![CareDx®]

AlloMap®

The Standard of Care for the Management of Heart Transplant Recipients

Overview for Health Care Professionals

Mohan M., heart transplant recipient

Introduction

AlloMap Testing

AlloMap® is an innovative diagnostic test that helps physicians care for their heart transplant recipients.

When used in conjunction with standard clinical assessments, AlloMap helps identify patients with stable allograft function who have a low probability of moderate to severe acute cellular rejection (ACR) at the time of testing.

AlloMap is a panel of 20 gene assays, 11 informative and 9 used for normalization and/or quality control, which produces gene expression data used in the calculation of an AlloMap test score – an integer ranging from 0 to 40. Compared with patients in the same post-transplant period, the lower the score, the lower the probability of acute cellular rejection at the time of testing.

The clinician uses the AlloMap score, along with other standard clinical assessments, to evaluate the patient's probability of rejection and the need for additional diagnostic evaluations.

AlloMap is performed at the CLIA-certified and CAP-accredited clinical laboratory at CareDx® in Brisbane, California.

The Standard of Care for Managing Heart Transplant Recipients

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Intended Use

AlloMap 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 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.

Indicated for use in patients:
- 15 years of age or older
- At least 2 months (≥55 days) since transplantation

Special Considerations

AlloMap performance characteristics were established with samples from patients at least 15 years of age.

Effect of corticosteroid dosage
Systemic corticosteroid dosage of >20 mg/day of prednisone or equivalent may artificially decrease the AlloMap score [Starling et al., 2006].

Following rejection therapy
The CARGO 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.

Following transfusion
The CARGO 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.

AlloMap and the Management of Heart Transplant Recipients

Moderate to severe acute cellular rejection remains a prominent issue despite the success of current immunosuppression regimens in decreasing its overall prevalence among heart transplant patients. Current management protocols illustrate the priority placed on identifying rejection based on the multiplicity, diversity and frequency of using complementary modalities, such as endomyocardial biopsy, echocardiography, and AlloMap testing.

Non-invasive Procedure

AlloMap is performed using a blood sample obtained by routine phlebotomy, providing the physician with a non-invasive test as part of the overall management of cardiac transplant patients. Blood samples for AlloMap can be obtained at specific facilities trained in CareDx protocols for test sample collection and preparation. This optimizes patient convenience and enables physicians to order AlloMap as frequently as necessary based on clinical need.

High Negative Predictive Value

The utility of AlloMap in identifying a low probability of ACR is based on its negative predictive value. The negative predictive value (NPV) is the probability, expressed as a percent, that the patient is not experiencing rejection[1] at the time of testing. Clinicians select a desired NPV based on their clinical experience and specific patient populations.

Quantitative and Reproducible Technology

Quantitative real-time polymerase chain reaction (qRT-PCR) is the gene expression technology that provides the basis of AlloMap. Since qRT-PCR measures individual copies of RNA from cells, it provides sensitive, specific and reproducible measurements of the expression levels of the 20 genes in the test [Bustin, 2000].

As part of the development of the AlloMap test, precision studies were performed in the clinical laboratory at CareDx to verify that the same score was obtained, within a specified range, upon multiple repetitions of the analytical process.

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1 The term "rejection" will be used to mean "moderate to severe acute cellular rejection (ACR)" throughout this document.

Science and Technology of AlloMap

The application of gene expression technologies to measure and analyze differences in the expression levels of individual genes involved in cardiac allograft rejection is the scientific basis for both the development and implementation of the AlloMap.

Genomics Approach

AlloMap represents a breakthrough achievement in the field of multivariate molecular diagnostics, which relies upon information from the human genome and advances in molecular techniques to provide novel insights into human diseases. Development strategies for molecular diagnostics involve sensitive assays for DNA, RNA or proteins. These intracellular molecules govern cellular functions and responses, with RNA being the dynamic intermediate between the static DNA blueprint and the downstream production of diverse structural proteins that define a cell's unique functions.

The number of copies of an RNA molecule reflects the expression level of an individual gene, which may change in a given cell in different states of activity. A complement of genes may demonstrate coordinated changes in their RNA levels in association with a specific clinical condition or state, thus constituting a recognizable "gene expression profile" for a cell contributing to disease. AlloMap uses a 20-gene algorithm based on the gene expression profile of 11 genes associated with acute cellular rejection and 9 genes used for normalization and quality control.

AlloMap techniques involve the qRT-PCR use multiple specific primers and probes and distinct cycle amplification to enable specific and highly quantitative measurement of individual copies of RNA for a specific gene. AlloMap takes advantage of the sensitivity, specificity and reproducibility of qRT-PCR to yield expression levels that are converted to a test score ranging from 0 to 40 for use by clinicians.

Bioinformatics

The development of the AlloMap test algorithm required sophisticated bioinformatics analytical strategies which blend the principles of biology, computer science and statistics to yield novel relationships between molecular and clinical parameters. Bioinformatics played a critical role in all phases of the test's development, including the identification of candidate genes from the microarray data; the selection of genes correlating individually with the ACR endpoint based on qRT-PCR data; and the derivation of the test algorithm, including its final constituent genes and an assignment of a coefficient, or relative importance, for each gene.

Gene Identification Analysis
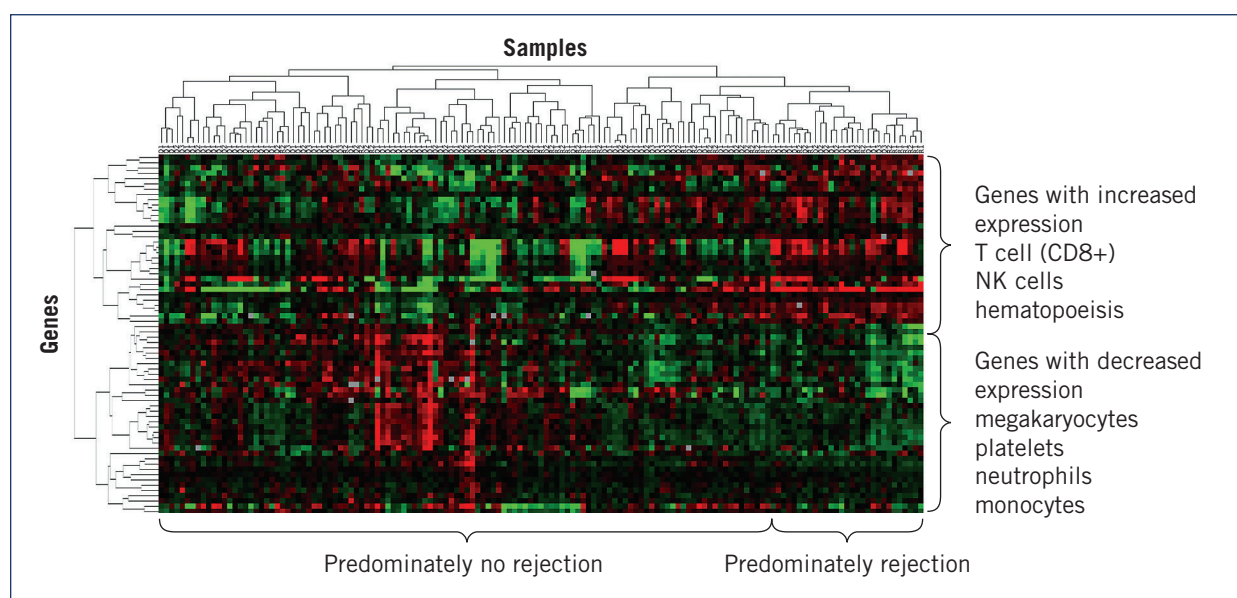
Heat Map of Rejection Genes: qRT-PCR measurements of 68 genes whose differential expression can distinguish between the presence and absence of acute cellular rejection. Red indicates increased expression of the gene. Green indicates decreased expression of the gene.

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Development of AlloMap

Scientists at CareDx and their clinical collaborators at leading cardiac transplant centers in the United States postulated that:

- Sensitivity and specificity of genomic technologies could identify differences in the expression levels of PBMC genes in association with the clinical endpoint of ACR;
- Bioinformatics techniques could be used to identify the optimal combination of genes in a unique mathematical algorithm to assess the clinical endpoint of ACR;
- Profile of identified genes would reflect multiple molecular pathways implicated in the temporal, biological, and population-dependent immune responses of heart transplant patients;
- Rigorous laboratory standards could enable the reliable and reproducible implementation of qRT-PCR and the test algorithm to generate test scores suitable for routine clinical use.

The CARGO Study

The Cardiac Allograft Rejection Gene expression Observational (CARGO) Study provided the collection of clinical information and the concurrent endomyocardial biopsy data and blood samples at various times post-transplant for gene expression profiling studies focused on the clinical endpoint of acute cellular rejection. Nine leading U.S. heart transplant centers representing more than 20% of the domestic cardiac transplant volume enrolled 737 heart transplant recipients. This group reflected the clinical spectrum of the national heart transplant population and participated in 5,834 clinical encounters during the study.

Gene Profiling Clinical Endpoint

The CARGO Study investigators selected moderate to severe (ISHLT grade $\geq$3A (2R)) ACR as the primary clinical endpoint based on its use as the clinical threshold for initiating rejection therapy. Since the published literature had previously recognized variations in the interpretation of endomyocardial biopsies and the assignment of the ISHLT grades, the CARGO Study implemented an enhanced biopsy interpretation procedure by utilizing a three-member study pathology panel that reviewed the original biopsy slides without any knowledge of associated clinical data. The CARGO Study pathology review process evaluated 1,356 endomyocardial biopsy samples and substantiated the published data by establishing a maximal interpretive concordance for grades $\geq$3A(2R) between the center and study pathologists of 40%.

The inclusion criteria for blood samples in gene profiling studies required their assignment to either the Rejection or No Rejection groups based on the following definitions [CareDx Laboratory Services Guide]:

- Rejection: A local biopsy grade $\geq$3A that was also assigned grade $\geq$3A by at least one of the three panel pathologists ("confirmed rejection")
- No rejection: All samples that did not qualify as rejection

Gene Identification

The development of a diagnostic algorithm starts with a large number of candidate genes and iteratively selects subsets of genes whose composite expression levels correlate best with the clinical endpoint.

The identification of candidate genes utilized microarrays first for an unbiased assessment of leukocyte genes. The subsequent phase used qRT-PCR quantification of the expression levels of genes in the microarray-identified candidate pool that had been supplemented by genes previously implicated in rejection biology based on a search of the published literature.

qRT-PCR assays of each of the 252 candidates guided the selection of 68 genes demonstrating a statistically significant correlation with the primary clinical endpoint of ISHLT grade ≥3A(2R) ACR. This gene cohort provided the candidates for the final bioinformatics steps in the algorithm development process.
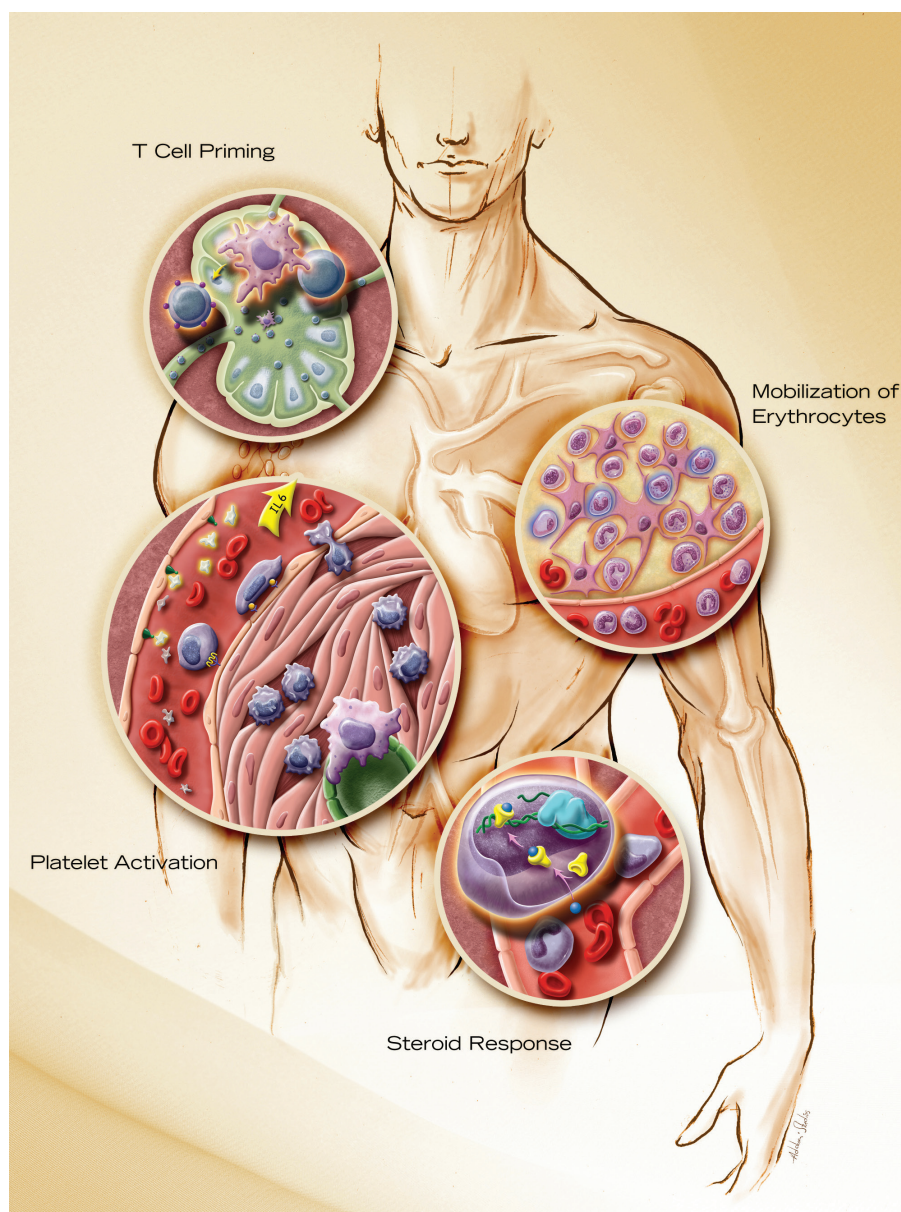
Algorithm Development

The AlloMap test is based on an algorithm that is a mathematical equation consisting of a series of terms for the expression level of 11 genes, including a coefficient, or relative weighting, for each term. Bioinformatics strategies determined the selection of these genes by using a blend of statistics and computer science known as "machine learning" to identify the gene combination that best correlated with the endpoint of grade ≥3A(2R) ACR. The application of linear discriminant analysis, a machine learning method, yielded an equation consisting of 11 of the 68 candidate genes that best distinguished between Rejection and No Rejection samples. Each term of the equation comprises the expression level of a single gene, and a multiplier coefficient that represents not only the relative importance of the gene, but also the directional change in expression of the specific gene, i.e. increased or decreased RNA levels.

In addition to the 11 genes identified by bioinformatics methodologies, an additional 9 genes were added to the test algorithm for quality control analyses, including a subset of 6 genes that function as normalization genes. To facilitate the clinical use of the algorithm, the final step in the test service involves a statistical transformation of the algorithm output to yield an integer value between 0 and 40 that constitutes the AlloMap test score range.

Targeting Specific Genes for the AlloMap Test
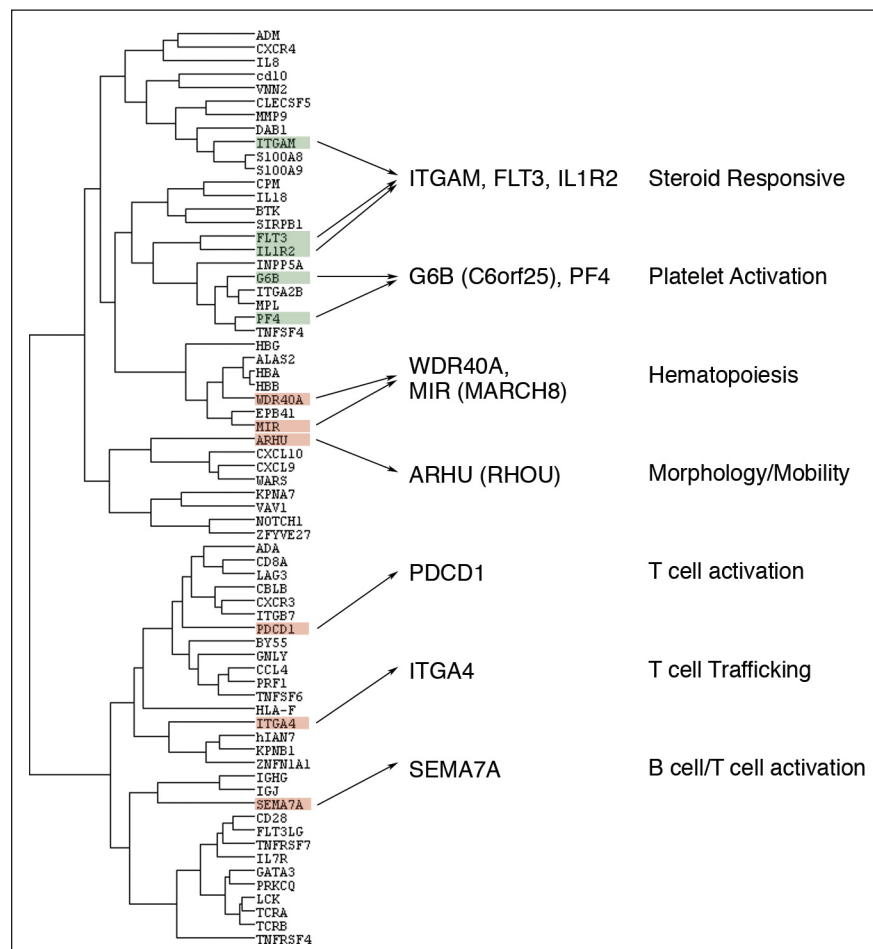
Using linear discriminant analysis, the expression levels of 11 genes were selected for the calculation of the AlloMap score (integer 0–40). This set of genes best distinguished between the presence and absence of rejection in the development of the AlloMap test.

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T Cell Priming

Mobilization of Erythrocytes

Platelet Activation

Steroid Response

Genes Represented in AlloMap Test Score: Multiple Rejection Pathways

Transplant rejection is initiated when the immune system of the recipient is stimulated by the genetically dissimilar allograft. Immune cells leave the circulation and infiltrate the graft setting up an inflammatory response that ultimately leads to rejection. Many of the AlloMap test genes are associated with various biological pathways involved in the rejection process.

T Cell Priming

Specialized dendritic cells carry antigens from the allograft to the lymph nodes. When recipient T cells in the lymph nodes recognize the allograft antigens, they begin to proliferate and to express different genes. These primed T cells re-enter the peripheral blood.

Two AlloMap test genes are associated with T cell priming; in validation studies their signals were increased in rejection samples:

PDCD1. Expression of this gene is induced during antigen presentation. It has recently been shown that this gene is expressed in circulating, antigen-specific T cells only during the course of an active immune response [Miller et al., 2008].

ITGA4. Priming increases the expression of this gene, which encodes a protein needed by T cells to infiltrate at sites of inflammation [Springer, 1994].

Proliferation and Mobilization of Erythrocytes

When the primed T cells arrive at the allograft, they are activated by allograft antigens. They leave the blood and infiltrate the heart tissue, stimulating localized inflammation. Inflammatory mediators from the rejecting heart, such as IL-6, can induce erythropoiesis and mobilization of immature erythrocytes [Ulich et al., 1989].

Two AlloMap test genes, *MARCH8* and *WDR40A*, are expressed in immature erythrocytes; their signals were increased in rejection samples [Goh et al., 2007].

Platelet Activation

ACR is associated with increased platelet activation, possibly another consequence of the inflammatory response [Segal et al., 2001].

Two AlloMap test genes, *PF4* and *C6orf25*, are expressed predominantly in platelets and their signals were reduced in rejection samples [McRedmond et al., 2003].

Steroid Response

Three AlloMap test genes, *IL1R2*, *ITGAM* and *FLT3*, were co-coordinately expressed and correlated with glucocorticosteroid dosage. Their signal was decreased in rejection samples, possibly reflecting an inadequate response to this immunosuppressive agent.

Unknown Role in ACR

The signals from both *SEMA7A* and *RHOU* genes were increased in rejection samples, but their role in ACR is unknown. The *SEMA7A* gene is expressed in T cells, B cells and immature granulocytes. The *RHOU* gene is a member of the Rho GTPase family, involved in the modulation of cytoskeletal organization.

Differential Expression of AlloMap Genes in Rejection Samples

Pathway and Gene	Gene Expression Level
T cell priming	
ITGA4 **Integrin alpha-4** α subunit of VLA-4; involved in T cell trafficking and adhesion	⬆
PDCD1 **Programmed cell death** T cell costimulatory molecular (inhibitory); CD28 family	⬆
Proliferation and mobilization of erythrocytes	
MARCH8 **Cellular mediator of immune response (MIR)** E3 ubiquitin ligase	⬆
WDR40A **WD repeat domain 40A** Uncharacterized protein of the WD-repeat protein family	⬆
Platelet activation	
PF4 **Platelet factor 4** Chemokine-like molecule expressed in platelets	⬇
C6orf25 **G6b inhibitory receptor** Putative inhibitory receptor of the Ig superfamily expressed in platelets	⬇
Steroid response	
IL1R2 **Interleukin-1 receptor type II** IL-1 decoy receptor inhibits cytokine signaling; steroid-dependent expression	⬇
ITGAM **Integrin alpha-M** α subunit of MAC-1; involved in cell trafficking	⬇
FLT3 **FMS-like tyrosine kinase** Signaling molecule expressed in monocytes	⬇
Unknown role	
SEMA7A **Semaphorin 7A** Expressed by T cells, B cells, and immature granulocytes	⬆
RHOU **Ras homolog gene family, member U** Member of the Rho GTPase family involved in the modulation of cytoskeleton organization	⬆

The Invasive Monitoring Attenuation through Gene Expression (IMAGE) Study

Study Design

The Invasive Monitoring Attenuation through Gene Expression (IMAGE) Study was a prospective, multi-center, randomized clinical trial comparing AlloMap and Endomycardial Biopsy for heart transplant rejection surveillance. Thirteen leading US heart transplant centers enrolled over 600 heart transplant recipients over 5 years.

This multi-center trial was a non-inferiority comparison of time to primary clinical events (outcomes). Heart transplant recipients underwent randomized allocation to two parallel rejection surveillance methods — Gene Expression Profiling (GEP) or Endomyocardial Biopsy (EMB).

The study inclusion criteria were heart transplant recipients >6 months to 5 years post-transplant, age ≥18 years, stable outpatient being seen for routine monitoring of rejection, and left ventricular ejection fraction >45%.

Primary and Secondary Outcome Measures:

Primary Outcome Measures (Time from Study Enrollment to):
- Rejection with hemodynamic compromise or
- Graft dysfunction due to other causes or
- Death or retransplantation

Secondary Outcome Measures:
- Number of deaths and cause of death
- Number of biopsies planned and performed
- Number of biopsy-related complications

The IMAGE study demonstrated non-inferiority of clinical outcomes for patients monitored with the AlloMap test compared to patients monitored with the traditional biopsy method.

AlloMap is the only non-invasive test to monitor for acute cellular rejection to have completed a large randomized, controlled clinical outcomes study, and is recommended in the first ISHLT guidelines for the care of heart transplant recipients [Costanzo et al., 2010].

The results from this landmark study, published in the New England Journal of Medicine in May 2010, are considered an important advance in the assessment of non-invasive methods for monitoring rejection after heart transplantation [Pham et al., 2010].

Understanding the Distribution of Scores Relative to a Reference Score*
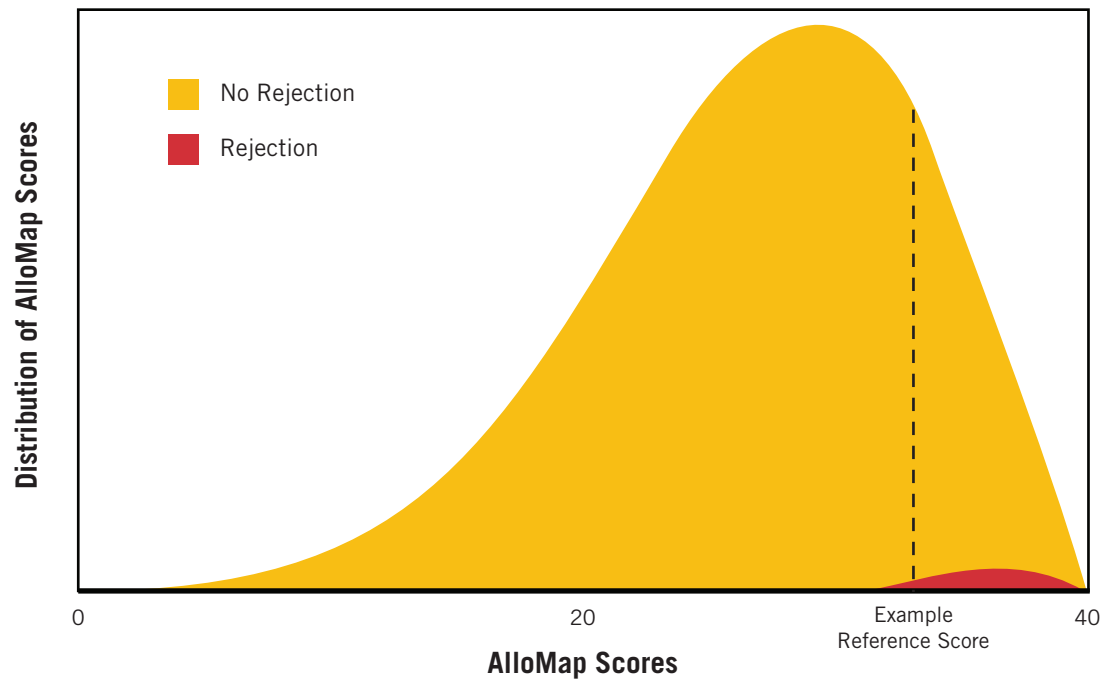
AlloMap scores can be evaluated against a selected reference score to help identify the probability of acute cellular rejection (ACR) at the time of testing for an individual patient. When used in conjunction with standard clinical assessments, scores below the reference point can help indicate a lower probability of ACR; scores above the reference score can help indicate an increased probability of ACR.

*The distribution of scores in this figure is intended to be for graphic illustration purposes based upon a typical distribution of AlloMap Test scores and Rejection from a general transplant population.

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Using AlloMap to Aid in Evaluating Probability of Rejection

To help assess the probability of rejection, the physician compares the AlloMap test score on the AlloMap Test Report with their pre-selected reference score. Results below the reference test score suggest a lower probability of rejection.

Selecting a Reference Score

Physicians use their clinical experience to select a "reference score" from the AlloMap test score scale of 0 to 40 for their patient population. This choice is based on the: 1) NPV or desired probability for the absence of rejection, and 2) the estimate of test scores expected to be lower than the reference test score, i.e. the "percent below." (Refer to Clinical Performance Characteristics of AlloMap Testing, page 13, for the performance characteristics calculated for each value of the AlloMap test score).

Clinical considerations for reference score selection may include the following: elapsed time post-transplant, treatment regimen, and an individual patient's history.
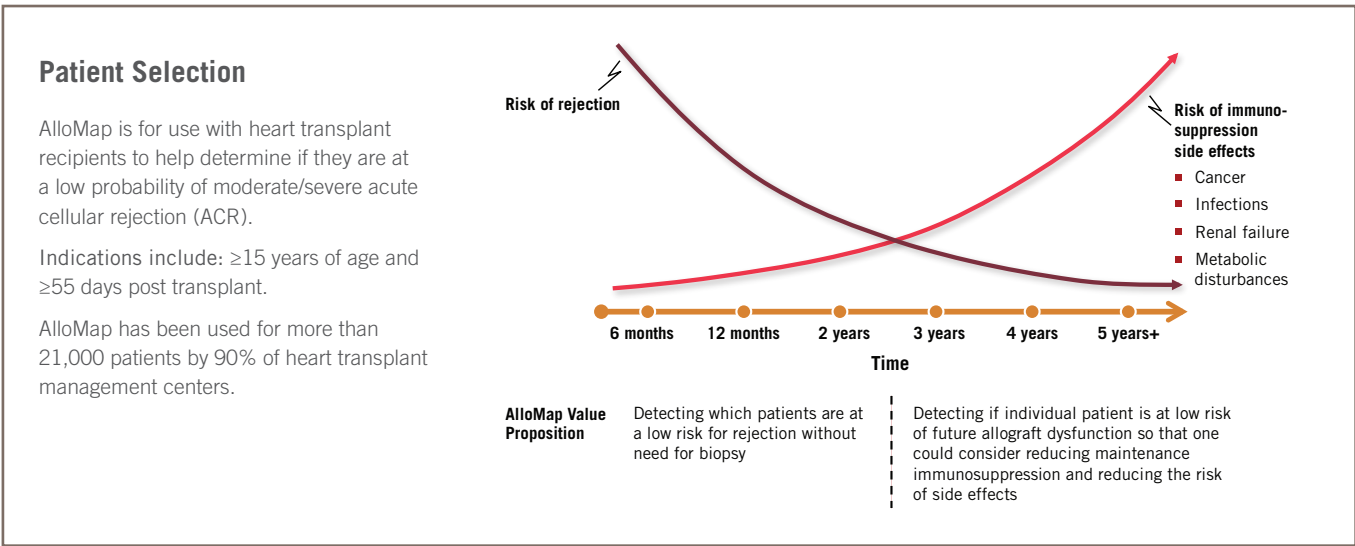
Comparing Results Against a Reference Test Score

The clinical value of the AlloMap test result is to aid in identifying patients at low probability of rejection at the time of testing. If the current result is below the reference test score, there is a lower probability of rejection.

For example, a clinician receives an AlloMap Test Report showing a result of 28. For her clinic patients in the 2 to 6 month period, she has chosen a reference score of 30. She desired an NPV of 98.6% with a frequency of results below this value of 77.2% (the "percent below" value). Since the result of 28 is less than 30, it suggests a lower probability of current rejection.

Evaluating the AlloMap Test Score in Conjunction with Other Clinical Assessments

Since the AlloMap test is one component available to assess rejection, clinicians interpret the AlloMap score in conjunction with other standard clinical assessments. These might include, among others, the history and physical examination, and the results of echocardiography, endomyocardial biopsy or other diagnostic tools used to evaluate the probability of rejection. This comprehensive assessment enables clinicians to choose an appropriate management plan.

Patient Selection

AlloMap is for use with heart transplant recipients to help determine if they are at a low probability of moderate/severe acute cellular rejection (ACR).

Indications include: ≥15 years of age and ≥55 days post transplant.

AlloMap has been used for more than 21,000 patients by 90% of heart transplant management centers.

Risk of rejection

Risk of immuno-suppression side effects
- Cancer
- Infections
- Renal failure
- Metabolic disturbances

6 months   12 months   2 years   3 years   4 years   5 years+

Time

AlloMap Value Proposition

Detecting which patients are at a low risk for rejection without need for biopsy

Detecting if individual patient is at low risk of future allograft dysfunction so that one could consider reducing maintenance immunosuppression and reducing the risk of side effects

The Standard of Care for Managing Heart Transplant Recipients

AlloMap Testing Clinical Performance Characteristics*+

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

Lower probability of ACR

+ (AlloMap Laboratory Services Guide - LQ-10004)

* The performance characteristics of the AlloMap test were established with samples from patients at least 15 years of age. The Clinical Performance Characteristics table for AlloMap testing has been updated to reflect the data utilized for FDA clinical validation studies in 2008. The original table contained the negative predictive values (NPV's) and positive predictive values (PPV's) for three different time periods and was based on the data utilized for the original 2005 launch. This dataset represents patient samples used to independently validate final performance of the AlloMap Test and support the Indications for Use statement. In addition, this table also includes standard errors, expressed as a percentage, and defines the NPV's and PPV's in two time periods, rather than the original three time periods, reflecting current clinical practice and experience gained since 2005. For additional information on AlloMap testing before and after FDA clearance, please go to www.allomap.com and download AlloMap Testing Bridging Letter.

** In general, the NPV values increase as the AlloMap scores decrease. This relationship, however, shows fluctuations due to the sampling of data set. The NPV is calculated as the (weighted) ratio of cardiac allograft recipients without ACR (numerator) to all recipients below a specified score (denominator). As one calculates this estimation for sequential scores, the estimate can move either up or down depending on the relative change in the numerator and denominator.

Interpreting the AlloMap Test Score as Part of Overall Clinical Assessments

The AlloMap Test Report shows the score result with its 95% confidence interval (95% C.I.). Physicians interpret this result by comparing it to an AlloMap test reference score they pre-selected based on a desired negative predictive value (NPV). In addition to the current result, the report also provides the patient's scores during the preceding 12 months. *NOTE: The performance characteristics of the AlloMap test were established with samples from patients at least 15 years of age.*

How to Read the AlloMap Test Report

A AlloMap Test Score

The AlloMap test algorithm transforms the measured expression levels of 20 genes into an integer value ranging from 0 to 40. In the example report, the current sample has an AlloMap test score of 28 for a patient in the 2 to 6 months period.

B 95% Confidence Interval

The 95% confidence interval (C.I.) provides a measure of score reproducibility. For example, the 95% C.I. of 24.7 to 31.3 for an AlloMap test score of 28 indicates that if the test were repeated 100 times, 95 of the test scores would be expected to fall between 24.7 and 31.3. The AlloMap Test Report provides the physician with the current AlloMap test score as well as the patient's historical scores to aid in the overall management of the patient.

C Post-transplant Periods

Since the prevalence of ISHLT grade 3A (2R) rejection is highest immediately post-transplantation and decreases through the first post-transplant year, the performance metrics of the AlloMap test score values are reported for two periods: 2 through 6 months and beyond 6 months post-transplant. This difference in rejection prevalence yields different negative predictive values (NPV) and positive predictive values (PPV) for the AlloMap test in the two time periods, with NPVs generally being higher for each AlloMap test score in the latter time period.

D Negative Predictive Value (NPV)

Each AlloMap test score is associated with an NPV that is the probability that the patient does not have current rejection. For example, an AlloMap test score of 28 is associated with an NPV of 98.5% that indicates a 98.5% estimated probability that the patient is not experiencing current rejection.

E Positive Predictive Value (PPV)

Similarly, each AlloMap test score is associated with a PPV that is the probability that the patient does have current rejection. The AlloMap test has a comparatively low PPV (relative to its NPV), and therefore, an AlloMap test score should not be used to "rule in" ACR.

AlloMap Test Score Frequencies Expressed as "Percent Below"

In addition to the NPV and PPV, the Clinical Performance Characteristics (see page 13) table provides additional information described as "percent below." For each AlloMap test score, the table provides the estimated percentage of test results that will be below this score. This enables the clinician to anticipate how often a score will be below (or above) a reference score selected for its associated NPV.

For example, a clinician desires an NPV of 98.6% in the 2 to 6 months period and chooses an AlloMap Reference Score of 30. This score of 30 has a "percent below" value of 77.2%, indicating that 77.2% of all results would be expected to be lower than 30. By contrast, 22.8% of score results would be expected to be higher than 30. Therefore, the "percent below" parameter enables the clinician to refine the reference score selection. While NPV provides the probability of the absence of rejection, the "percent below" enables estimation of the frequency of results expected to be below (or above) the chosen reference score.

The Meaning of Standard Error

To define the statistical confidence for the PPV and NPV values, the Clinical Performance Characteristics table also provides the standard error for each value.

The standard error of NPV and PPV values is shown in the Clinical Performance Characteristics (see table, page 13). The standard error of a calculation is the estimated standard deviation of the error in that calculation. Specifically, it estimates the standard deviation of the difference between the estimated value and the true value.

AlloMap Test Report

CareDx, Inc.
Lab Directors: Patrick Joseph, MD
Judith C. Wilber, PhD, D(ABMM)
CLIA No.:   05D1029609
CAP No.:   7193868

AlloMap®

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Brisbane, CA 94005
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Fax: (415) 287-2456
www.allomap.com

Patient Name:	Jane Doe
Medical Record No.:	7
Date of Birth:	13 Oct 1964
Transplant Date:	28 Aug 2007
Referring Facility Sample ID:	

Test Results

Sample Date:
15 Jan 2009
AlloMap Score:
(A) 37
(B) % CI: 36.5-38.3)
(range 0-40)

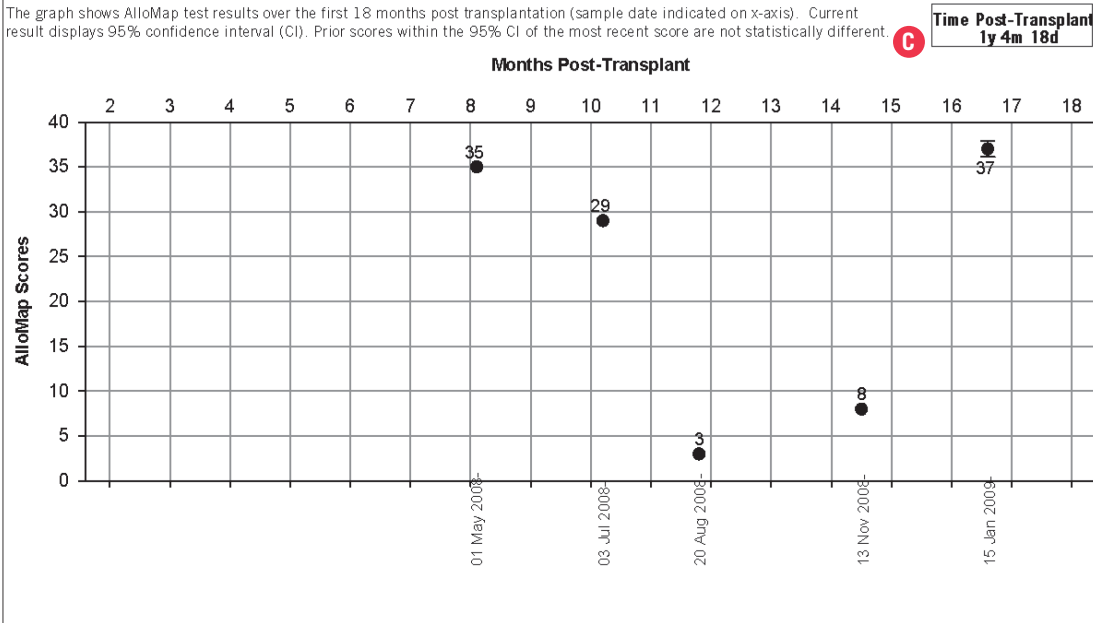Accession ID No.: **TEST01**   Report ID NO.: **TEST01**

Ordered By:	Jane Doctor
Client:	Example Clinic
Test Comments:	

Longitudinal Results - First 18 Months

The graph shows AlloMap test results over the first 18 months post transplantation (sample date indicated on x-axis). Current result displays 95% confidence interval (CI). Prior scores within the 95% CI of the most recent score are not statistically different.

(C) Time Post-Transplant
1y 4m 18d

Months Post-Transplant

(Graph: AlloMap Scores (y-axis 0–40) vs Months Post-Transplant (2–18))
- 35 — 01 May 2008
- 29 — 03 Jul 2008
- 3 — 20 Aug 2008
- 8 — 13 Nov 2008
- 37 — 15 Jan 2009

(D) Interpretation of AlloMap Score (E)

AlloMap Score	Negative Predictive Value (NPV)*	Positive Predictive Value (PPV)**
37	98.4%	Not Determined

The performance characteristics of the AlloMap test were established in patients who are 15 years of age or older, and at least 55 days post-transplant.

* The NPV is the probability of the absence of ISHLT grade >=3A (2R) acute cellular rejection for the AlloMap score below this score. The standard error for this NPV is 0.2%.
** The PPV is the probability of the presence of ISHLT grade >=3A (2R) acute cellular rejection for AlloMap scores at or above this score. The standard error for this PPV is Not Determined.

Special Considerations:
- Systemic corticosteroid dosage of >20 mg/day of prednisone or equivalent may artificially decrease the AlloMap score (Starling. et al. 2006).
- 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.
- 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.

INDICATIONS FOR USE

AlloMap Molecular Expression Testing 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.

Note: Additional information about the AlloMap test, including performance characterstics, can be found at www.allomap.com.

AlloMap is a registered trademark of CareDx, Inc. AlloMap molecular expression testing is a service provided by CareDx, Inc.
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CareDx   Final Report Date: 18 Jul 2018

Legend: (A) AlloMap Score (B) 95% Confidence Interval (C) Post-Transplant Periods (D) NPV (E) PPV

The Standard of Care for Managing Heart Transplant Recipients

The AlloMap Testing Service

Sample Collection and Preparation

The AlloMap test requires a blood sample obtained by routine phlebotomy and additional processing steps that enable the extraction and stabilization of RNA from peripheral blood mononuclear cells (PBMCs). As components of the immune system, PBMCs reflect the body's responses to the transplanted organ and have a distinct gene expression profile (i.e. individual RNA levels for each gene) associated with rejection that is assessed by the AlloMap test. After blood is collected, it is centrifuged to isolate the PBMCs. Further processing of the PBMCs releases the RNA from the cells and preserves it to ensure the recovery of high quality RNA for testing. The preserved sample is shipped together with the completed test requisition form to the clinical laboratory at CareDx.

Customer Care
1-888-ALLOMAP
1-888-255-6627
customercare@caredx.com

CareDx Customer Care is available to answer questions about AlloMap testing and to help resolve any problems regarding sample preparation, shipping, or test results.

AlloMap Testing Process at the Clinical Laboratory at CareDx

The testing procedure involves sequential steps beginning with purification of RNA from the sample received and finishing with the reporting of the AlloMap test score to the clinician. The intervening steps include analysis of the purified RNA by qRT-PCR, a proven methodology that yields sensitive, specific and reproducible gene expression measurements [Bustin, 2000]. The clinical laboratory at CareDx has optimized and standardized the performance of the AlloMap test processes. Comprehensive quality control ensures the reliability of the gene expression measurements used in the calculation of the AlloMap test score.

Testing Procedure

After purification, RNA is reverse transcribed into complementary DNA (cDNA), which is added to each of 60 wells containing gene-specific primers and probes. The expression of each gene is then measured by amplification and fluorescence detection using a qRT-PCR instrument.

This procedure is performed in triplicate and normalized to provide the integrity and accuracy of the sample.

Quality Control and Normalization

The relative expression of the quality control genes used in AlloMap testing provides the data to assess the quality of all of the testing process. These include:
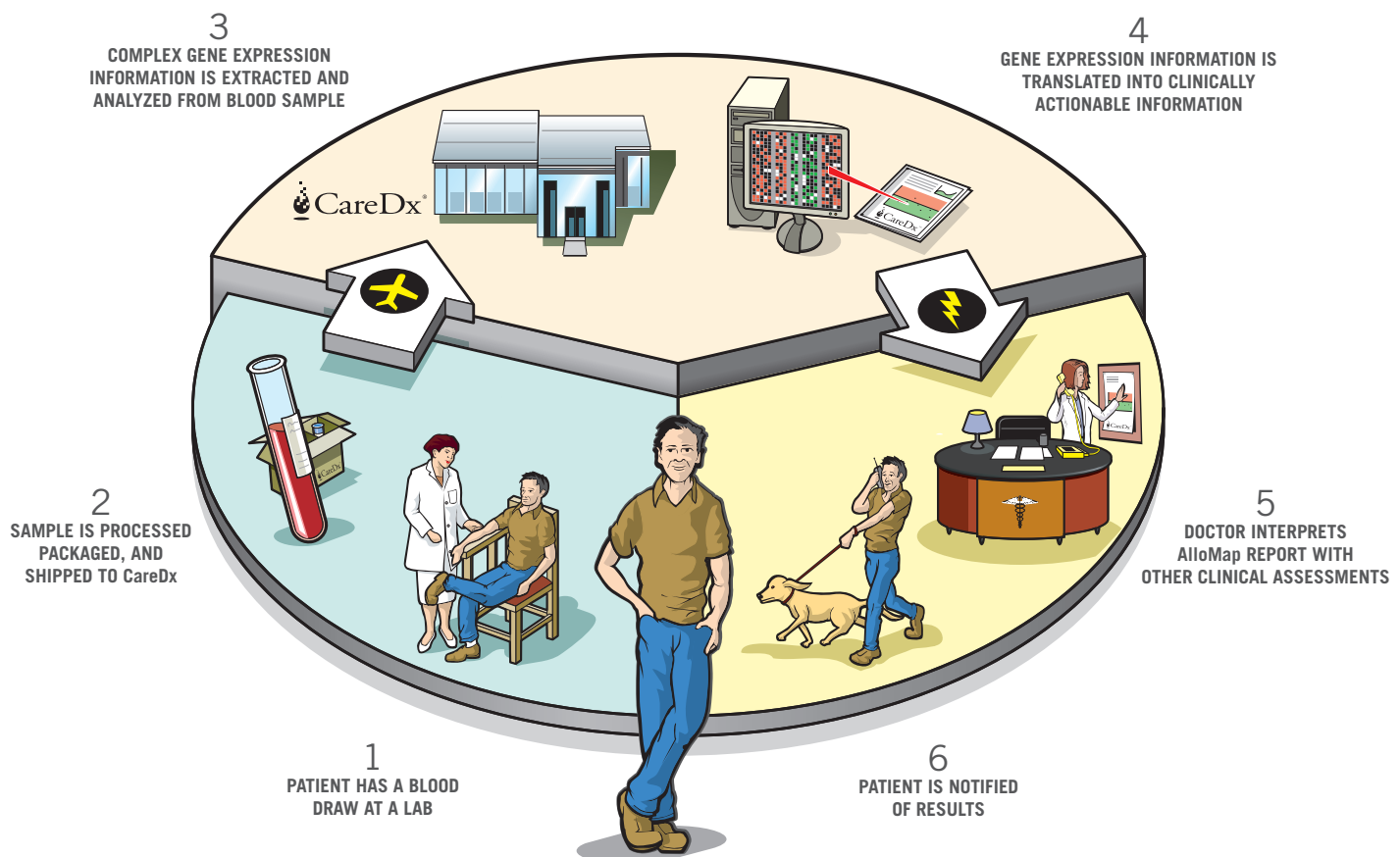
- Gene-specific measurement ranges
- Efficiency of the qRT-PCR
- Precision
- Accuracy and consistency

Generation of the AlloMap Test Score

A proprietary mathematical algorithm combines the measured expression values for each gene into a single integer value between 0 and 40 that is reported as the AlloMap test score. The clinician uses this score in the overall assessment of the probability of rejection at the time of testing.

Reporting Results

The clinical laboratory at CareDx reports the AlloMap test score to the ordering physician within 1 to 2 business days after receipt of the sample at its Brisbane, CA facility. Upon receipt of the test report from CareDx by fax, the physician interprets the report as part of the patient's overall clinical assessment. For additional details, please refer to the AlloMap Test Report on page 15.

3
COMPLEX GENE EXPRESSION INFORMATION IS EXTRACTED AND ANALYZED FROM BLOOD SAMPLE

4
GENE EXPRESSION INFORMATION IS TRANSLATED INTO CLINICALLY ACTIONABLE INFORMATION

2
SAMPLE IS PROCESSED PACKAGED, AND SHIPPED TO CareDx

5
DOCTOR INTERPRETS AlloMap REPORT WITH OTHER CLINICAL ASSESSMENTS

1
PATIENT HAS A BLOOD DRAW AT A LAB

6
PATIENT IS NOTIFIED OF RESULTS

AlloMap® in ISHLT Guidelines for Heart Transplant Care

Excerpt from ISHLT Guidelines

Topic 1: Rejection Surveillance. Recommendations for the Non-Invasive Monitoring of Acute Heart Transplant Rejection:

Class IIa:

1. In centers with proven expertise in ventricular evoked potentials (VER) monitoring, intramyocardial electrograms recorded non-invasively with telemetric pacemakers can be used for rejection surveillance in patients at lower risk for rejection.

   Level of Evidence: C.

2. Gene Expression Profiling (**AlloMap**) can be used to rule out the presence of ACR of grade 2R or greater in appropriate low-risk patients, between six months and five years after heart transplant.

   Level of Evidence: B.

Class IIb:

1. Use of echocardiography as primary monitoring modality for acute heart allograft rejection in infants can be considered as an alternative to surveillance EMB.

   Level of Evidence: C.

Classes of Recommendations and Levels of Evidence in ISHLT Guidelines

The classes of recommendations and the levels of evidence are graded as follows:

Class	Description
Class I	Evidence and/or general agreement that a given treatment or procedure is beneficial, useful and effective;
Class II	Conflicting evidence and/or divergence of opinion about the usefulness/efficacy of the treatment or procedure;
Class IIa	Weight of evidence/opinion is in favor of usefulness/efficacy;
Class IIb	Usefulness/efficacy is less well established by evidence/opinion;
Class III	Evidence or general agreement that the treatment or procedure is not useful or effective and in some cases may be harmful.
Evidence	**Description**
Level of Evidence A	Data derived from multiple randomized clinical trials or meta-analyses
Level of Evidence B	Data derived from a single randomized clinical trial or large non-randomized studies
Level of Evidence C	Consensus of opinion of the experts and/or small studies, retrospective studies, registries

Indicates recommendations for AlloMap

The use of the AlloMap test is described in the recommendations for the non-invasive monitoring of acute heart transplant rejection in the first evidence-based clinical practice guidelines for the care of heart transplant recipients issued by the International Society for Heart and Lung Transplantation (ISHLT).

Background

ISHLT convened experts in all areas of heart transplantation to develop practice guidelines for the care of heart transplant recipients. After involving 40 writers from nine countries worldwide, the ISHLT Guidelines for the Care of Heart Transplant Recipients were published in the Journal of Heart and Lung Transplantation on August 2010 [Constanzo et al., 2010].

Recommendation for AlloMap

AlloMap received the highest grade evidence-basis of invasive or non-invasive rejection monitoring technologies. The classification for the recommendation for AlloMap is:

"Gene Expression Profiling (AlloMap) can be used to rule out the presence of ACR of grade 2R or greater in appropriate low-risk patients, between six months and five years after heart transplant."

The level of recommendation for AlloMap is equal to or higher than the recommendation level for any methodology for monitoring for rejection, including endomyocardial biopsy, which has been the traditional standard for 40 years.

AlloMap Grading:

- **Recommendation: Class IIa** (IIa = weight of evidence/opinion is in favor of usefulness/efficacy)
- **Level of Evidence: B** (B = data derived from randomized controlled trial)

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CareDx Customer Care is available to answer
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help resolve any problems regarding sample
preparation, shipping, or test results.

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