HeartCare®

Go Beyond Surveillance Biopsies with HeartCare

The first and only multi-modal test with studies demonstrating a significant reduction in surveillance biopsies and revolutionizing post heart transplant care

Sam D, heart transplant recipient, and his wife

Test Description

HeartCare is a Multi modality surveillance solution comprised of two noninvasive 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 polymerase chain reaction (PCR). 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.

HeartCare®

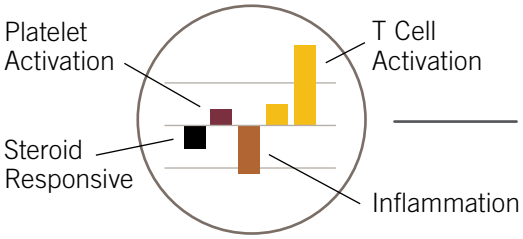
AlloMap® HEART

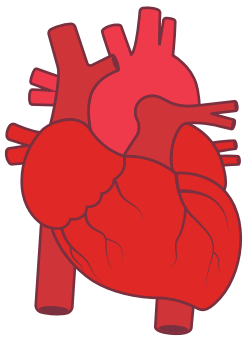
Gene Expression Profiling

AlloSure® HEART

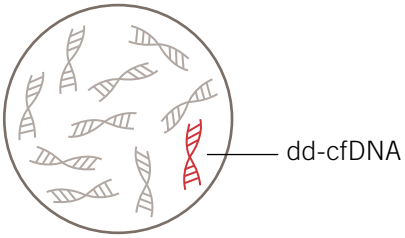
Donor-Derived Cell-Free DNA

Recipient Immune Activity





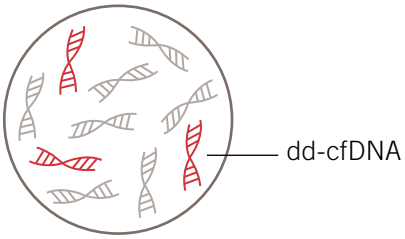
dd-cfDNA No Graft Injury



Genes in the AlloMap Signature

- **Lymphocyte Activation:** SEM7A
- **Cell Migration:** RHOU
- **T Cell Priming:** PDCD1, ITGA4
- **Inflammation (Hematopoietic Proliferation):** MARCH8, WDR40A
- **Steroid Sensitive:** IL1R2, FLT3, ITGAM
- **Platelet Activation:** PF4, C6orf25

dd-cfDNA During Graft Injury



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.

Indications For Use

AlloMap Is Indicated For Use In Patients:

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

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
- 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 histology. 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.

Clinical Validity

Clinical validity of AlloMap and AlloSure were established in CARGO II and D-OAR, respectively. The studies demonstrate that both AlloMap and AlloSure can discriminate 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 and correlated with endomyocardial biopsy results to identify allograft rejection.

Reimbursement

AlloSure® Heart, AlloMap® Heart, and HeartCare® are identified as covered services by Medicare when coverage criteria are met. Commercial and other coverage varies.

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 Patient Financial Assistance Program.

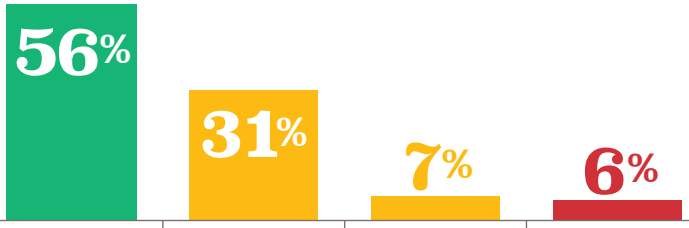
Questions, please email the CareDx Reimbursement Team at financialassistance@caredx.com or call at (866) 383-1924 (hours 8:00 AM–7:00 PM ET / 5:00 AM–4:00 PM PT).

Examples of Clinical Interpretation of HeartCare Test Results

Based on data from the SHORE 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.

Fig. 1

Test Result Frequency In SHORE



AlloMap	Low	High	Low	High
AlloSure Heart	Low	Low	High	High
Considerations	Low probability of rejection	A biopsy is unlikely to reveal ACR	Possibility of injury: AMR, ACR, cardiac allograft vasculopathy, or other non-rejection injury	Highest PPV: rejection may be present

Definitions:

Low AlloMap: a score <30, if patient is <6 months post-transplant, or <34, for patient ≥6 months post-transplant (based on the NPV and PPV for patients in these two groups).

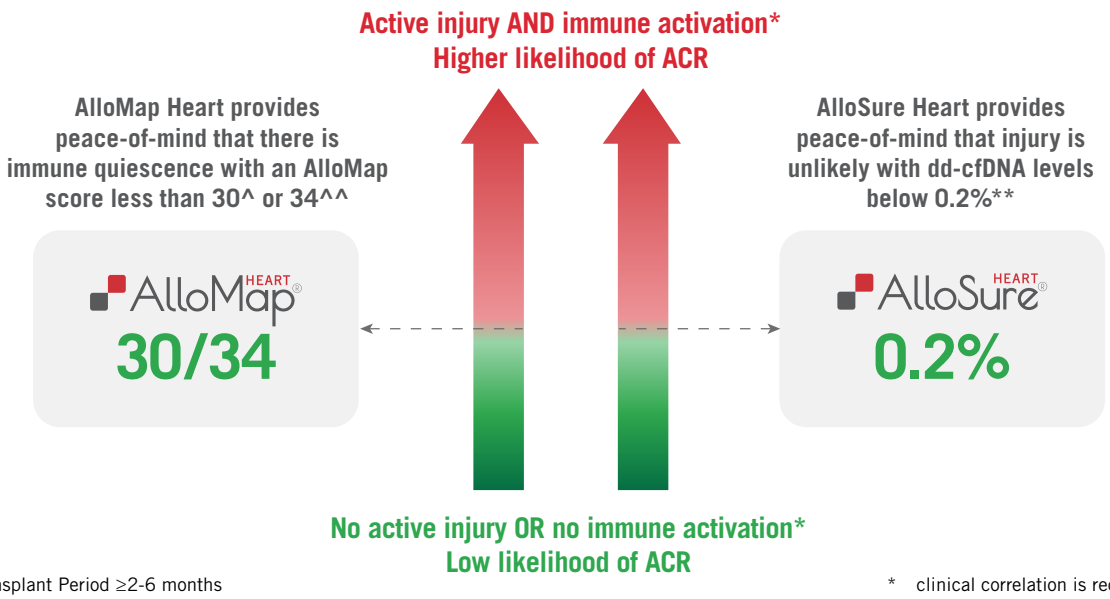
High AlloMap: a score ≥30, if the patient is ≤6 months post transplant, or ≥34 if the patient is >6 months post-transplant (based on the NPV and PPV for patients in these two groups).

Low AlloSure Heart: dd-cfDNA 0.2%

High AlloSure Heart: dd-cfDNA 0.2%

HeartCare Provides Peace-Of-Mind During Surveillance

Fig. 2



^ Post-transplant Period ≥2-6 months
^^ Post-transplant period >6 months

* clinical correlation is required
** as defined by their institutional protocol

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.

Fig. 3

The table is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, current guidelines, and assessment of the patient. Please refer to publications for detailed clinical discussion. Clinical interpretation of AlloSure in Heart Transplantation graph is based on data from all commercial samples.

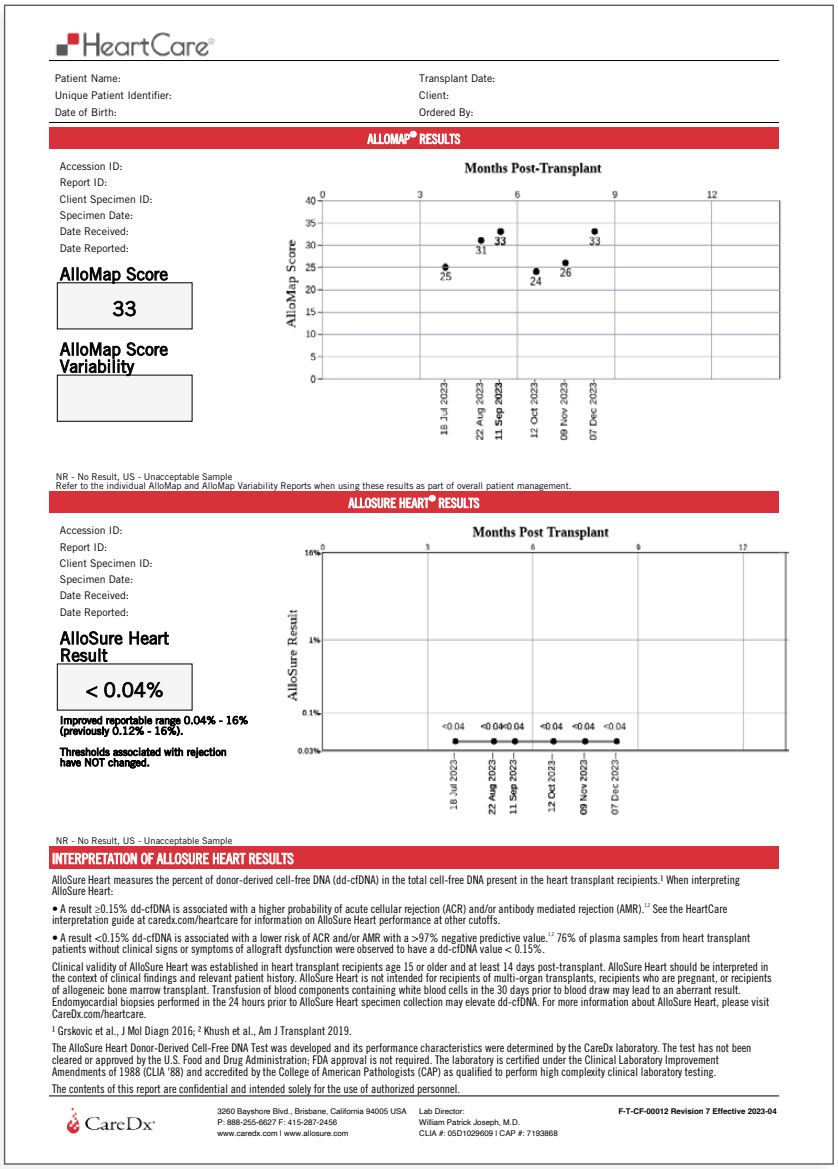
This table is designed for the context of surveillance testing for ACR; For patients that are at risk of AMR or being tested in other clinical context, different guidance may apply. 0.05 in High AlloMap is ≥30 for ≥2-6 months or ≥34 for >6 months | High AlloSure is ≥0.2%

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 in Fig. 4 below:

Fig. 4



HeartCare®

Patient Name:
Unique Patient Identifier:
Date of Birth:

Transplant Date:
Client:
Ordered By:

ALLOMAP® RESULTS

Accession ID:
Report ID:
Client Specimen ID:
Specimen Date:
Date Received:
Date Reported:

AlloMap Score

33

AlloMap Score Variability

Months Post-Transplant

AlloMap Score

25, 31, 33, 24, 26, 33

18 Jul 2023, 22 Aug 2023, 11 Sep 2023, 12 Oct 2023, 09 Nov 2023, 07 Dec 2023

NR - No Result, US - Unacceptable Sample
Refer to the individual AlloMap and AlloMap Variability Reports when using these results as part of overall patient management.

ALLOSURE HEART® RESULTS

Accession ID:
Report ID:
Client Specimen ID:
Specimen Date:
Date Received:
Date Reported:

AlloSure Heart Result

< 0.04%

Improved reportable range 0.04% - 16% (previously 0.12% - 16%).

Thresholds associated with rejection have NOT changed.

Months Post Transplant

AlloSure Result

<0.04, <0.04, <0.04, <0.04, <0.04, <0.04

18 Jul 2023, 22 Aug 2023, 11 Sep 2023, 12 Oct 2023, 09 Nov 2023, 07 Dec 2023

NR - No Result, US - Unacceptable Sample

INTERPRETATION OF ALLOSURE HEART RESULTS

AlloSure Heart measures the percent of donor-derived cell-free DNA (dd-cfDNA) in the total cell-free DNA present in the heart transplant recipients.[1] When interpreting AlloSure Heart:

- A result ≥0.15% dd-cfDNA is associated with a higher probability of acute cellular rejection (ACR) and/or antibody mediated rejection (AMR).[1,2] See the HeartCare interpretation guide at caredx.com/heartcare for information on AlloSure Heart performance at other cutoffs.
- A result <0.15% dd-cfDNA is associated with a lower risk of ACR and/or AMR with a >97% negative predictive value.[1,2] 76% of plasma samples from heart transplant patients without clinical signs or symptoms of allograft dysfunction were observed to have a dd-cfDNA value < 0.15%.

Clinical validity of AlloSure Heart was established in heart transplant recipients age 15 or older and at least 14 days post-transplant. AlloSure Heart should be interpreted in the context of clinical findings and relevant patient history. AlloSure Heart is not intended for recipients of multi-organ transplants, recipients who are pregnant, or recipients of allogeneic bone marrow transplant. Transfusion of blood components containing white blood cells in the 30 days prior to blood draw may lead to an aberrant result. Endomyocardial biopsies performed in the 24 hours prior to AlloSure Heart specimen collection may elevate dd-cfDNA. For more information about AlloSure Heart, please visit CareDx.com/heartcare.

[1] Grskovic et al., J Mol Diagn 2016; [2] Khush et al., Am J Transplant 2019.

The AlloSure Heart Donor-Derived Cell-Free DNA Test was developed and its performance characteristics were determined by the CareDx laboratory. The test has not been cleared or approved by the U.S. Food and Drug Administration; FDA approval is not required. The laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) and accredited by the College of American Pathologists (CAP) as qualified to perform high complexity clinical laboratory testing.

The contents of this report are confidential and intended solely for the use of authorized personnel.

CareDx®

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F-T-CF-00012 Revision 7 Effective 2023-04

Issuing of the HeartCare Report

AlloMap results (Fig. 5) are mostly issued within 24 hours of receipt of the specimen, while AlloSure results (Fig. 6) take approximately 48 hours following receipt of the specimen (estimated times due to possible need for repeat testing).

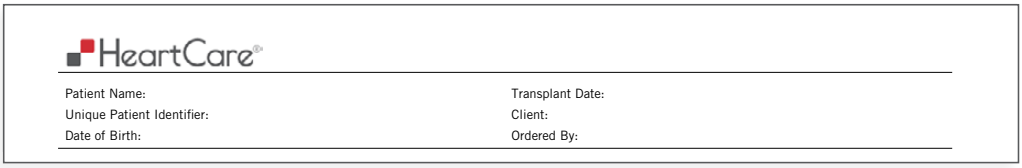
If the patient had the two tests drawn at different times:

- 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.


HeartCare®

Patient Name:	Transplant Date:
Unique Patient Identifier:	Client:
Date of Birth:	Ordered By:

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.

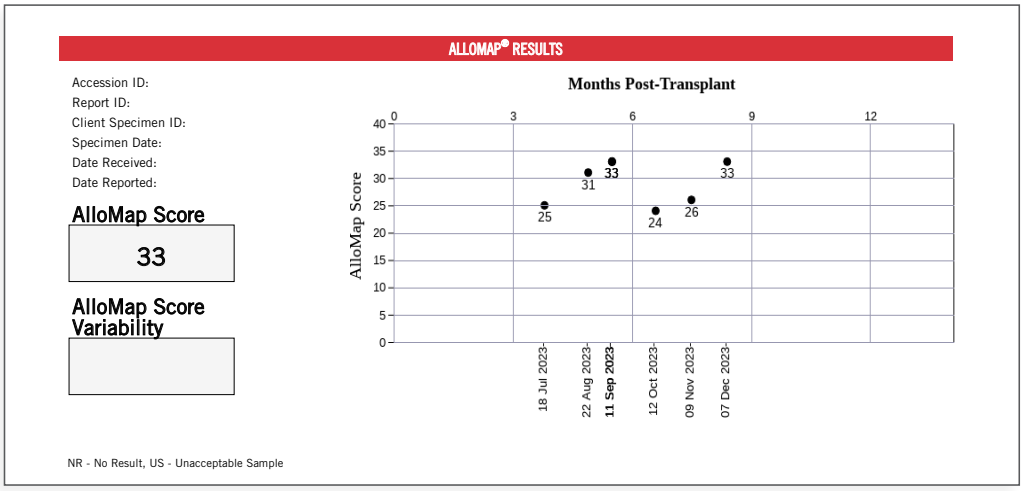
The AlloMap test is derived from a panel of 20 gene assays, 11 informative and 9 genes used for normalization and quality control, which produces gene expression data used in the calculation of an AlloMap score that ranges from 0-40. This score is associated with activity of the recipient's immune system, 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 (AMV), an opt-in consulting service by CareDx. The AlloMap score and the AMV 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.

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

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 AlloMap tests, 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.

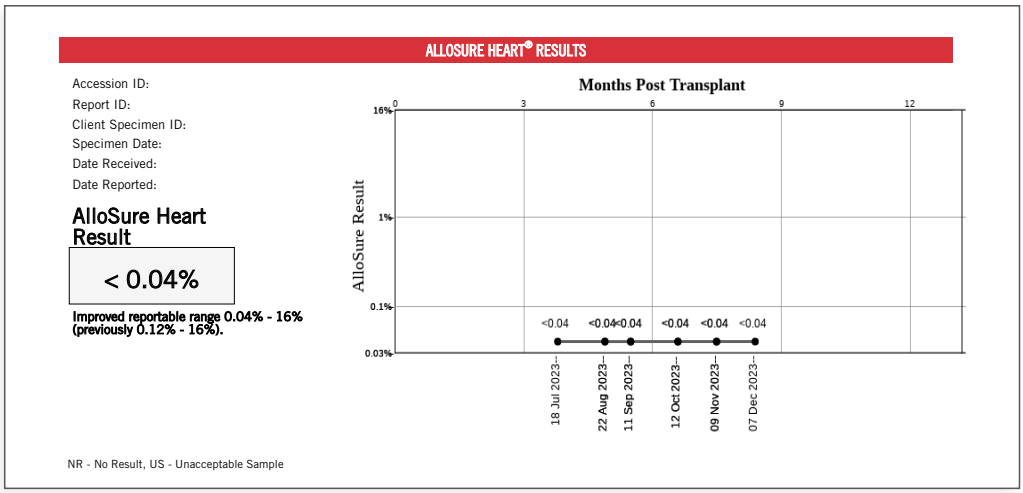
Consideration of cytomegalovirus (CMV) serologic status and/or CMV infection: CMV serologic status and or active infection has also been shown to be associated with higher AlloMap scores. Thus, interpreting an individual patient's AlloMap score should include consideration of the patient's prior scores,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

AlloSure Heart Result

The AlloSure Heart result is shown, in Fig. 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 0.04% 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 0.04%, levels below this are reported as 0.04%. 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

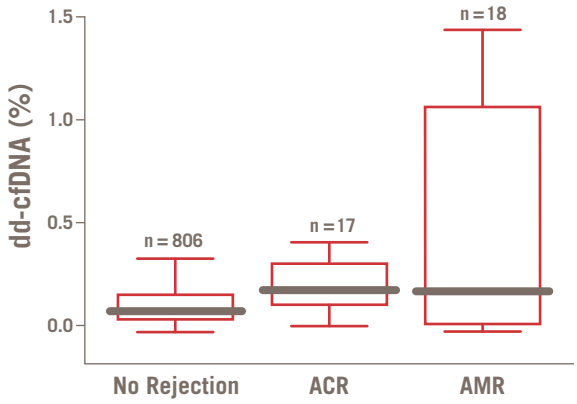
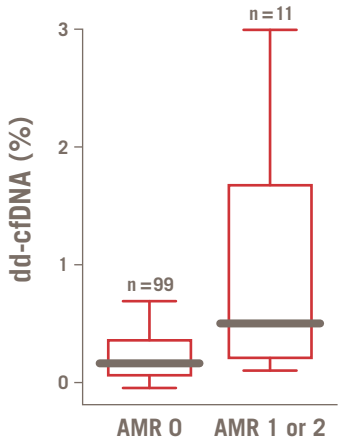


Fig. 8



The D-OAR study found the median dd-cfDNA level in the stable reference population was 0.07%, while for both the AMR and ACR groups the median dd-cfDNA levels were 0.17% (Fig. 7). Patients with AMR grade 1 or higher were also shown to have significantly elevated median dd-cfDNA levels vs those with AMR grade 0 (0.50% vs 0.16%) (Fig. 8).

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				>6 months			
	Sensitivity	Specificity	PPV ≥ 3A(2R)	NPV < 3A(2R)	Sensitivity	Specificity	PPV ≥ 3A(2R)	NPV < 3A(2R)
19	100.0%	22.8%	≤ 2.7 %	**100.0 %**	100.0%	5.3%	≤ 1.8 %	**100.0 %**
20	100.0%	24.7%	2.8 %	**100.0 %**	100.0%	8.3%	1.8 %	**100.0 %**
21	75.0%	34.0%	2.5 %	**98.8 %**	100.0%	9.8%	1.9 %	**100.0 %**
22	75.0%	39.3%	2.7 %	**98.9 %**	100.0%	11.4%	1.9 %	**100.0 %**
23	75.0%	42.3%	2.9 %	**99.0 %**	100.0%	14.4%	2.0 %	**100.0 %**
24	75.0%	48.1%	3.2 %	**99.1 %**	100.0%	18.9%	2.1 %	**100.0 %**
25	75.0%	56.8%	3.8 %	**99.3 %**	100.0%	22.7%	2.2 %	**100.0 %**
26	66.7%	62.0%	3.8 %	**99.0 %**	100.0%	27.3%	2.3 %	**100.0 %**
27	66.7%	64.2%	3.4 %	**98.7 %**	66.7%	31.8%	1.9 %	**98.7 %**
28	50.0%	68.7%	3.3 %	**98.5 %**	66.7%	39.4%	2.1 %	**98.9 %**
29	50.0%	74.2%	4.0 %	**98.6 %**	66.7%	40.9%	2.1 %	**99.0 %**
30	50.0%	77.8%	4.6 %	**98.6 %**	50.0%	50.8%	2.1 %	**98.7 %**
31	33.3%	81.5%	3.3 %	**98.2 %**	50.0%	54.5%	2.3 %	**98.8 %**
32	25.0%	85.8%	2.9 %	**98.0 %**	50.0%	63.6%	2.9 %	**99.0 %**
33	25.0%	89.6%	4.0 %	**98.1 %**	50.0%	72.7%	3.8 %	**99.1 %**
34	25.0%	92.0%	5.0 %	**98.2 %**	50.0%	79.5%	4.1 %	**98.9 %**
35	25.0%	94.5%	5.7 %	**98.1 %**	50.0%	84.7%	4.0 %	**98.7 %**
36	0.0%	97.5%	7.6 %	**98.1 %**	33.3%	90.8%	5.4 %	**98.7 %**
37	0.0%	98.1%	9.5 %	**98.1 %**	0.0%	100.0%	–	**98.4 %**
38	0.0%	100.0%	–	**97.9 %**	0.0%	100.0%	–	**98.2 %**
39	0.0%	100.0%	–	**97.9 %**	0.0%	100.0%	–	**98.3 %**

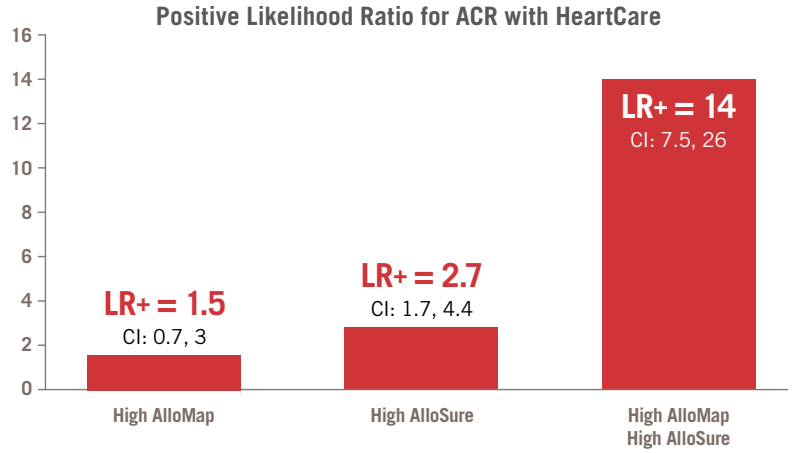
Clinical Performance Characteristics of AlloSure Test

Threshold	Sensitivity	Specificity	PPV	NPV
0.150 %	55.9 %	71.5 %	7.8 %	97.4 %
0.200 %	44.1 %	80.4 %	8.9 %	97.1 %
0.250 %	32.4 %	85.5 %	8.8 %	96.7 %

High HeartCare Results Increased the Odds of ACR by ~14X

Data derived from the combination of two independent single center studies

The chance of a biopsy revealing ACR are greater with a high HeartCare than with either a high AlloMap or high AlloSure alone



Positive Likelihood Ratio gives the change in the odds of having a diagnosis in patients with a positive test.

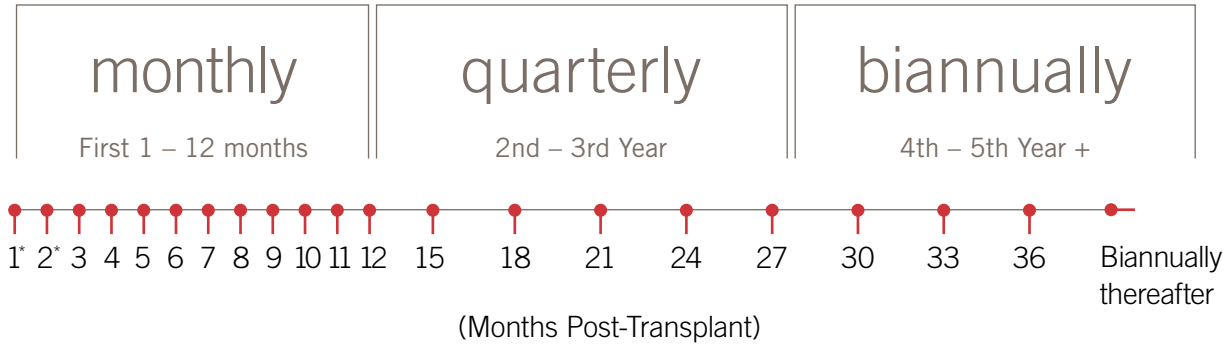
High AlloMap is: ≥30 for ≥2-6 months or ≥34 for >6 months | High AlloSure is ≥0.2%

A LR+ is mathematically defined as sensitivity / (1-specificity);
For HeartCare Sensitivity = 50.0% (22.4%, 77.6%); Specificity: 96.4% (94.3%, 97.9%)

Heart Allograft Routine Testing Schedule (HARTS)

The Heart Allograft Routine Schedule is based on the recommended use of AlloMap in 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. 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
- De Vlaminck 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 KK, Patel J, Pinney S, et al. Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. *Am J Transplant*. 2019;19(10):2889-2899. doi:10.1111/ajt.15339. ; D-OAR is a sub-study of the Outcomes AlloMap Registry (OAR).
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