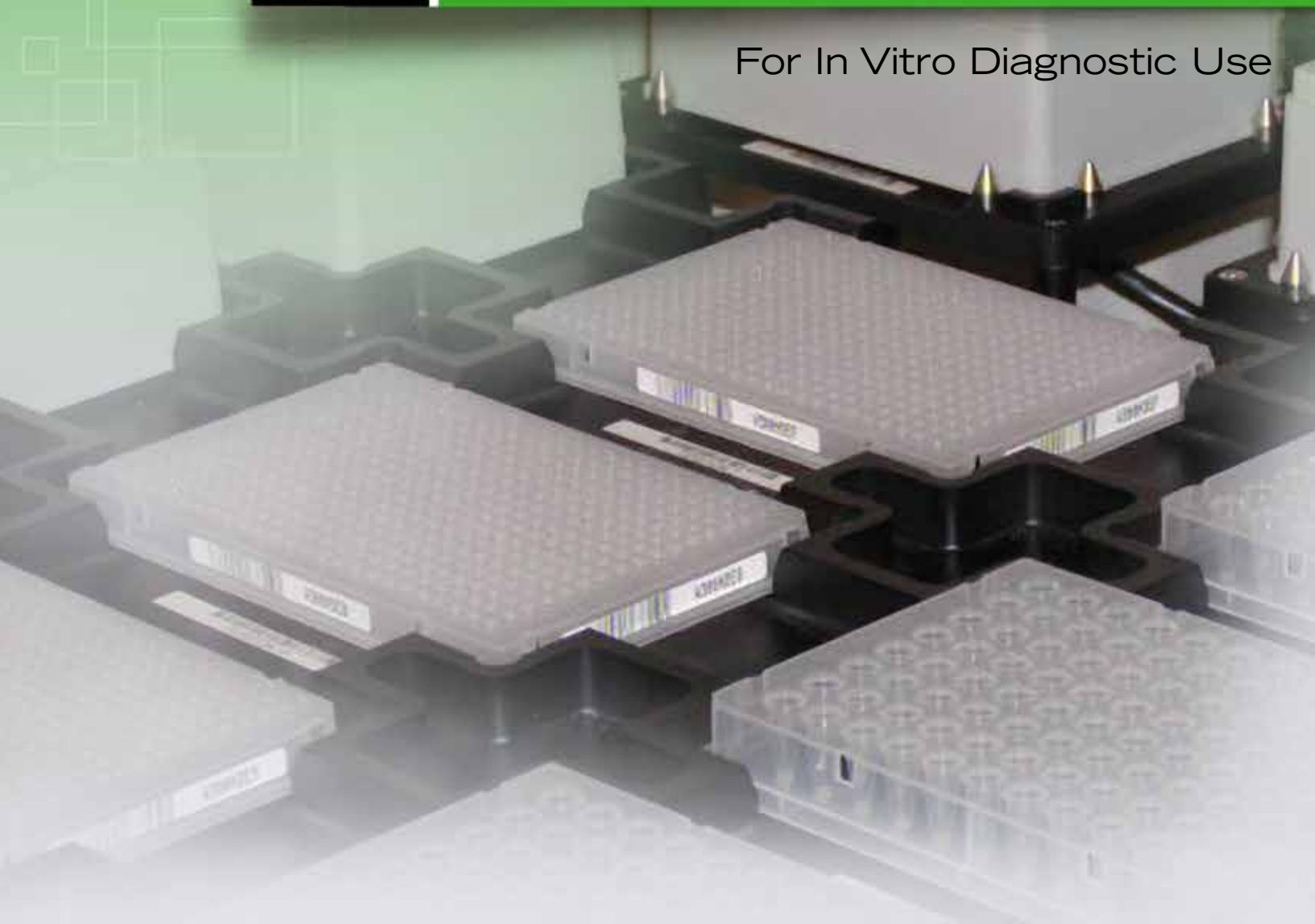
CareDx®

AlloMap® Testing

Laboratory Services Guide

For In Vitro Diagnostic Use



AlloMap®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LABORATORY SERVICES GUIDE
AlloMap Testing

I. INTENDED USE

AlloMap Molecular Expression Testing (the AlloMap Test) is an In Vitro Diagnostic Multivariate Index Assay (IVDMIA) test service, performed in a single laboratory, assessing the gene expression profile of RNA isolated from peripheral blood mononuclear cells (PBMC). AlloMap Testing is intended to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection (ACR) at the time of testing in conjunction with standard clinical assessment.

The AlloMap Test is indicated for heart transplant recipients:

- 15 years of age or older
- At least 2 months (≥55 days) post-transplant

The performance characteristics of the AlloMap Test were established with samples from heart transplant recipients at least 15 years of age and at least 2 months (≥ 55 days) post-transplant at the time samples were collected.

II. INTRODUCTION

Since the first successful cardiac transplant in 1967, more than 100,000 such operations have been recorded by the Registry of the International Society for Heart and Lung Transplantation. Improved immunosuppressive agents have resulted in significant reductions in acute rejection rates and improved survival. However, even with modern immunosuppressive therapy, acute cellular rejection can still lead to graft dysfunction, and remains a significant cause of morbidity and mortality.

CareDx is a molecular diagnostics company focused on the discovery, development and commercialization of non-invasive gene expression testing in the areas of transplant medicine and autoimmunity. The company has developed a proprietary method of measuring gene expression that provides additional options to current patient management by physicians specializing in these disease areas.

Principle of the Test

Performed solely in the CareDx CAP-accredited clinical laboratory, the AlloMap test is a service that is intended to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection (ACR) at the time of testing, in conjunction with standard clinical assessment.

AlloMap Molecular Expression Testing Process

Figure 1 illustrates the process for AlloMap Molecular Expression Testing. Blood is collected from the patient and subsequently processed, packaged and shipped frozen to the CareDx laboratory. At the CareDx laboratory, gene expression levels are determined via real time PCR for each of the test and control genes and converted to the AlloMap Test Score. The AlloMap Score is then reported to the physician, who determines the next steps for the patient.

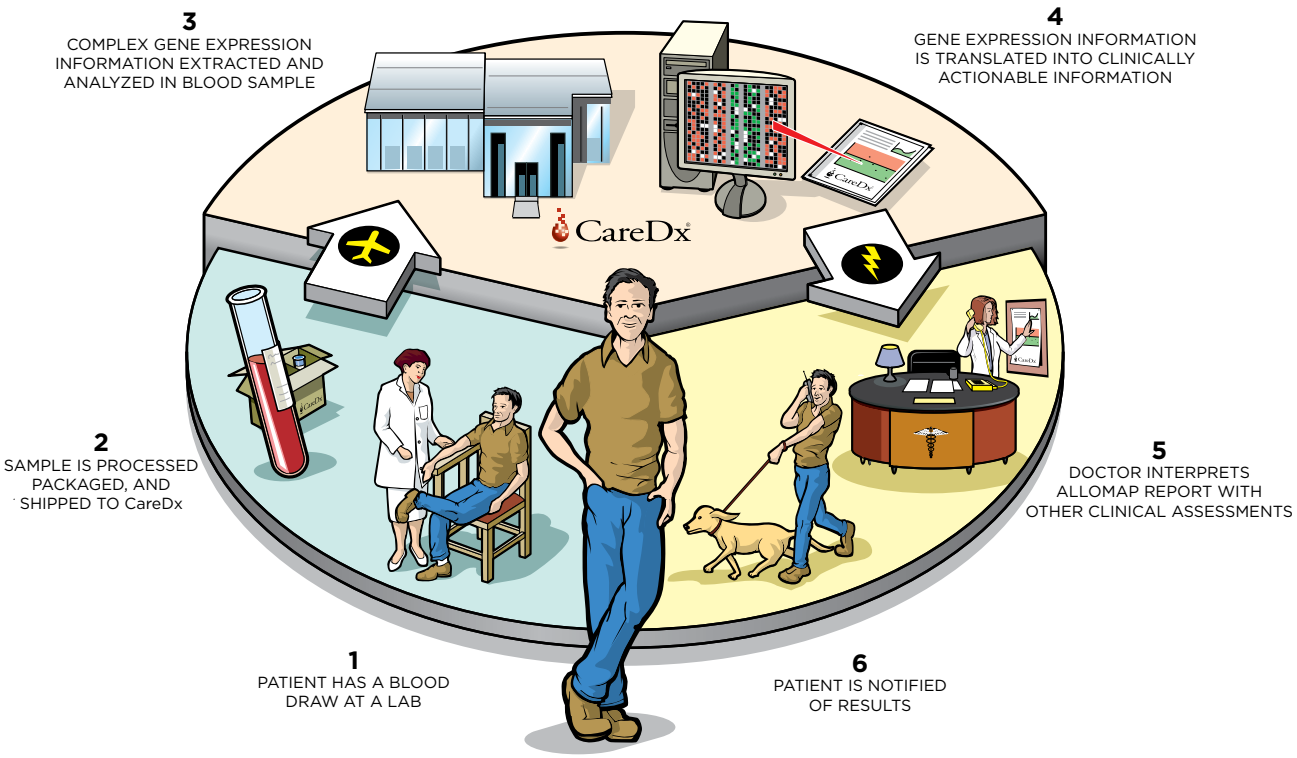

Figure 1: AlloMap Molecular Expression Testing Process

III. SUMMARY AND EXPLANATION OF THE TEST

The AlloMap test is based on standard quantitative real-time polymerase chain reaction methodology (qRT-PCR)[1] using RNA isolated from peripheral blood mononuclear cells (PBMC). A whole blood sample is collected in a BD Vacutainer® CPT™ Cell Preparation tube (Becton Dickinson, NJ) with sodium citrate anticoagulant (CPT tube). PBMC are isolated, lysed and the released RNA is stabilized and frozen (PBMC lysate). Following shipment to the CareDx CAP-Accredited laboratory in Brisbane, CA, RNA is then purified from the PBMC lysate. The cDNA is subsequently generated from the RNA for use in the AlloMap test. The AlloMap test is performed using general laboratory equipment and commercially available thermal cycling instruments configured for qRT-PCR testing. The AlloMap test is a panel of 20 gene assays, 11 informative and 9 used for normalization and quality control, which produces gene expression data used in the calculation of an AlloMap Score that ranges from 0-40.

IV. CUSTOMER CARE CONTACT INFORMATION

CareDx Customer Care

Telephone	1-888-255-6627 (1-888-ALLOMAP)
Fax	1-415-287-2456
Email	CustomerCare@CareDx.com

Ordering

Telephone	1-888-255-6627 (1-888-ALLOMAP)
Fax	1-415-287-2456
Email	OrderSupplies@CareDx.com

Hours of operation: 6 AM to 5 PM Monday through Friday, 8 AM to 3 PM Saturday, Pacific Time

V. EQUIPMENT REQUIRED – CENTRIFUGE

CareDx provides to each laboratory processing AlloMap samples either a **Drucker 755VES** centrifuge (Drucker) or a **Thermo Electron Centra CL2** centrifuge (CL2), and all required accessories.

Note: if, for any reason, an alternate centrifuge must be used to process a sample, call CareDx Customer Care for assistance with centrifuge specifications and RCF settings. Samples that are processed outside of CareDx specifications cannot be tested.

Centrifuge specifications:

- Swing-out bucket rotor; Note: **Do not** use a fixed-angle rotor
- Tube bucket that accommodates the 16mm x 125mm CPT tube

  Note: There must be sufficient room between the rotor and the CPT tube when it is inserted into the tube carrier, so that the stoppered end of the tube does not come into contact with the rotor during centrifugation causing the tube to break.
- Relative centrifugal force (RCF): 1796-1970 x g

Initial Set Up

Set up the centrifuge following the manufacturer's recommended procedures found in the "Drucker Model 755VES Operator's Manual" or the "Centra CL2 Instruction Manual" that is shipped with the centrifuge unit. Both centrifuges should be set up on a flat and level surface with enough space around the unit to allow for adequate ventilation and airflow (approximately 3 inches on each side and 4 inches at the back of the unit).

Drucker: To assemble the tube holders, place the flat, black rubber disk in the bottom of the green tube holder **first** and then insert the black plastic adapter inside tube holder. The black adapter stabilizes the tube during centrifugation, and the black rubber disk will cushion the tube to minimize the risk of tube breakage. During use, press the cone-shaped clear plastic lid down firmly on the tube holder to contain any aerosols that may be created during centrifugation.

CL2: To assemble the tube buckets, insert one brown tube adapter into each tube holder **first**, and then drop one black rubber disk down through the adapter to the bottom of the holder-adapter assembly. The brown adapter stabilizes the tube during centrifugation, and the black disk will cushion the tube to minimize the risk of tube breakage. During use, screw the dome shield (lid) onto the tube holder to contain any aerosols that may be created during centrifugation.

Daily Use

Before using the centrifuge, ensure that the speed of the centrifuge is set appropriately (see Section X Specimen Processing Procedure).

Cleaning

Keep your centrifuge, tube holders, adapters and lids clean to ensure good operation and to extend its life.

To clean the sample chamber, use a damp sponge, warm water and a mild liquid detergent suitable for washing dishes by hand. Do not use caustic detergents or detergents that contain chlorine ions, since these attack metals. Remove stubborn stains with a plastic scrub pad. Do not use steel wool, wire brushes, abrasives, or sandpaper, since they create corrosion sites. Never pour water directly into the sample chamber. After cleaning any part of the centrifuge, dry it properly, preferably using a clean, absorbent towel.

Broken Tubes / Blood Spill

Follow your laboratory's safety precautions for handling and disposal of biohazardous contaminated broken glass when carrying out these steps.

1. If a tube breaks during centrifugation, or if blood otherwise becomes aerosolized, do not open the centrifuge for 30 minutes.
2. After opening the centrifuge, disconnect it from its power source.
3. To remove the broken glass and blood, follow your laboratory's usual safety precautions (such as use of personal protective equipment and engineered safety devices) and carefully remove broken glass from the bowl of the centrifuge.
4. Remove the buckets and dispose of broken glass/blood.
5. Ensure all broken glass is removed, since fragments of broken tubes may lead to additional breakage.
6. Decontaminate bowl, buckets, rotor, lid, and any other grossly contaminated area with 10% sodium hypochlorite. To reduce the chance of corrosion, always decontaminate for the minimum recommended time. After decontaminating, follow the cleaning procedure, above.

Corrosion

Rotors and structural accessories are manufactured and finished to give maximum resistance to corrosion. However, maximum equipment life requires that you continually inspect the rotor cavities for corrosion, especially after using chlorine ion solutions, such as sodium chloride (saline), and sodium hypochlorite (household bleach). These solutions attack most metals. Follow the Cleaning Procedure and clean the rotor, rotor chamber, and accessories (particularly the sample compartments and bucket cups) thoroughly after each use of sodium hypochlorite. Inspect all surfaces under bright light for corrosion; small crevices will grow deeper and cause failure.

If you see signs of corrosion, contact CareDx Customer Care at CustomerCare@CareDx.com

Power Cord Replacement

Any sign of damage to the power cord should be reported immediately to CareDx Customer Care. Do not use a centrifuge with signs of wear or damage to the power cord.

Decontaminating Equipment before Shipping

Follow the decontamination instructions included in the return shipping box provided by CareDx.

VI. SUPPLIES REQUIRED AND SUPPLIED BY CareDx

1. **BD Vacutainer® CPT™ Cell Preparation Tubes with Sodium Citrate (CPT). 8.0 mL**
   - Store tubes at room temperature (18 to 25° C). Protect tubes from direct light.
   - Shelf life at 18 to 25° C is printed on each individual tube. Check expiration date of each tube before use.
2. **CareDx Frozen Shipper Pack**
   - Materials required to properly ship the specimen to CareDx for testing.
3. **AlloMap Sample Processing Reagents Kit**
   Consult Instructions for Use (REF LQ-10016)

Contents of the Kit

Each AlloMap Sample Processing Reagent Box includes:

PBS 10 centrifuge tubes containing 5.0 mL phosphate buffered saline

LDX 10 tubes containing 1.8 mL of LyseDx™ lysing reagent:

- 1% Beta-Mercaptoethanol in buffer RLT (Contains guanidinium thiocyanate)

PIP 10 sterile disposable transfer pipettes

PI 1 Package insert with instructions for use

QRC 1 Quick reference card

Reagent Storage and Stability

- Sample Processing Reagents: Store sample processing reagents at room temperature (18 to 25° C). Check expiration date printed on each reagent tube before use.
- Sample processing reagents are single use only. Keep reagent tubes closed until use. Once reagent tubes are opened, use immediately. Do not recap and store for later use.

Ordering Supplies

To order AlloMap testing supplies, contact CareDx Customer Care at OrderSupplies@CareDx.com. Please allow at least a two week lead time for replenishing supplies.

VII. WARNINGS AND PRECAUTIONS

- When drawing blood for use in the AlloMap test, follow your laboratory's universal precautions for bloodborne pathogens.
- Follow your laboratory's policy for personal protective equipment (PPE) during sample preparation.
- AlloMap Sample Processing reagents are single use only.
- LyseDx™ lysing reagent contains beta-mercaptoethanol and guanidinium thiocyanate which can cause skin and eye irritation and is considered toxic. Avoid contact with skin, mouth, eyes and clothing.
- Do not mix LyseDx lysing reagent with sodium hypochlorite (chlorine bleach). When cleaning LyseDx reagent spills, the area must first be wiped down with soapy water, after which 10% sodium hypochlorite may be used, if necessary.
- **Do not mix reagents between lots.**

IMPORTANT: *Gene expression continues to change in live cells; therefore, the time from phlebotomy to freezing the cell lysate must be no longer than **3 hours**.*

References http://allomap.com/healthcare-providers/allomap-sample-preparation-training/

Safety Data Sheet, Phosphate Buffered Saline

Safety Data Sheet, LyseDx

Safety Data Sheet, Dry Ice (CO2)

VIII. AlloMap TEST REQUISITION FORMS (TRF) AND TEST ORDERS

AlloMap Test Requisition Forms (TRF) are pre-printed with your lab information and can be ordered at OrderSupplies@CareDx.com. If you receive a TRF used to order an AlloMap test that has a different lab name on it, please cross out the lab name on the form and add your lab name.

AlloMap tests may be ordered in a number of ways including (examples below):

- AlloMap TRF which has tube labels attached to be used for labeling the AlloMap specimens.
- Order sheet, lab script or faxed AlloMap TRF

AlloMap TRF completed by transplant center: If the patient presents with a completed AlloMap test requisition form or the transplant center has provided it to your site, use that form to identify the patient, record specimen collection and processing information and label the specimen. **Send all paperwork you receive with the processed specimen to CareDx.**

Test is ordered using an alternate method (not on an AlloMap TRF): If the patient does not arrive with the AlloMap TRF and the transplant center has not provided it, (i.e., phone orders, faxed orders, lab script or test order form), use a blank TRF and write the patient's first and last name, date of birth, and medical record number on the AlloMap test requisition. Use this TRF to identify the patient, record specimen collection and processing information and label the specimen. **Send all paperwork you receive with the processed specimen to CareDx.**

Specimen Collection and Processing Information: Specimen collection date, specimen collection time, and specimen in freezer time MUST BE FILLED OUT FOR EVERY TEST.

Comments: Enter any comments relevant to the specimen collection, processing or patient status.

The TRF must accompany the patient specimen into your processing area, because a barcode label MUST be affixed to the specimen sent to CareDx. Extra barcode labels are provided on the TRF for labeling paperwork if necessary. The barcode labels uniquely identify a patient's specimen. DO NOT use the same barcode label for more than one patient's specimen. Improperly labeled and unlabeled specimens will not be tested.

Requisitions must accompany patient specimens when they are sent to CareDx. Keep the yellow copy of the TRF for your records, and send the white copy to CareDx with the processed patient specimen.

IX. SPECIMEN COLLECTION PROCEDURE

IMPORTANT NOTE:

*All processing must be completed at room temperature and the lysate from processed specimens must be frozen **within 3 hours of blood collection**. Freeze the lysate at -15° C or colder, or on dry ice, and ship as soon as possible, either on the day the specimen was collected or the next business day, to CareDx for testing.*

1. Identify the patient according to your laboratory's patient identification policy.
2. Prepare the patient for phlebotomy according to your standard procedure.
3. Select a CPT tube and additional supplies needed to perform venipuncture. Check the tube's expiration date before use.
4. If collecting additional specimens from the same venipuncture, follow the recommendations of your laboratory for the correct order of collection for citrate tubes. If your laboratory does not have a recommended draw order, draw tubes in the following order:
   - Blood cultures - SPS
   - Citrate tubes, including CPT
   - BD Vacutainer SST Gel Separator Tube, Serum Tube (glass or plastic)

- Heparin tube, BD Vacutainer PST Gel Separator Tube with heparin
- EDTA tube
- Fluoride (glucose) tube

5. Collect 8 mL of blood into the CPT tube. Allow the tube to fill completely - flow may slow at the end of the draw.

   Note: *Minimum blood volume for the 8.0 mL CPT tube is 6 mL. CPT tubes with less than 6 mL of blood are **not** acceptable for AlloMap testing. Do **not** process CPT tubes containing less than 6 mL of blood; collect a new specimen for use.*

6. Gently invert the tube at least 10 times to fully mix the blood with anticoagulant.
7. Label the tubes according to your laboratory's procedure for specimen identification.
8. Place a bar-coded label from the AlloMap Test Requisition Form (TRF) on the CPT tube.
9. On the TRF, fill in the specimen collection date and time.
10. Immediately send the specimen and the TRF to the processing area.
11. Do not refrigerate the specimen.

X. SPECIMEN PROCESSING PROCEDURE

IMPORTANT NOTES:

*Use only AlloMap Sample Processing Reagents; do not substitute other general-purpose reagents or supplies. Do not mix reagents between lots. Check the expiration date on each reagent tube before use. The centrifuge speed settings listed in these instructions are for the **Drucker 755VES** and the **Thermo CL2** centrifuges only.*

If, for any reason, an alternate centrifuge must be used to process a specimen, refer to section V. EQUIPMENT REQUIRED – CENTRIFUGE for centrifuge specifications and call CareDx Customer Care for assistance to ensure that centrifuge specifications and RCF settings are met. Specimens that are processed outside of CareDx specifications cannot be tested.

1. Following specimen collection, make sure the CPT tube is mixed well by inverting it at least 10 times prior to centrifugation. Process specimens at room temperature (18 to 25° C). Centrifuge the CPT tube at 3400 rpm for 15 minutes in the Drucker or CL2 centrifuge. Be sure the centrifuge is balanced by centrifuging an even number of specimens or by using a balance tube.
2. From the AlloMap Sample Processing Reagents Box, obtain the centrifuge tube containing phosphate buffered saline. (PBS)
3. Label the centrifuge tube with a barcode label from the patient's AlloMap TRF. Place the barcode label in a vertical orientation in the designated space on the PBS reagent label, indicated by: →|#

   Note: *For best results, perform the next two steps immediately following centrifugation. Delay may result in the time from specimen collection to freezing to exceed three hours.*

4. After centrifugation, invert the CPT tube at least 10 times to resuspend the separated mononuclear cells into the plasma.
5. Pour the plasma/mononuclear cell mixture (the layer above the gel) from the CPT tube into the labeled centrifuge tube that contains PBS. Cap and invert at least 10 times to mix.
6. Centrifuge for 5 minutes at 3400 rpm in the Drucker or CL2 centrifuge. Be sure the centrifuge is balanced by centrifuging an even number of specimens or by using a balance tube.

7. The mononuclear cells will form a pellet in the bottom of the tube. Open the tube and place the cap **open end up** on a clean surface. Pour off and discard the supernatant. Remove as much supernatant as possible by touching the rim of the tube to a clean paper towel or absorbent pad to get the last drop. The cell pellet is sticky and will not easily fall out of the tube.
8. Pipette all of the contents from the LyseDx lysing reagent tube (LDX) onto the cell pellet, using the transfer pipette supplied. (PIP) Vigorously pipette the cell pellet and the LyseDx lysing reagent up and down until the cells have completely disappeared and the lysate is clear. If you are doing this step properly, you will have a foamy mix of cells and reagent, which becomes clear when all cells are lysed. If the pellet is drawn into the bulb of the pipette, draw the LyseDx into the bulb to rinse the pellet down into the tube. Continue mixing until the pellet has been completely lysed and no visible cellular material sticks to the pipette.

   Note: *Do not vortex.*

9. When lysis is complete, replace and tighten the cap on the centrifuge tube, freeze upright at -15° C or colder until shipped to CareDx for AlloMap testing.
10. On the TRF, fill in the time the processed specimen was put in the freezer, or into the dry ice if specimen is to be packaged immediately for shipping.

XI. SPECIMEN ACCEPTANCE CRITERIA

Specimens

To be acceptable for AlloMap molecular expression testing, a lysate specimen must meet the following acceptance criteria:

1. Minimum labeling requirements: The centrifuge tube containing the lysate must be labeled with:
   - The CareDx accession number that corresponds to the accompanying TRF, or
   - Patient name, or a unique patient identifier, such as the medical record number, and the date and time the specimen was drawn. Inadequately labeled specimens will not be tested and will be discarded.
2. Specimen age and condition: The specimen must be:
   - Prepared from at least 6 mL blood drawn in BD Vacutainer® CPT™ Cell Preparation Tubes with Sodium Citrate. Refer to section titled “Specimen Collection Procedure”.
   - Collected from a patient who is 2 months (at least 55 days) post-transplant.
   - Processed with in-date AlloMap Sample Processing Reagents as indicated by the expiration date printed on the individual reagent tubes.
   - Processed according to Section X. Specimen Processing Procedure and frozen at -15° C or colder within three hours of collection.
   - Shipped to and received at CareDx frozen on dry ice.

   Note: *Specimens that do not meet the conditions above will not be tested and will be discarded.*

Test Order / Test Requisition Form (TRF)

1. No testing will be performed without an authorized test order.
2. All test orders must include:
   - Patient first and last name, or other patient identifier
   - A second identifier such as medical record number, or other unique identifying number
   - Name and address of the authorized person (physician or transplant coordinator) requesting the test or his/her authorized designee
   - Name, address and phone number of the laboratory submitting the specimen
   - Date and time of specimen collection
   - Date and time lysate was frozen at -15° C or colder
   - Diagnosis information (ICD-9 code)
   - Any additional information relevant and necessary for accurate reporting of the AlloMap test result
3. The following information must be either on the test order or previously provided to CareDx:
   - Date of birth
   - Gender
   - Transplant date
4. Customer Care will contact the laboratory or transplant center to obtain any required information that is missing from the TRF. All communication(s) required to obtain the missing information will be documented by the CareDx representative.

Unacceptable Specimen

1. CareDx Customer Care or designee will contact the requesting facility and referring laboratory before discarding a specimen that is unacceptable for testing.
2. If the specimen is to be discarded and not used for testing, a new specimen will be requested.
3. A written report stating the reason for rejection and discard will be sent to the transplant center.

XII. SPECIMEN SHIPPING PROCEDURE

IMPORTANT SAFETY NOTES:

The frozen lysate is derived from blood. Follow your laboratory's universal precautions for bloodborne pathogens.

When handling dry ice, follow your laboratory's safety procedures.

- Never handle dry ice with your bare hands. Dry ice has a temperature of -109° F (-78° C), cold enough to freeze skin cells and cause an injury similar to a burn. When handling dry ice, always use insulated gloves.
- Use dry ice in a ventilated area.
- Store dry ice in an appropriate dry ice storage chest. Do not use airtight containers as these may provide an explosive hazard.
- Dry ice is a skin and eye irritant. Avoid contact with skin, mouth, eyes, and clothing.
- Use personal protective equipment – gloves, eye protection, and lab coat or apron.

Equipment, Materials, and Supplies

CareDx supplies the Frozen Shipper Pack which includes the following:

- Aqui-Pak 6-Bay absorbent pouch
- 95 kPa Specimen Transport Bag
- Dry ice label filled out with 2.3 kg in all sections and affixed to outer shipping box
- List of Contents card
- Cooler
- Outer shipping box
- Monday through Thursday FedEx Shipping Packet containing:
  - Priority Alert Airbill, pre-printed for Priority or First Overnight service, depending upon service level required
- Friday FedEx Shipping Packet containing:
  - Priority Alert Airbill, pre-printed for Saturday HOLD service
  - Saturday Delivery stickers
  - HOLD Sticker addressed to 900 Gateway Blvd., South San Francisco, CA 94080
- 9 x 12 plastic zip bag
- Instructions for Shipping Card

Your laboratory will provide the following:

1. Dry ice – pellet form is recommended, approximately 1.5 cm x 4 cm
2. Packing tape for outer box

Packaging and Shipping

IMPORTANT:

Once you begin this process, work quickly. Do NOT let the specimens thaw. Do not expect dry ice to last more than 24 hours. While frozen specimens will keep for a short time, ship specimens daily to ensure the quickest turnaround time for results.

1. Prepare the cooler by removing the contents (absorbent pouch, bags, labels, etc.).
2. Have the dry ice ready; keep the specimen tube(s) in the freezer as long as possible.
3. Insert the frozen specimen tube(s) into the Aqui-Pak 6-Bay absorbent pouch. The pouch can hold a maximum of 6 specimen tubes.
4. Roll the pouch with the tube(s) and put it into the 95 kPa Specimen Transport Bag. Seal this bag closed. Each bag holds one absorbent pouch with up to 6 tubes.
5. The cooler can hold up to 6 specimens. If you have more than 6 specimens, use a second Frozen Shipper Pack.
6. Place the Transport Bag with the specimen(s) in the cooler and pack with pellets or small chunks of dry ice. Snow-like dry ice will not last 24 hours. Fill the cooler with as much dry ice as possible; generally, a cooler with up to 6 specimens holds approximately 5 pounds (2.3 kgs) of dry ice. If there are more than 6 tubes to ship, use a second Frozen Shipper Pack.
7. Place the lid on the cooler. DO NOT tape the lid shut.
8. Fold the test requisition forms in half, place them in the 9 x 12 plastic zip bag and place it on top of the lid.
9. Place the List of Contents card on top of the plastic zip bag.
10. Close the outer box and seal with packing tape.

11. The Dry Ice Shipping label has been filled in with your laboratory name and the maximum amount of dry ice in kilograms that will fit in the box. This label has already been affixed to the shipping box.
12. To expedite specimens, CareDx uses the FedEx Priority Alert service. With this service, FedEx tracks each shipment and ensures that it arrives at the destination on time. Be sure to use only the special, Priority Alert Airbills that come with the CareDx Frozen Shippers. Priority Alert cannot be used with FedEx Online, so please follow these instructions. Based on the day of the week that you are shipping, use the appropriate FedEx Shipping Packet:

For FedEx shipments sent Monday through Thursday:

Select the Packet marked "USE **OVERNIGHT DELIVERY** PACKETS ONLY ON **MONDAY—THURSDAY** SHIPMENTS TO CAREDx".

i. Ensure that the Priority Alert FedEx airbill, a multi-part paper form, is marked in Section 4a for **FedEx Priority or First Overnight service**. Follow the instructions below for proper completion of the airbill.

For FedEx shipments sent Friday:

Select the Packet marked "USE **SATURDAY DELIVERY** PACKETS ONLY ON **FRIDAY** SHIPMENTS TO CAREDx**".**

i. Place the **yellow and white Saturday Delivery** stickers on the outside of the box. Do not cover any of the markings on the box.
ii. Place the **white and orange HOLD sticker** on top of the shipper box, next to the airbill. Ensure that the address on the sticker is 900 Gateway Blvd., South San Francisco, CA 94080.
iii. Ensure that the Priority Alert FedEx airbill, a multi-part paper form, is marked in Section 3 for **HOLD Saturday at FedEx Location**. Follow the instructions below for proper completion of the airbill.

13. **Completion of the Airbill for all shipments using the multi-part paper Priority Alert FedEx airbill:**

a. Enter the shipment date and enter or check that the correct sender information is entered in Section 1.
b. Enter or check that the correct recipient information is entered in Section 3.
c. Enter or check that the Dry Ice Section is marked and it includes the weight of the dry ice in kilograms (2.3 kg for 5 lbs of dry ice) in Section 6. The weight entered must match the weight on the Dry Ice Shipping Label.
d. Enter or check that the number of packages and total weight (3 kgs) is entered in Section 7 (2.3 kgs of dry ice + 0.7 kg for the shipper, tubes and paperwork).
e. When finished, remove and keep the top copy of the airbill for your records.
f. Affix the airbill to the top of the shipper box.
g. Arrange for FedEx Express shipping pickup.

References

DOT Hazardous Materials Regulations for Diagnostic Specimens, sec. 173.199: diagnostic specimens and used health care products.

IATA Package Instruction 650

IATA Packing Instruction 904 – Dry Ice

XIII. INTERPRETATION OF RESULTS

An AlloMap Score between 0 and 40 is calculated based on the gene expression levels of the test and control genes. The score and additional data are provided to the clinician on a Test Report. The range of AlloMap scores observed in 1002 CARGO samples tested was 2 to 39 (see Performance Characteristics section below).

The performance characteristics of the AlloMap Test were established with samples from heart transplant recipients at least 15 years of age and at least 2 months (≥ 55 days) post-transplant at the time samples were collected.

The use of AlloMap scores in the management of heart transplant patients is defined for each patient by the physician based on their clinical experience and knowledge of their patient populations. Expected Negative Predictive Values (NPV) will be dependent upon the threshold selected by the physician.

XIV. PERFORMANCE CHARACTERISTICS

The CARGO Study[2]

Polymerase Chain Reaction (PCR) technology provides a sensitive, specific, and reproducible measurement of gene expression. The emergence of this technology along with the development of statistical and database tools capable of storing and analyzing gene expression data made it possible for CareDx to develop the AlloMap test for use in monitoring cardiac allograft rejection. This technology was used in the CARGO (Cardiac Allograft Rejection Gene Observational) study, a multi-center collaboration that provided the basis for the development of the AlloMap test. CARGO was a prospective, observational, multi-center protocol active from 2001 to 2005 whose prime objectives were to develop:

- A sample archive of RNA samples prepared from peripheral blood obtained concurrently during endomyocardial biopsy (EMB) procedures
- A database of clinical information collected at the time of EMB. The study enrolled 737 subjects at 9 heart transplant centers in the United States and yielded an archive of data and RNA samples for 5834 clinical visits. Clinical data was entered into the study database.

CareDx sequenced 25000 clones from leukocyte cDNA libraries and incorporated more than 8000 probe sequences representing these clones into custom microarrays and used them to examine gene expression. Patient blood samples were obtained at the time of biopsy and the expression levels of these 8000 genes were determined and compared to the biopsy result. A subset of more than 250 candidate genes showed promise as markers to discriminate rejection from no rejection.

The next phase of the CARGO study used RT-PCR to measure gene expression levels of the candidate genes. These studies provided highly quantitative and reproducible measures of expression levels for each gene and were correlated with the biopsy readings and overall clinical results.

CLINICAL VALIDATION

Clinical validation was performed to evaluate the performance characteristics of the AlloMap test and statistical informativeness of the AlloMap test in cardiac transplant patients. Samples used for the study were derived from the CARGO study. The objective of the clinical validation was to estimate predictive values for the AlloMap test for ACR in the intended clinical population.

DIAGNOSTIC CLASSIFICATIONS

This study used the following definition for rejection: a local biopsy grade ≥3A that was also assigned grade ≥3A by at least one of the three panel pathologists ("confirmed rejection"). All local ≥3A biopsies were graded by the central pathologists. The "no rejection" class included all samples that did not qualify as rejection.

SAMPLES AND PATIENTS

A total of 300 samples, from 154 patients enrolled in CARGO were assayed with the AlloMap test but were not used to develop the AlloMap test algorithm. These samples conformed to the following inclusion criteria:

- At least 55 days since transplantation, and
- More than 30 days after administration of immunosuppressive therapy for treating rejection.

STATISTICAL ANALYSIS

The specific objectives of the clinical validation were:

- To test the informativeness of the test as measured by Receiver Operator Characteristic (ROC) curve analysis, specifically Area Under the Curve (AUC)
- To estimate the clinical performance of the test for all integer AlloMap test scores (0-40) and indicated patients in two time periods post-transplant: 55-182 days ("2-6 months") and ≥183 days (">6 months").

CLINICAL PERFORMANCE METRICS ESTIMATED:

- Percentage of AlloMap test scores below a specific AlloMap test score
- Negative Predictive Value (NPV) for ACR for scores below a specific AlloMap test score
- Positive Predictive Value (PPV) for ACR for scores at or above a specific AlloMap test score

RESULTS AND CONCLUSIONS

The AUC calculated from the 300 samples from 154 patients was 0.67 with 95% confidence interval from 0.56 to 0.78 calculated by bootstrap. The AUC for the 55-182 days post-transplant period was 0.71 with 95% confidence interval from 0.56 to 0.84. The AUC for the ≥183 days post-transplant period was 0.67 with 95% confidence interval from 0.50 to 0.88.

Clinical Performance Characteristics

The following table provides the clinical performance characteristics for two to six months post transplant and greater than 6 months post-transplant.

Table 1: Clinical Performance Characteristics of the AlloMap Test

AlloMap Score	>2 – 6 months (n=166 samples)					>6 months (n=134 samples)					AlloMap Score
	% Pts Below	PPV ≥3A(2R)	PPV Std. Err.	NPV <3A(2R)	NPV Std. Err.	% Pts Below	PPV ≥3A(2R)	PPV Std. Err.	NPV <3A(2R)	NPV Std. Err.	
≤19	<22.4	≤2.7%	≤0.1%	**100.0%**	0.0%	≤5.4	≤1.8%	0.0%	**100.0%**	0.0%	**≤19**
20	24.3%	2.8%	0.2%	**100.0%**	0.0%	8.1%	1.8%	0.1%	**100.0%**	0.0%	**20**
21	33.6%	2.5%	0.4%	**98.8%**	0.8%	9.8%	1.9%	0.1%	**100.0%**	0.0%	**21**
22	38.8%	2.7%	0.5%	**98.9%**	0.7%	11.0%	1.9%	0.1%	**100.0%**	0.0%	**22**
23	41.8%	2.9%	0.5%	**99.0%**	0.6%	14.1%	2.0%	0.1%	**100.0%**	0.0%	**23**
24	47.5%	3.2%	0.6%	**99.1%**	0.6%	18.4%	2.1%	0.1%	**100.0%**	0.0%	**24**
25	56.0%	3.8%	0.7%	**99.3%**	0.5%	22.1%	2.2%	0.1%	**100.0%**	0.0%	**25**
26	61.4%	3.8%	0.9%	**99.0%**	0.5%	26.8%	2.3%	0.1%	**100.0%**	0.0%	**26**
27	63.6%	3.4%	1.0%	**98.7%**	0.5%	31.6%	1.9%	0.4%	**98.7%**	0.9%	**27**
28	68.3%	3.3%	1.1%	**98.5%**	0.5%	39.1%	2.1%	0.5%	**98.9%**	0.7%	**28**
29	73.7%	4.0%	1.3%	**98.6%**	0.4%	40.8%	2.1%	0.5%	**99.0%**	0.7%	**29**
30	77.2%	4.6%	1.6%	**98.6%**	0.4%	50.6%	2.1%	0.6%	**98.7%**	0.6%	**30**
31	81.0%	3.3%	1.6%	**98.2%**	0.4%	54.1%	2.3%	0.7%	**98.8%**	0.6%	**31**
32	85.6%	2.9%	2.0%	**98.0%**	0.3%	63.1%	2.9%	0.9%	**99.0%**	0.5%	**32**
33	89.4%	4.0%	2.7%	**98.1%**	0.3%	72.4%	3.8%	1.3%	**99.1%**	0.4%	**33**
34	91.7%	5.0%	3.5%	**98.2%**	0.3%	79.1%	4.1%	1.7%	**98.9%**	0.4%	**34**
35	94.5%	5.7%	4.8%	**98.1%**	0.2%	84.1%	4.0%	2.2%	**98.7%**	0.4%	**35**
36	97.3%	7.6%	13.8%	**98.1%**	0.2%	90.2%	5.4%	3.2%	**98.7%**	0.3%	**36**
37	97.8%	9.5%	21.1%	**98.1%**	0.2%	91.7%	—	–	**98.4%**	0.2%	**37**
38	100.0%	—	–	**97.9%**	0.0%	96.5%	—	–	**98.2%**	0.0%	**38**
39	100.0%	—	–	**97.9%**	0.0%	97.7%	—	–	**98.3%**	0.0%	**39**

Reproducibility/Precision[3]

Reproducibility for the AlloMap test process was established for the following variables:

- Run to Run
- Operator to Operator
- Lot-to-lot, plate-to-plate and plate section-to-section

Reproducibility of AlloMap scores was analyzed in healthy donor and patient samples from the Specimen Handling and Testing Reproducibility study. Run-to-run and operator-to-operator variability was analyzed in Arm A data. Lot-to-lot, plate-to-plate and section-to-section variability was analyzed in Arm C data. The AlloMap test process demonstrated acceptable precision, summarized below in **Tables 2-16**, with respect to coefficients of variation (CV's). Summary of maximum CV's are:

- Overall CV's (**Tables 2, 3, 4, and 5**):
  - 4 Donor Samples (Arm A): ≤6.3%
  - 6 Patient Samples (Arm A): ≤10.4%
  - Donor Sample (Arm C): 3.8%
  - Pooled Patient Sample (Arm C): 4.4%

- Run-to-run CV's (**Tables 4, 5, 6**)
  - Donor Samples: ≤9.2%
  - Patient Samples: ≤11.9%
- Operator-to-operator CV's (**Tables 7, 8**)
  - Donor Samples: ≤8.3%
  - Patient Samples: ≤11.7%
- Lot-to-lot CV's (**Tables 11, 12**)
  - Donor Samples: ≤4.7%
  - Patient Samples: ≤4.5%
- Plate-to-plate CV's (**Tables 13, 14**)
  - Donor Samples: ≤5.1%
  - Patient Samples: ≤4.5%
- Section-to-section CV's (**Tables 15, 16**)
  - Donor Samples: ≤5.7%
  - Patient Samples: ≤5.8%

Coefficients of variation are acceptable and comparable between donors and patients.

Table 2: Overall statistics for 4 donor samples (D1-D4), 4 runs per sample, 4 operators (Op1-Op4) per run and 2 reagent plates per operator

Donor	n	Mean	SD	CV(%)
D1	28	30.047	1.654	5.5%
D2	28	30.678	1.105	3.6%
D3	30	28.365	1.771	6.2%
D4	30	30.139	1.897	6.3%

Table 3: Overall statistics for 6 patient samples (Stanford1-Stanford3 and UCLA1-UCLA3), 2 runs per sample, 2 operators (Op1 and Op2) per run and 2 reagent plates per operator. NOTE: no run 2/operator 2 data obtained for UCLA samples

Patient	n	Mean	SD	CV(%)
Stanford1	8	27.456	1.642	6.0%
Stanford2	8	34.481	0.598	1.7%
Stanford3	8	31.656	2.014	6.4%
UCLA1	6	33.720	0.295	0.9%
UCLA2	4	34.858	1.262	3.6%
UCLA3	6	24.263	2.534	10.4%

Table 4: Descriptive statistics for run to run assessment for donor samples

Run:	1				2			
Donor	n	Mean	SD	CV(%)	n	Mean	SD	CV(%)
D1	8	30.915	0.773	2.5%	8	29.959	1.406	4.7%
D2	8	30.763	0.996	3.2%	8	30.143	0.867	2.9%
D3	8	27.847	1.974	7.1%	8	28.695	1.259	4.4%
D4	8	30.016	1.332	4.4%	8	29.992	1.457	4.9%

Table 5: Descriptive statistics for run to run assessment for donor samples (cont.)

Run:	3				4			
Donor	n	Mean	SD	CV (%)	n	Mean	SD	CV (%)
D1	8	29.175	1.752	6.0%	8	29.702	2.284	7.7%
D2	8	32.306	0.648	2.2%	8	30.314	0.887	2.9%
D3	8	28.071	2.594	9.2%	8	28.740	1.418	4.0%
D4	8	29.105	2.607	9.0%	8	31.185	1.986	6.4%

Table 6: Descriptive statistics for run to run assessment for patient samples

Run:	1				2			
Patient	n	Mean	SD	CV(%)	n	Mean	SD	CV(%)
Stanford1	4	27.911	1.878	6.7%	4	27.001	1.487	5.5%
Stanford2	4	34.748	0.442	1.3%	4	34.214	0.670	2.0%
Stanford3	4	32.868	1.410	4.3%	4	30.444	1.887	6.2%
UCLA1	4	33.730	0.354	1.0%	2	33.700	0.241	0.7%
UCLA2	2	34.320	0.319	0.9%	2	35.396	1.875	5.3%
UCLA3	4	23.556	2.804	11.9%	2	25.679	1.585	6.2%

Table 7: Descriptive statistics for operator to operator assessment for donor samples

Operator:	Op1				Op2			
Donor	n	Mean	SD	CV(%)	n	Mean	SD	CV(%)
D1	6	30.631	1.896	6.2%	6	30.947	1.294	4.2%
D2	6	29.957	0.971	3.2%	6	31.198	0.437	1.4%
D3	6	28.649	1.241	4.3%	8	27.557	2.110	7.7%
D4	6	30.072	2.499	8.3%	8	29.671	2.399	8.1%

Operator:	Op3				Op4			
Donor	n	Mean	SD	CV(%)	n	Mean	SD	CV(%)
D1	8	30.258	0.829	2.7%	8	28.723	1.748	6.1%
D2	8	30.932	1.507	4.9%	8	30.574	0.931	3.0%
D3	8	28.759	1.437	5.0%	8	28.567	2.082	7.3%
D4	8	31.342	0.838	2.7%	8	29.453	1.240	4.2%

Table 8 : Descriptive statistics for operator to operator assessment for patient samples

Operator:	Op1				Op2			
Patient	n	Mean	SD	CV(%)	n	Mean	SD	CV(%)
Stanford1	4	26.843	1.552	5.8%	4	28.069	1.697	6.0%
Stanford2	4	34.459	0.851	2.5%	4	34.502	0.328	1.0%
Stanford3	4	31.722	1.396	4.4%	4	31.590	2.740	8.7%
UCLA1	4	33.663	0.162	0.5%	2	33.835	0.562	1.7%
UCLA2	4	34.858	1.262	3.6%	0	—	—	—
UCLA3	4	23.644	2.771	11.7%	2	25.503	2.114	8.3%

Table 9: Overall statistics for 1 donor sample using 3 reagent lots (made from 3 distinct lots of raw materials), 4 plates per lot and 4 sections per plate

N	Mean	SD	CV(%)
48	31.630	1.214	3.8%

Table 10: Overall statistics for 1 pooled patient sample using 3 reagent lots (made from 3 distinct lots of raw materials), 4 plates per lot and 4 sections per plate

N	Mean	SD	CV(%)
48	28.875	1.273	4.4%

Table 11: Descriptive statistics for reagent lot assessment for donor samples

Lot	n	Mean	SD	CV(%)
1	16	31.279	1.470	4.7%
2	16	31.258	0.920	2.9%
3	16	32.354	0.878	2.7%

Table 12: Descriptive statistics for reagent lot assessment for patient samples

Lot	n	Mean	SD	CV(%)
1	16	28.611	1.299	4.5%
2	16	28.582	1.284	4.5%
3	16	29.431	1.114	3.8%

Table 13: Descriptive statistics for plate to plate assessment for donor samples

Plate	n	Mean	SD	CV(%)
1	12	31.428	1.594	5.1%
2	12	31.864	0.960	3.0%
3	12	31.467	1.010	3.2%
4	12	31.762	1.289	4.1%

Table 14: Descriptive statistics for plate to plate assessment for patient samples

Plate	n	Mean	SD	CV(%)
1	12	28.834	1.116	3.9%
2	12	29.359	1.323	4.5%
3	12	28.958	1.304	4.5%
4	12	28.349	1.287	4.5%

Table 15: Descriptive statistics for plate section to section assessment for donor samples (Note: UL=upper left quadrant of plate; LL=lower left quadrant of plate; UR=upper right quadrant of plate; LR=lower right quadrant of plate)

Sections	n	Mean	SD	CV(%)
UL	12	31.771	1.035	3.3%
LL	12	31.072	1.764	5.7%
UR	12	31.735	1.026	3.2%
LR	12	31.942	0.759	2.4%

Table 16: Descriptive statistics for plate section to section assessment for patient samples (Note: UL=upper left quadrant of plate; LL=lower left quadrant of plate; UR=upper right quadrant of plate; LR=lower right quadrant of plate)

Sections	n	Mean	SD	CV(%)
UL	12	29.244	0.997	3.4%
LL	12	28.558	1.645	5.8%
UR	12	28.896	1.036	3.6%
LR	12	28.801	1.373	4.8%

XV. LIMITATIONS OF PROCEDURE

Patient Population

The AlloMap test has not been validated for use in patient populations other than heart transplant recipients who are at least 15 years old and at least 55 days post-transplant.

Corticosteroid dosage

Systemic corticosteroid dosage of >20 mg/day of prednisone or equivalent may artificially decrease the AlloMap score.[4]

Following rejection therapy

The CARGO Clinical Validation study excluded all samples from patients who had received rejection therapy within the past 21 days. The performance characteristics of AlloMap testing in such samples, therefore, have not been established.[2]

Following transfusion

The CARGO Clinical Validation study excluded all samples from patients who had received a transfusion within the past 30 days. The performance characteristics of AlloMap testing in such samples, therefore, have not been established.[2]

Dual Organ Transplant Populations

The performance characteristics of AlloMap testing in a recipient of a cardiac allograft and another non-cardiac organ transplant (e.g. kidney) have not been established.

XVI. INTERFERING SUBSTANCES

Assessment of Endogenous and Exogenous Interferents

Table 17 outlines endogenous and exogenous substances and concentrations tested. No interference was observed at the concentrations tested for any of the substances evaluated.

Table 17: Interference testing of endogenous and exogenous substances[3]

	Material	Concentrations Tested	Recommended CLSI Test Concentrations*
Endogenous	Hemoglobin (HgB)	2.0 mg/mL	2.0 mg/mL
	Bilirubin	20 mg/dL	342 µmol/L (20 mg/dL)
	Triglyceride	37 mmol/L	37 mmol/L
Exogenous	Heparin	13.3 U/mL	0.35 – 1.0 U/mL
	Acetylsalicylic Acid	3.62 mmol/L	3.62 mmol/L
	Acetaminophen	1324 µmol/L	1324 µmol/L

** Clinical and Laboratory Standards Institute, CLSI EP7-A2 (Vol.25, No 27, 2005) recommended test concentrations.*

Genomic DNA Contamination[3]

Genomic DNA (gDNA) was evaluated for potential interference on AlloMap test Scores. Results demonstrate that samples may contain up to 25% gDNA without interfering with the AlloMap test score and that CareDx sample processing and QC procedures provide adequate control over potential gDNA interference with the AlloMap test results.

Cytomegalovirus Infection[3]

To determine if immune system activation associated with cytomegalovirus (CMV) viremia affected the performance of the AlloMap test, AlloMap test scores were compared between CMV viremic patients and CMV non-viremic patients. Results indicated that the immune system activation associated with CMV infection present in peripheral blood samples at the time of sample collection did not interfere with the performance of the AlloMap test.

Assessment of Immunosuppression Therapy[3]

To determine if immunosuppression therapy affected the performance of the AlloMap test, serum drug levels for cyclosporine A, tacrolimus and sirolimus were evaluated from 700 clinical samples. The level of concordance between AlloMap test scores and biopsy grades were compared in samples with therapeutic drug levels versus those samples with drug levels greater than the therapeutic range. Results show there was no detectable affect of drug level on AlloMap test scores.

XVII. REFERENCES

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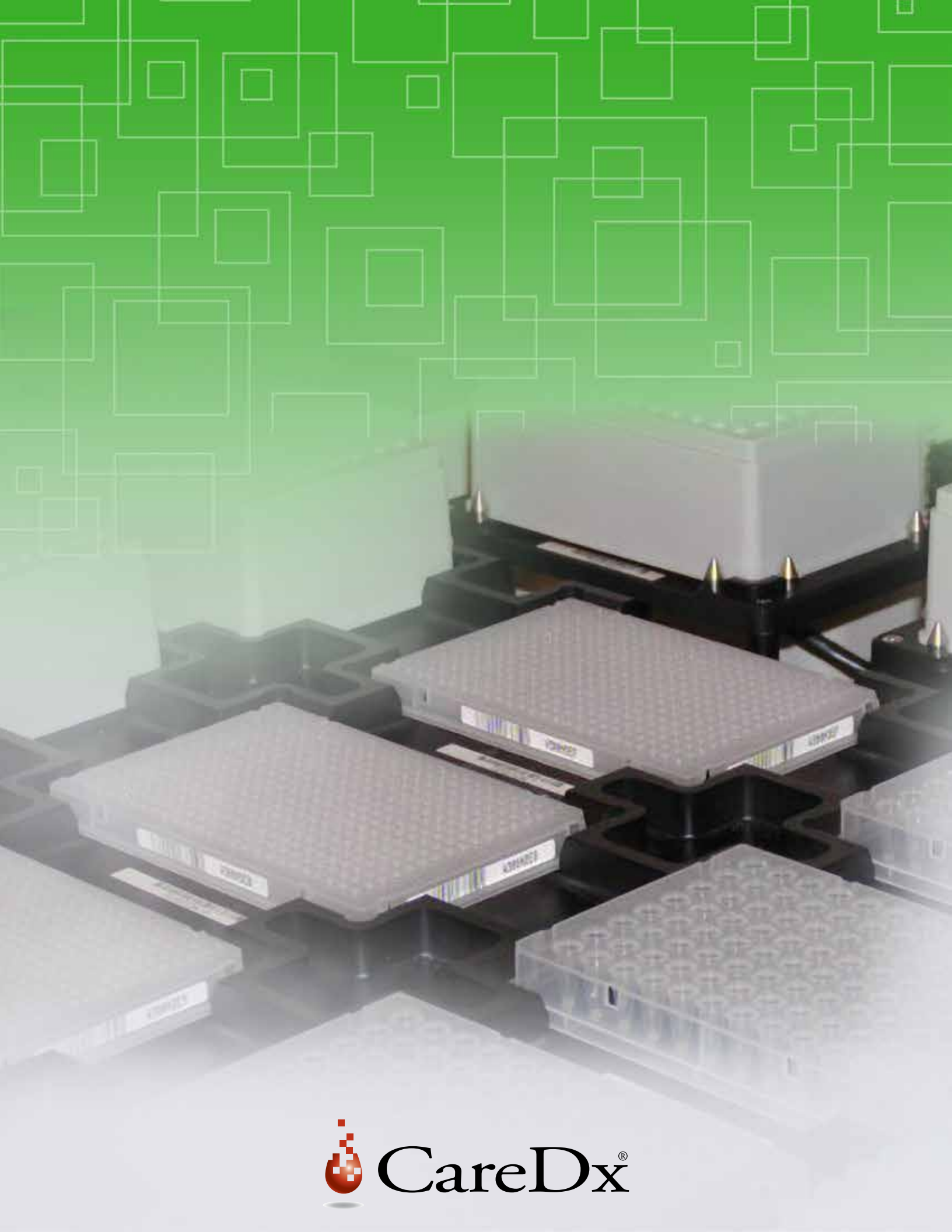

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