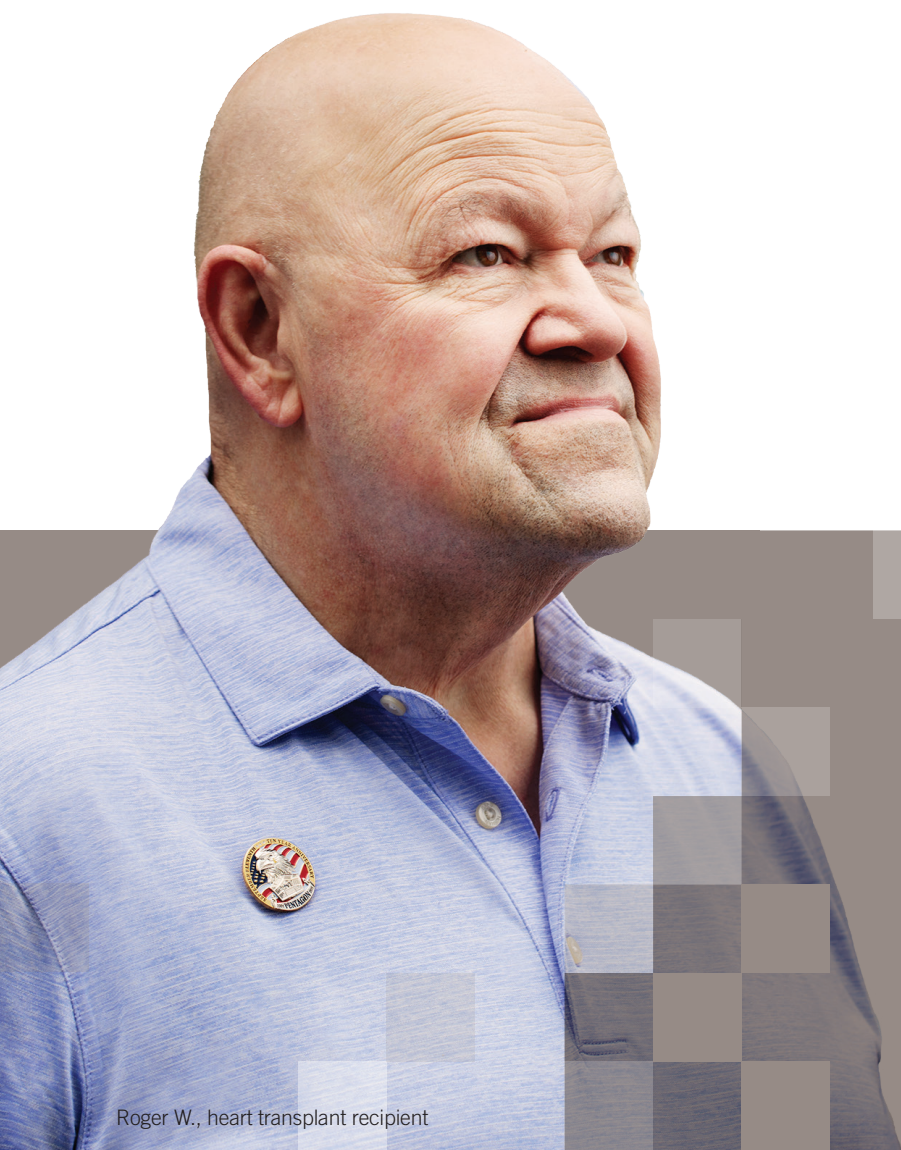




HeartCare Interpretive Guide

Broadly Available for Heart Transplant Patients



Roger W., heart transplant recipient

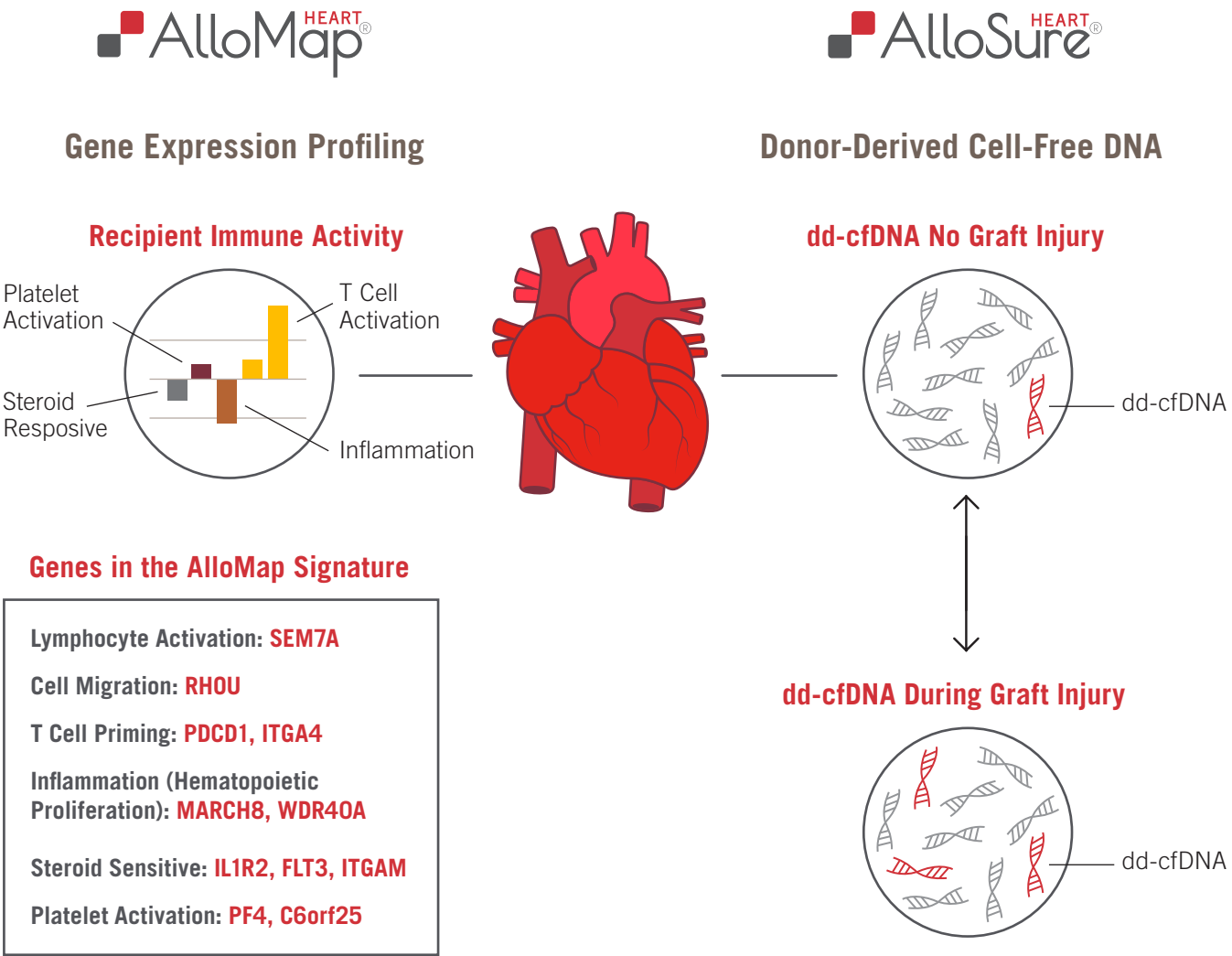


Test Description

HeartCare is a multi-modality surveillance solution comprised of two non-invasive blood tests: AlloMap and AlloSure Heart.

AlloSure Heart utilizes targeted, next-generation sequencing (NGS) to quantify donor-derived cell-free DNA (dd-cfDNA) in the plasma of recipients and is a biomarker of allograft injury and rejection.

AlloMap is a gene expression profiling (GEP) test of a recipient’s peripheral blood mononuclear cells utilizing real-time PCR (polymerase chain reaction). The AlloMap score is correlated with the probability that the allograft is quiescent (lack of clinically-significant acute cellular rejection (ACR)) and is associated with the level of a recipient’s immune system activity.



Indications For Use

AlloMap is indicated for use in patients:

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

AlloSure Heart is covered by Medicare for patients that are:

- At least 2 months (≥55 days) since transplantation
- Receiving a concomitant AlloMap test
- Meet all other criteria as outlined in the applicable Local Coverage Determination*

Contraindications

AlloMap should not be used:

- Less than 30 days after a blood transfusion that contains white blood cells (leukocyte-depleted red blood cell transfusion is acceptable).
- Corticosteroid dosage >20 mg/day: systemic corticosteroid dosage of >20 mg/day of prednisone or equivalent may result in a decreased AlloMap score.
- 21 days following rejection therapy with steroids: AlloMap performance characteristics have not been established for patients who have received rejection therapy in the 21 days prior to testing.

AlloSure Heart should not be used for:

- Recipients of multiple transplanted organs originated from the same donor
- Recipients of a bone marrow transplant
- Recipients who are pregnant
- Less than 24 hours following an endomyocardial biopsy

Limitations

HeartCare does not provide information on specific allograft histomorphology. All HeartCare results must be considered in the context of a patient’s overall clinical presentation, including other diagnostic findings, history, and examination of the patient. There may be within and between individuals, differences in biological variability of baseline values of dd-cfDNA and/or GEP scores. Damage to the graft caused by invasive procedures such as endomyocardial biopsy may cause a short-term elevation of dd-cfDNA. Until definitive studies are completed, HeartCare should not be performed on patients within 24h following an endomyocardial biopsy. HeartCare does not provide information on specific allograft histomorphology.

Clinical Validity

Clinical validity of HeartCare has been established in the multi-center observational CARGO II and D-OAR studies, which demonstrate that using AlloMap and AlloSure together can discriminate clinically significant ACR from no rejection. Test results from prospectively collected blood specimens were correlated with clinically indicated or surveillance endomyocardial biopsies performed according to center protocols. dd-cfDNA and GEP were measured using HeartCare and correlated with endomyocardial biopsy results to identify allograft rejection.

Reimbursement

AlloSure® Heart is covered by Medicare when used in conjunction with AlloMap® to assess the probability of allograft rejection in heart transplant recipients with clinical suspicion of rejection and to inform clinical decision-making about the necessity of a heart biopsy in such patients at least 55 days post-transplant in conjunction with standard clinical assessment.

Together, AlloSure Heart dd-cfDNA and AlloMap GEP **provide complementary information about the probability of acute cellular or antibody-mediated rejection and the status of the heart allograft.** When used in conjunction with standard clinical assessments, HeartCare may help to inform clinical management decisions and may be used along with clinical information when determining the necessity of performing endomyocardial biopsy in patients.

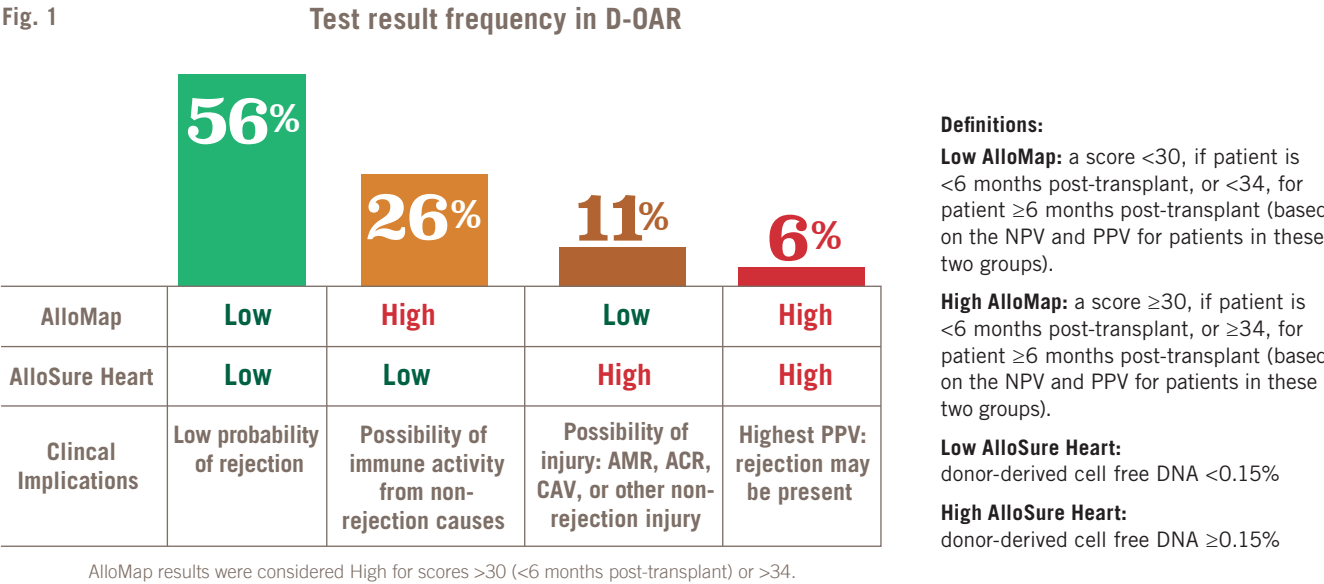


For patients who are uninsured, under-insured, or have insurance that does not cover the cost of HeartCare testing, and who meet financial eligibility criteria, CareDx offers a financial assistance program.

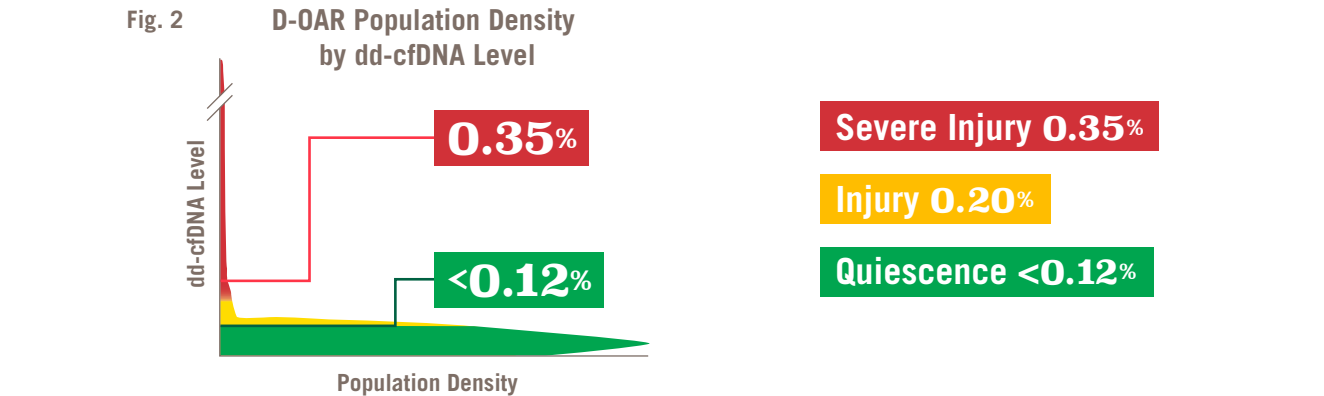
Questions, please email the CareDx Reimbursement Team at financialassistance@caredx.com.

Examples of Clinical Interpretation of Heartcare Test Results

Based on data from the D-OAR registry, the chart below (Fig. 1) provides examples of clinical interpretation of HeartCare results. In clinical practice, HeartCare results must be considered in the context of all other relevant clinical information for each individual patient.



Clinical Interpretation of AlloSure in Heart Transplantation**



Note: Graphs for illustrative purposes, please refer to publications for detailed clinical discussion
**Interpretation of AlloSure in Heart Transplantation graph is based on data from the D-OAR and SHORE studies

Considerations for HeartCare Results



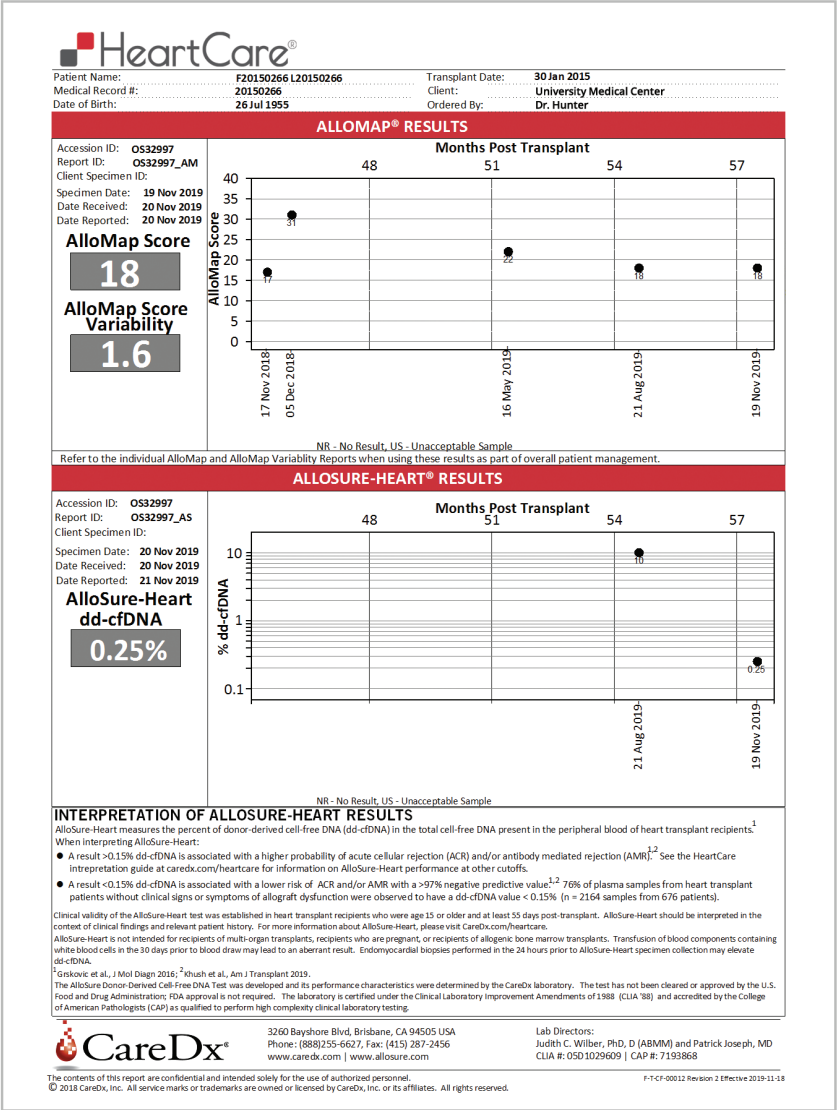
This panel (Fig. 3) is a consideration based on current data. This is not an enforced recommendation, CareDx takes no liability for the interpretation of HeartCare. All results should be interpreted by the ordering physician using their best clinical judgment.

*Kanwar, Manreet K., et al. The Journal of Heart and Lung Transplantation (2021)
ACR - acute cellular rejection AMR - antibody mediated rejection DSA - donor specific antibody CAV - cardiac allograft vasculopathy

The HeartCare Report

The HeartCare report provides both AlloMap and AlloSure Heart results. An example report is provided below:

Fig. 4



Issuing of the HeartCare Report

AlloMap results are mostly issued within 24 hours of receipt of the specimen, while AlloSure takes approximately 48 hours following receipt of the specimen (estimated times due to possible need for repeat testing).

If the patient had the two tests drawn at different times:

- AlloMap drawn following AlloSure Heart — the HeartCare report will contain the most recent AlloSure Heart result with all historic AlloMap and AlloSure Heart results on the longitudinal graphs. The new AlloMap result will be sent separately on the AlloMap report and will appear on subsequent HeartCare reports on the graph of longitudinal results. The comprehensive HeartCare Report will not be routinely re-issued.

- AlloMap drawn before AlloSure Heart — the HeartCare Report will show the AlloSure Heart result on the left side in the current results section and on the graph of longitudinal results. The AlloMap result will have been previously reported on an AlloMap report and will appear on the longitudinal graph of historic results.

Recipient Information

Recipient information, including date of birth, demographic data, date and time of blood draw, and type of organ transplant is included.



AlloMap Result

AlloMap gene expression profiling 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.

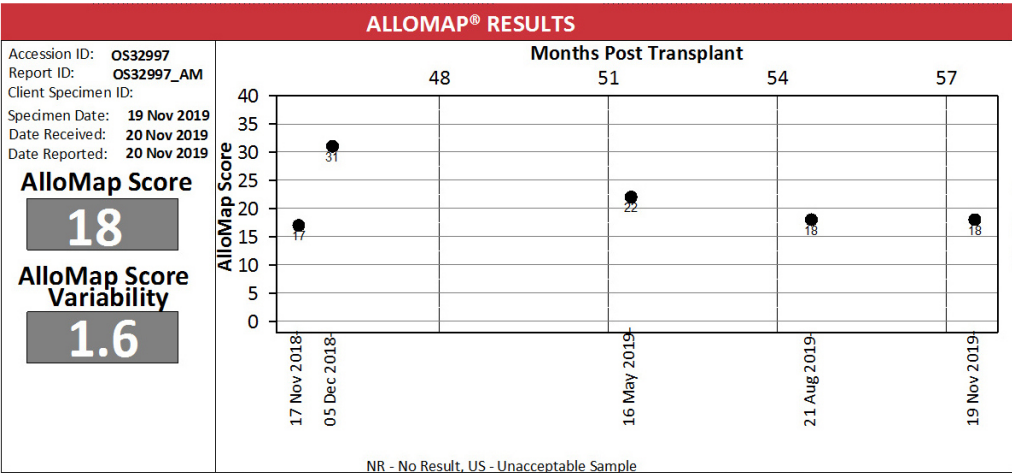
The AlloMap test is derived from a panel of 20 gene assays, 11 informative and 9 genes used for normalization and quality control, which produces gene expression data used in the calculation of an AlloMap score that ranges from 0-40. This score is associated with activity of the recipient's immune system, and a lower score is associated with a quiescent allograft. The four most recent AlloMap scores in a patient may be used to compute the AlloMap Score Variability, an opt-in consulting service by CareDx. The AlloMap score and the AlloMap Score Variability are shown on the left of the panel below, in bold with the most current specimen collection date. If prior results were obtained, these are provided on the graph, with corresponding prior test dates, to allow visualization of the longitudinal test result pattern in the patient.

AlloMap score variability is used to predict clinical events in heart transplant recipients. Increased variability of gene expression profiling scores from an individual may help predict the risk of clinically defined future allograft dysfunction or graft loss in the individual.

GEP score variability (AlloMap score variability, AMV) was defined as the standard deviation of the four consecutive scores collected ≥315 days post-transplantation. The time interval between the first and fourth score for the AMV should be ≥85 days and ≤780 days. For this calculation, the individual scores used were the direct output of the GEP linear discriminate algorithm (LDA) prior to the step that transforms the LDA score to the non-linear GEP score that fits within the 0–40 scale used for the AlloMap report.

A low variability of sequential GEP scores (≤0.6), rendered NPVs of ≥97.0 % indicating clinical utility of GEP score variability in the identification of patients at a low risk for future clinical events of greatest concern. This finding may have important clinical implications in the longer-term management of heart transplant recipients as the low risk patients may be good candidates for optimization (i.e. reduction) of their immunosuppressive drugs.

Fig. 5



AlloMap scores in acute rejection

It is recommended to consider the change in AlloMap score that is dependent on the time post-transplant in the general heart transplant population. The table below (Fig. 9) details AlloMap PPV and NPV generated from the CARGO study. The mean and median AlloMap scores in the general heart transplant recipient cohorts from the OAR study and from the database of all commercially ordered AlloMaps, rise most sharply from 2 to 6 months post-transplant, and more gradually between 7 to 12 months post-transplant. One cause of this upward score trend is that the AlloMap test includes three corticosteroid sensitive genes and the majority of patients undergo tapering or withdrawal of steroids during the first 6 months post-transplantation.

Consideration of cytomegalovirus (CMV) serologic status and/or CMV infection: CMV serologic status and or active infection has also been shown to be associated with higher AlloMap scores. Thus, interpreting an individual patient’s AlloMap score should include consideration of the patient’s prior scores, time post-transplantation, current corticosteroid use, and CMV infection or CMV serologic status.

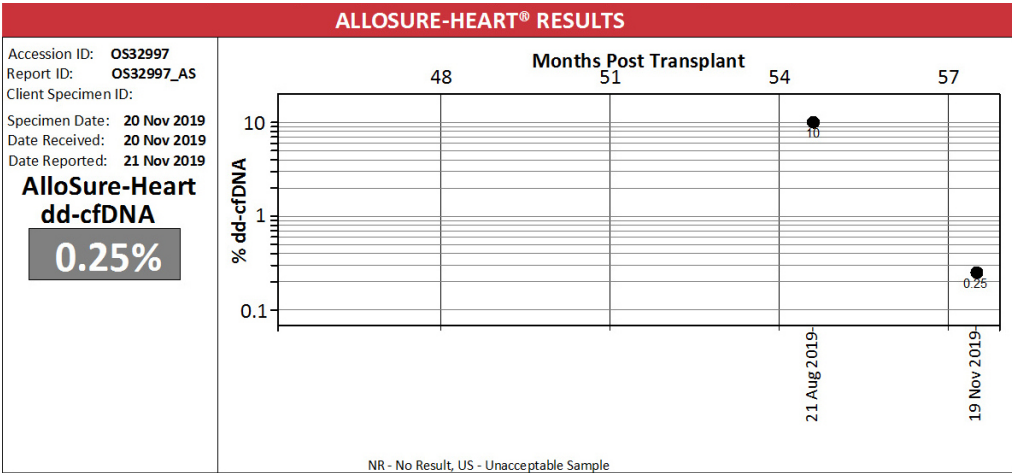
The mean and median score of AlloMap associated with biopsy proven ACR 2R is 28 compared to 25 for samples with no rejection found on biopsy (scale of 0-40 AlloMap score). Conversely, the high negative predictive value of test results below these thresholds are interpreted to indicate a relatively low likelihood of ACR.

AlloSure Heart result

The AlloSure Heart result is shown, in figure 6, as the percent of dd-cfDNA of the total cell-free DNA (cfDNA) in the results box and plotted on the graph along with the numerical value. The possible result range is from 0.12% to 16%, plotted against an ascending scale, where the probability of injury increases with higher dd-cfDNA levels. Based on current literature, elevated AlloSure is suspicious of active injury and possible rejection. The active test result is shown on the left of the panel, in bold with the specimen collection date. If serial specimens have been taken, results are displayed sequentially, with specimen date to allow correlation of trends, important in surveillance.

The minimum quantifiable dd-cfDNA level for unrelated donor is 0.12%, levels below this are reported as <0.12%. Although highly improbable for heart transplantation, if there is a donor-recipient genetic relationship (e.g. the recipient is a 1st degree relative of the donor), the AlloSure Heart score is adjusted and the minimum reportable level is 0.18%. In all recipients, the upper quantifiable limit is 16%, with levels above this recorded as >16%.

Fig. 6



AlloSure Heart test in acute rejection

Fig. 7

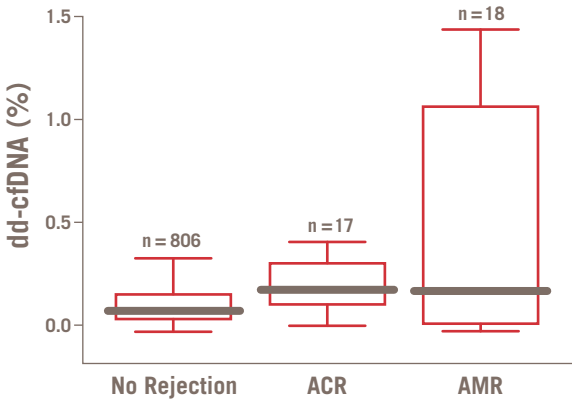
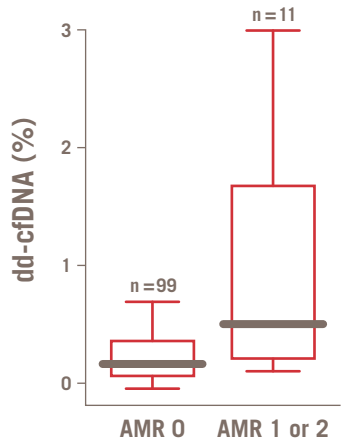


Fig. 8



Khush K, Patel J, Pinney S, Kao A et al. AJT Mar 5 2019

Additional Information to Assist with the Interpretation of the Results

Clinical Performance Characteristics of the AlloMap Test

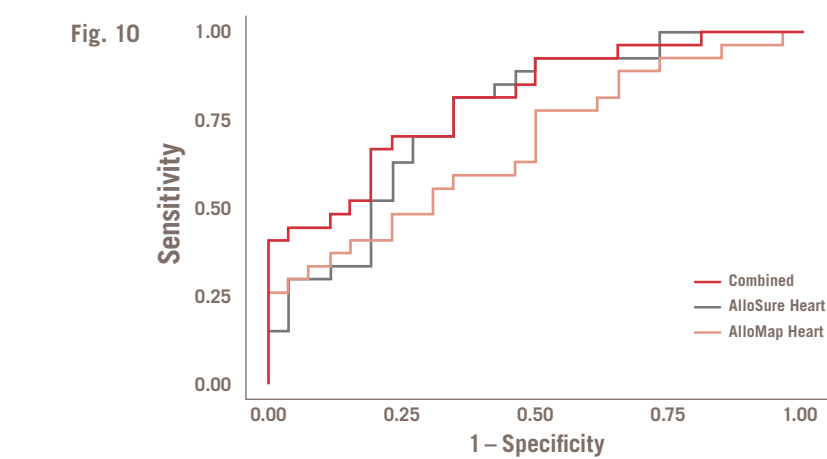
The following table (Fig. 9) provides the clinical performance characteristics for two to six months post-transplant and greater than 6 months post-transplant. The AUC calculated from the 300 samples of 154 patients was 0.67 with a 95% confidence interval from 0.56 to 0.78 calculated by bootstrap. The AUC for the 55-182 days post-transplant period was 0.71 with a 95% confidence interval from 0.56 to 0.84. The AUC for the ≥183 days post-transplant period was 0.67 with a 95% confidence interval from 0.50 to 0.88.

Fig. 9

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

The distribution of positive or negative GEP and dd-cfDNA test scores, and events of no biopsy-based rejection (NR), acute cellular rejection (ACR) and antibody mediated rejection (AMR) in a multicenter registry study of long term surveillance of heart transplant recipients (D-OAR).

Rationale for Combined AlloSure and AlloMap

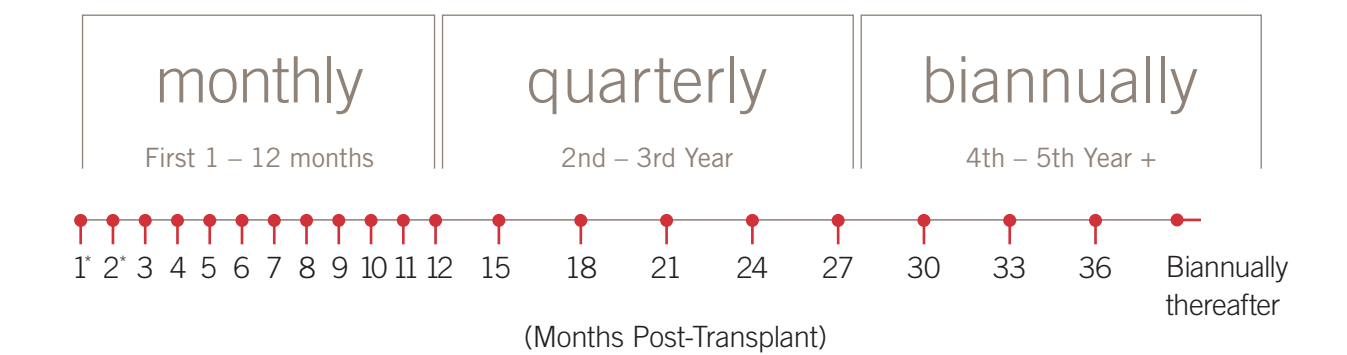


Interpretation of AlloSure in Heart Transplantation graph is based on data from the D-OAR and SHORE studies

Heart Allograft Routine Testing Schedule (HARTS)

The Heart Allograft Routine Schedule is based on a recommendation from the IMAGE trial. All testing should be performed when medically necessary, in accordance with a physician’s guidance. The SHORE registry study is assessing the clinical utility of this testing interval.

HARTS Timeline



*Under FDA-cleared labeling, AlloMap is indicated for use in heart transplant patients who are ≥15 years old and ≥55 days post transplant. AlloSure Heart is covered by Medicare after 55 days when ordered in conjunction with AlloMap and with clinical suspicion of rejection. All specimens submitted for testing and billed to third party payers must support the medical necessity of testing when a test is ordered, consistent with clinical judgment and applicable payer policies.

Key publications

Crespo-Leiro MG, Stypmann J, Schulz U et al. *Clinical usefulness of gene-expression profile to rule out acute rejection after heart transplantation: CARGO II*. Eur Heart J. 2016; 37(33):2591-601

Crespo-Leiro MG, Stypmann J, Schulz U et al. *Performance of gene expression profiling test score variability to predict future clinical events in heart transplant recipients*. BMC Cardiovasc Disord. 2015;15:120

Deng MC, Eisen HJ, Mehra MR et al. *Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling*. Am J Transplant. 2006; 6(1):150-60

Deng MC, Elashoff B, Pham BX, et al. *Utility of gene expression profiling score variability to predict clinical events in heart transplant recipients*. Transplantation 2014; 97(6):708-714

DePasquale et al – *Combination of cell-free DNA with gene-expression profiling in the diagnosis of acute rejection* – Submitted

De Vlamincq I, Valantine HA, Snyder TM et al. *Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection*. Sci Transl Med. 2014; 6: 241ra77

Pharm MX, Teuteberg JJ, Kfoury AG et al. *Gene expression profiling for rejection surveillance after cardiac transplantation*. N Engl J Med. 2010; 362(20):1890-900

Kobashigawa JA, Pinney S, Khush KK et al. *Donor derived cell free DNA in D-OAR, a real-world clinical use heart transplant population*. J Heart Lung Transplant. 2018; 37(4): S149-150

Khush K, Patel J, Pinney S, Kao A et al. *Non-invasive Detection of Graft Injury after Heart Transplantation Using Donor-Derived Cell-Free DNA: A Prospective Multi-Center Study* AJT Mar 5 2019

Kanwar, Manreet K., et al. *Impact of cytomegalovirus infection on gene expression profile in heart transplant recipients*. The Journal of Heart and Lung Transplantation 40.2 (2021): 101-107.



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