Medical Coverage Policy

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Laboratory Testing for Transplantation Rejection

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Heart, Lung, and Heart-Lung Transplantation
Magnetic Resonance Imaging (MRI), Cardiac

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Overview

This coverage policy addresses the clinical indications for laboratory testing for heart and kidney transplantation rejection (i.e., AlloMap®, AlloSure®).

Coverage Policy

Genetic expression profile (i.e., AlloMap®) is considered medically necessary when ALL of the following criteria are met:

- age 15 years or older
- six months to five years post-heart transplantation
- performed in lieu of routinely scheduled endomyocardial biopsies
- result will be used to determine the need for subsequent endomyocardial biopsy to clarify rejection status
- heart allograft function is stable as demonstrated by ALL of the following:
  - absence of signs or symptoms of congestive heart failure
  - current echocardiogram with left ventricular ejection fraction (LVEF) ≥ 45%
  - absence of severe cardiac allograft vasculopathy (CAV)
- low probability of moderate or severe rejection as demonstrated by BOTH of the following:

    - no history or evidence of acute cellular rejection that required treatment
    - no history or evidence of antibody mediated rejection
- no history of genetic expression profile (i.e., AlloMap) that did not correlate with endomyocardial biopsy

Genetic expression profile (i.e., AlloMap) for any other indication is considered experimental, investigational or unproven.

Donor-derived cell-free DNA (i.e., AlloSure®-Kidney and AlloSure®-Heart) testing is considered experimental, investigational or unproven.

General Background

Organ transplant recipients are at risk for allograft rejection, even with modern immunosuppressive therapies. Traditionally, diagnosis of allograft rejection has relied on nonspecific biochemical markers and histologic examination of the grafted tissue. Since this requires an invasive tissue biopsy, there is great interest among those in the field of transplantation medicine to develop a noninvasive method of detecting organ transplant rejection (Verhoeven, et al., 2018).

AlloMap (Genetic Expression Profile)

Heart transplantation is the treatment of choice in selected patients with end-stage heart failure. The first year after transplantation is the most critical in terms of rejection. Although the risk of rejection decreases over time, late rejection does occur. The current standard for identifying rejection is endomyocardial biopsy (EMB). Using the International Society for Heart and Lung Transplantation (ISHLT) grading system, EMB samples can be classified as no rejection (Grade 0R), mild rejection (Grade 1R), moderate rejection (Grade 2R) or severe rejection (Grade 3R). These classifications help to establish and maintain the management of patients following transplantation. While published data evaluating the accuracy of EMB are lacking, no other proposed modalities for detecting rejection (e.g., echocardiography, magnetic resonance imaging, breath testing) have proven to be as accurate or clinically useful as EMB (International Society of Heart and Lung Transplantation [ISHLT], 2010).

EMBs are initially performed weekly and then at decreasing intervals. At 12 to 24 months following transplantation, EMB may be performed every three to twelve months. The biopsy is necessary because rejection may not manifest any clinical signs or symptoms. However, the procedure is not without limitations. It is painful, invasive and does not detect rejection until it is actually present. Biopsy specimens may be difficult to obtain and/or inadequate due to poor venous access. Tissue samples may also be obscured by scarring. Reported complications of EMB include: hematoma, infection, arrhythmia, ventricular perforation, and fistulas. EMB is reported to be limited by suboptimal interobserver reproducibility and uniform interpretation, and there may be a lack of histological findings in patients with hemodynamic compromise (Mehra and Parameshwar, 2010; Cadeiras, et al., 2007; Fang, 2007; Renlund, et al., 2007; Patel and Kobashigawa, 2006; Starling, et al., 2006; Mehra, 2005). The limitations of EMB have prompted researchers to develop alternatives.

AlloMap® has evolved into an established alternative to EMB in a defined subgroup of heart transplant recipients. The blood test measures gene expression by quantifying the gene-specific messenger RNA (mRNA) that is present in the sample. The expression level of 11 informative genes and nine normalization genes is measured using quantitative real-time polymerase chain reaction (qRT-PCR). The results are reported as an integer ranging from 0–40, and the lower the score the less the likelihood that the patient will experience rejection (i.e., AlloMap detects a low risk of rejection). It is proposed that circulating peripheral blood mononuclear cells may be indicative of rejection earlier than changes seen at local sites.

AlloMap may be used to help identify patients, age 15 years or older, who are between six months and five years post-heart transplantation, have a stable heart allograft function, and are at low risk of moderate or severe rejection, have no history or evidence of acute cellular or antibody mediated rejection and therefore, may not need to undergo endomyocardial biopsies. The test is recommended for use in conjunction with standard clinical

evaluation and assessment (e.g., history and physical, echocardiography, endomyocardial biopsy) of graft function.

Stable heart allograft function is determined by the absence of congestive heart failure, left ventricular ejection fraction (LVEF) ≥◦45% and absence of severe cardiac allograft vasculopathy (CAV). Symptoms of congestive heart failure include exertional dyspnea, orthopnea, paroxysmal nocturnal dyspnea, edema, syncope, palpitation and/or arrhythmias. LVEF measures the amount of blood being pumped out of the left ventricle of the heart. A LVEF below 45 may be evidence of CHF or cardiomyopathy. Rejection can be associated with hemodynamic compromise as indicated by a decrease in the LVEF. LVEF can be assessed by echocardiography, cardiac catheterization, MRI, multiple gated acquisition (MUGA) scan, or ventriculography (Pham, et al., 2010; Yamani and Taylor, 2010).

Angiography or intravascular ultrasound (IVUS) is used to assess CAV. The classic feature of CAV is diffuse concentric narrowing with luminal stenosis. CAV may be diagnosed when there is stenosis of 50% within any major epicardial coronary vessel or branches on angiography, severe diffuse or distal vessel tapering on angiography, maximal intimal thickness ≥◦0.5 mm in any major epicardial coronary vessel at the time of intravascular ultrasound, evidence of recent ischemic injury on biopsy, graft dysfunction and/or an epicardial stenosis of <◦50% that does not respond to anti-rejection therapy. Pham, et al. (2010) identified severe CAV as ">◦50% left main stenosis; ≥◦50% stenosis in ≥ 2 primary vessels (proximal 1/3 or middle 1/3 of the left anterior descending or left circumflex, right coronary artery to takeoff of posterior descending artery in right-dominant coronary circulations) or isolated branch stenosis of >◦50% in all three systems (diagonal branches, obtuse marginal branches, distal 1/3 of left anterior descending or left circumflex, posterior descending artery, posterior lateral branch, and right coronary artery to takeoff of posterior descending artery in non-dominant systems)". Clinical manifestation of CAV may be silent, or occur as acute myocardial infarction, congestive heart failure, arrhythmias, and/or wall motion abnormalities (Pham, et al., 2010; Yamani and Taylor, 2010; Schmauss and Weis, 2008).

Acute cellular rejection (ACR), or cell-mediated rejection, is the clinical syndrome that occurs as the result of an alloimmune response against a transplanted organ and can be caused by either a cellular or humoral response. ACR is the most common form of rejection and occurs at least once in approximately 50% of heart transplant recipients during the first year. Patients with a low probability of moderate or severe acute cellular rejection have a histological International Society of Heart and Lung Transplantation (ISHLT) grade 0 or grade 1. Typically, these grades are not treated for rejection. Grades 2R (moderate) and 3R (severe) are indicative of rejection and treated per institutional protocol. The two key elements of acute cellular rejection are the presence of lymphocytes and myocyte injury. Low probability of moderate or severe acute cellular rejection has been defined as patients who do not have treatable rejection on two consecutive biopsies over a period of 3–9 months. Treated rejection includes the administration of anti-rejection therapy (e.g., steroids, antibody therapy) (Maleszewski and Burke, 2014; Acker and Jessup, 2011; ISHLT, 2010; Pham, et al., 2010).

Antibody mediated rejection (AMR), or humoral rejection, is initiated by antibodies rather than by T cells. AMR is manifested as graft dysfunction or hemodynamic compromise (e.g., shock, hypotension, decreased cardiac output, and/or a rise in pulmonary capillary wedge pressure) in the absence of cellular rejection on biopsy. The diagnosis is based on histologic findings indicative of acute myocardial capillary injury. Per Pham et al. (2010), AMR may be associated with hemodynamic compromise including: "LVEF ≤◦30% or at least 25% below baseline, cardiac index <◦2 L/min/m^2 or administration of inotropic agents to support circulation" (Berry, et al., 2013; Acker and Jessup, 2011; Pham, et al., 2010).

AlloMap has been proposed as an alternative to EMB in patients for whom biopsy is contraindicated or cannot be performed. One complication of biopsy is tricuspid regurgitation caused by repeated passing of the bioptome across the tricuspid valve into the right ventricular to obtain tissue specimens. Repeated biopsies may further damage the valve and increase regurgitation (Strecker, et al., 2013; Badiwala and Rao, 2007). Other contraindications for EMB include profound hemodynamic compromise, coagulopathy, and mechanical tricuspid prosthesis (Bennett and Tang, 2013). Lack of adequate vascular access, malignant arrhythmic events (e.g., unstable ventricular arrhythmias) or intracavitary mass or thrombus may also be contraindications to EMB. Clinical trials investigating AlloMap in this subset of patients are lacking.

AlloMap has not been validated for use in patients who demonstrate antibody-mediated rejection or noncellular rejection accompanied by hemodynamic compromise, pregnant women, patients who have recently (i.e., less than 30 days) received a blood transfusion, patients recently (i.e., less than 20 days) treated with high-dose steroids, patients recently treated for rejection, or patients who are being treated with ≥ 20 milligrams per day of prednisone or equivalent (Caideiras, et al., 2007; Mehra and Uber, 2007).

U. S. Food and Drug Administration (FDA)
In 2008, XDx Laboratories received 510(k) Class II approval for AlloMap Molecular Expression Testing "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 is indicated for use in heart transplant recipients who are 15 years of age or older and at least 2 months (≥ 55 days) post-transplantation (FDA, Nov 2008).

Literature Review
Clinical trials have reported that AlloMap was effective in identifying those patients with a low probability of rejection following heart transplantation at greater than six months following transplantation. Crespo-Leiro et al. (2016) conducted the Cardiac Allograft Rejection Gene Expression Observational II Study (CARGO II) to further clinically validate the GEP test performance using an independent study population. AlloMap testing was performed during post-transplant surveillance. Using a cutoff GEP score of 34 at six months post-transplantation, 95.5% (381/399) of GEP tests were true negatives, 4.5% (18/399) were false negatives, 10.2% (6/59) were true positives, and 89.8% (53/59) were false positives. Based on 938 paired biopsies, the GEP test score receiver operating characteristic curve (AUC-ROC) for distinguishing ≥ 3A rejection was 0.69 for 6 months post-transplantation. Depending on the chosen threshold score, the NPV and PPV ranged from 98.1%–100% and 2.0%–4.7%, respectively. In the CARGO (Cardiac Allograft Rejection Gene Expression Observational) study, Deng, et al. (2006) reported that the gene test significantly distinguished the absence of moderate/severe acute rejection from quiescence (p=0.0018). At a threshold of 30, the test predicted that patients with low molecular scores at or after one year following transplant were at low risk of current moderate/severe rejection (negative predictive value > 99%). Using CARGO samples (n=127), Mehra et al. (2008) reported that the use of AlloMap allowed for the identification and separation of patients into low-, intermediate-, and high-risk groups. Baseline scores were significantly higher for those who went on to reject, remained high during an episode of rejection, and dropped post-treatment for rejection (p<0.01). The Pham et al. randomized controlled trial (2010a; 2010b) (Invasive Monitoring Attenuation through Gene Expression [IMAGE]) compared outcomes of monitoring for rejection following heart transplantation using AlloMap gene-testing (n=297) compared to routine endomyocardial biopsy (n=305). Outcomes reported that this selected subpopulation of patients more than six months post transplantation were not at an increased risk of serious adverse outcomes and significantly fewer biopsies (p=0.001) were performed.

AlloMap Testing at Two to Six Months Following Transplantation: A prospective, observational, multi-center study, the Cardiac Allograft Rejection Gene Expression Observational II Study (CARGO II) (Crespo-Leiro, et al., 2016), was conducted to validate the clinical performance of AlloMap in an independent population. The performance of AlloMap was evaluated in two post-transplantation windows, ≥◦2–6 months and >◦6 months. Included samples were obtained at least 55 days post-transplantation, 30 days after transfusion of blood products, 21 days after administration of ≥ 20 mg/day of prednisone; and 60 days after treating a prior rejection. The main metric for validating the GEP test performance was the receiver operating characteristic curve (AUC-ROC). Values for the AUC-ROC range from 0.5 (uninformative) to 1.0 (perfect discrimination). All samples that had local biopsy grades of 1B, 2, and ≥◦3A were sent to an independent central pathology for grading. If more than one grade 0 or grade 1A local sample was obtained on a subject, one random sample was sent to central pathology. The ≥ 2–6 months post-transplantation specimens with histopathology slides selected for independent central panel pathology rejection grading included 328 patients with 480 GEP test scores. The mean receiver AUC-ROC for the GEP test scores in the ≥ 2–6 months post-transplantation period was 0.70 with a 95% confidence interval from 0.67 to 0.73. The mean AUC-ROC for the GEP test scores for the >◦6 months post-transplantation period was 0.69 with 95% confidence interval from 0.66 to 0.72. A GEP test score of ≥ 34 corresponded to histology-based grade ≥ 3A (2R) rejection with a PPV of 4.0% at months ≥ 2–6 post-transplantation vs. 4.3% at >◦6 months post transplantation. The negative predictive values (NPVs) were 98.4% at months ≥◦2–6 post-transplantation and 98.3% at > 6 months post-transplantation. The rates of rejection were similar in the early post-transplantation period (3.2% for months ≥ 2 to 6) compared with the >◦6 post-

transplantation population (3.2%). However, it was noted by the authors that the biopsies performed after month six post-transplantation may have been performed more often due to clinical suspicion of rejection, whereas biopsies performed prior to six months post-transplantation were probably conducted for surveillance of asymptomatic patients. There were several limitations of the study discussed by the authors including: 1) the study excluded GEP data and biopsies from visits earlier than 55 days post-transplantation; therefore, GEP performance is not known in this population; 2) patients treated for rejection within the prior 30 days were excluded which may have led to an underestimation of the incidence of rejection; 3) not all GEP tests had paired biopsy results; 4) because of the very small number and proportion of clinically suspected, biopsy confirmed, rejections of grade ≥◦3A (2R), rejections discovered during routine surveillance and rejections confirmed with biopsies performed for clinical suspicion were pooled together in the analyses; and 5) the central pathology feature of this study did not reflect practical clinical care. Additional studies are needed to validate the efficacy of AlloMap in the ≥ 2–6 months post-transplantation period.

Kobashigawa et al. (2015) conducted a single-center randomized controlled trial (n=60), the Early Invasive Monitoring Attenuation through Gene Expression (EIMAGE) study, to evaluate the safety and efficacy of gene expression profiling (GEP; AlloMap) (n=30), compared to endomyocardial biopsy (n=30), in monitoring heart rejection from day 55 and onward post-transplantation. Patients were ≥◦18 years of age, had undergone cardiac transplantation within the past 2–6 months (55–185 days), and in a clinically stable condition with an echocardiographic LVEF of ≥◦50%. Exclusion criteria included any clinical signs of declining graft function defined by symptoms of congestive heart failure at the first study surveillance visit, rejection therapy for biopsy-proven ISHLT Grade 2R (3A) or higher during the preceding two months, previous or current evidence of antibody-mediated rejection (AMR) defined according to the ISHLT 2004 guidelines, presence of donor-specific antibodies (DSAs), high corticosteroid doses of >◦20 mg at the time of the first study visit, receiving hematopoietic growth factors or blood transfusion during the previous 30 days, and pregnancy. A positive GEP was considered ≥◦30 at 2–6 months and ≥◦34 after six months and prompted a biopsy. The primary outcome was a composite of first occurrence of death/retransplant, rejection with hemodynamic compromise, or allograft dysfunction because of other causes ≤◦18 months post-transplant. There was no statistically significant difference in the primary outcome between the groups at 18 months follow-up. Intravascular ultrasound (IVUS) results showed no significant differences between the groups in the average change in maximal intimal thickness (p=0.944) or plaque burden in the first year. LVEF results at 18 months were not significantly different (p=0.522), nor were the cardiac index score (p=0.413) or other cardiac hemodynamics. The number of biopsy-proven ISHLT≥◦2R (3A) rejection episodes within the first 18 months post-transplant was not significantly different between the groups (p=0.31). At 18 months, 42 biopsies were performed in the GEP group, compared with 253 in the EMB group. There was no significant difference in prednisone weaning or average prednisone dosage between the two groups. Satisfaction of method rejection surveillance was statistically significant in the GEP group (p=0.003) but there were no significant differences in the SF-12 mental health and physical-health summary scores. The results of this pilot study should be viewed with caution due to the low risk, small patient population included in the trial. The authors noted that firm conclusions could not be made about noninferiority of GEP versus EMB, further large-scale clinical trials to confirm these findings are required; and the study was limited by the few primary end points experienced as only eight patients reached the composite primary end point.

Other indications: Studies have also been conducted to determine if AlloMap could identify different forms of rejection, identify patients who may develop Grade ≥ 3A rejection, what impact coronary allograft vasculopathy (CAV) and blood transfusion would have on AlloMap scoring. AlloMap has also been analyzed for the ability to predict clinical events ≥◦315 days following transplantation and as a predictor for long-term survival after heart transplant.

In a retrospective, single center study, Fujita et al. (2017) investigated AlloMap as a predictor of long-term survival following heart transplantation. Data on 46 patients from the CARGO II trial were analyzed. Patients who had survived for at least one year and in whom AlloMap scores were available at six, nine, 12 and 18 months after transplant were included. The primary outcome measure was long term all-cause mortality. Survival data were gathered by review of medical records and contact with the patient/family. Six to nine months following transplantation 23 patients showed a decreased AlloMap score and 23 showed an increased score. The scores at individual time points (6, 9, 12, 18 months) did not correlate with long-term survival. Patients with an increased score from six to nine months after transplant compared to a decreased score showed a significant elevation in

all-cause mortality (p=0.005). It was also noted that long-term mortality may have been associated with side effects or inadequate immunosuppression. Limitations of the study are the retrospective design, small patient populations and heterogeneity in baseline characteristics. Prospective studies with larger patient populations are required to validate the predictive value of AlloMap in identifying patients at high risk for death during long-term follow-up.

Crespo-Leiro et al. (2015) conducted a retrospective analysis (n=91) of an independent patient population from the Cardiac Allograft Rejection Gene Expression Observational (CARGO) II study to determine the prognostic utility of within patient variability of GEP scores in predicting future, significant clinical events. A second objective was to determine the negative predictive value (NPV) and the positive predictive value (PPV) of GEP score variability in predicting future significant clinical events. The GEP score variability was defined as the standard deviation of four GEP scores collected ≥◦315 days post-transplant. Analyzed clinical outcome data included CARGO II patients who had four AlloMap scores preceding a first clinical event (event group) or had four sequential GEP scores without a subsequent event (control group). Out of the 737 patients in the CARGO II study, 55 patients who did not have events and had at least a three year follow-up were chosen as controls and 36 patients who experienced at least one predefined cardiac event were included in the event group. There was no statistically significant difference between the groups in number of days between the first and the last AlloMap test. The estimated prevalence of events was 17%. Events occurred at a median of 391 days after the final GEP Test. The NPV increased from 87.4% at a score variability of 1.0 to 97% at a score variability of 0.6. The PPVs for the same score decreased from 26% at a score variability of 1.0 to 23.3% for a GEP score variability of 0.6. For a GEP score variability cutoff of 1.5, the estimated PPV was 35.4%. In the event group 58% of the patients died, 31% experienced graft failure and 11% underwent cardiac re-transplantation. The results of the study proposed that the AlloMap score variability may be useful in estimating the probability of future events of death, re-transplantation or graft failure in heart transplant recipients tested with AlloMap ≥◦315 days post-transplantation. The NPV ≥◦97% indicated that clinical utility of AlloMap score variability may help identify patients at a low risk for future clinical events. Given, the limitations of the study, the results should be viewed with caution. Limitations of the study include the retrospective study design, small patient population, potential selection bias of patients, imbalance of number of patients per group and heterogeneity of clinical conditions.

Bernstein et al. (2007) conducted a subanalysis of the CARGO study to determine if gene expression (GE) (i.e., AlloMap genetic testing) could distinguish different forms of mild heart transplant rejection. Inclusion criteria were met by 265 of the 737 adult and pediatric CARGO patients. Reinterpretation of the tissue identified: 176 grade 0 biopsies, 17 grade 1As, 12 grade 1Bs, 21 grade 2, and 24 grade 3As. The mean GE scores differentiated moderate-to-severe rejection (grades ≥ 3A) (32 ± 0.9) from grades 0 (25.3 ± 0.5), 1A (23.8 ± 2.1) and 2 (26.9 ± 1.5) ($p<0.00001$, $p<0.001$ and $p<0.01$, respectively). The mean GE score for grade 1B was indistinguishable from that for grades ≥ 3A, (29.8 ± 2.0 vs. 32.0 ± 0.9) (p=0.25). Based on a calculation of the fold-difference of each gene, grade 1B was identified as a subgroup of rejection with a peripheral gene expression profile that more closely resembled moderate-to-severe rejection. The study also analyzed whether or not the time from transplantation influenced the GE scores compared to the grades. For the two- to six-month period following transplantation, the mean GE score for grade ≥ 3A (30.8 ± 1.4) was not significantly different from that for grade 1B (28.5 ± 3.9) (p=0.49). The mean GE scores differentiated grades 0, 1A, and 2 from grades ≥ 3A. EMBs obtained more than six months following transplantation indicated grades ≥ 3A demonstrated mean GE scores similar to grade 1B scores (p=0.19). Mean GE scores for grades 0, 1A, and 2 were significantly lower than for grades ≥ 3A scores.

Mehra et al. (2007) also conducted a subanalysis of cardiac allograft recipients (n=104) from the CARGO study to determine if the AlloMap test could distinguish between rejection-free stable patients and patients who develop Grade ≥ 3A rejection within 12 weeks following transplantation. In addition, the study characterized the associations with rejection within 180 days of transplantation, identified individual classifier genes' associated with the risk of future rejection and explored the pathways and functions of the genes. Patients with grades 0 or 1A at baseline and free of ≥ grade 2 rejection for at least the first 12 weeks post-transplantation were designated as the matched control group (n=65). The rejection group included 39 patients, clinically stable at baseline, who experienced an episode of grade ≥ 3A within 12 weeks following sample collection. Data for the study was analyzed from blood samples and EMB obtained during the same visit. Analysis of the data demonstrated a significant difference in the mean GE score of 27.4 ± 6.3 for the study group and 23.9 ± 7.1 for the control group (p=0.01). The study also analyzed a subgroup of these patients who were ≤ 180 days post-transplant and

reported a significant difference in the mean GE score of 28.4 ± 4.9 for the study group (n=28) and 22.4 ± 7.5 for the control group (n=46) (p<0.001). To explore the molecular pathways associated with steroid sensitivity and T-cell activation, the expression levels of 33 additional genes were measured, and the data demonstrated that "transcriptional signals of genes regulated by corticosteroids or involved in T-cell activation in peripheral blood of heart transplant recipients are associated with the presence or absence of future clinically relevant rejection." The authors stated that the data from this study "must be interpreted with care and in the context of the case-control study in which they were derived." They further explained that case-control studies include "inherent spectrum bias, preventing generalization," and noted that milder rejection grades (i.e., 1B and 2) were not addressed.

In 2007, Yamani et al. conducted two retrospective reviews. The first study (Apr 2007a) included 69 patients and evaluated the impact of transplant coronary allograft vasculopathy (CAV) on AlloMap gene expression analysis. Evidence of CAV within 4.3 ± 3 months of AlloMap testing was demonstrated in 20 patients by coronary angiography. The control group had a mean AlloMap score of 26.1 ± 6.5 compared to > 32.2 ± 3.9 in the CAV group (p<0.001). Fifteen control patients and 14 CAV patients had an AlloMap score of greater than 30 (p=0.0026). CAV was associated with a significantly increased AlloMap score in the absence of significant rejection (p=0.0002). The second review (2007b) investigated the impact of early post-transfusion ischemic injury on subsequent AlloMap testing from data retrieved from a transplant database (n=67). The subjects were evaluated at a mean 34 ± 20 months following heart transplantation. Compared to the control group, the injury group demonstrated worse five-year freedom from vasculopathy, lower left ventricular ejection fraction (LVEF), and higher percentage of AlloMap scores. The presence of ischemic injury was associated with a significant increase in AlloMap scores (p<0.0001).

AlloSure® (Donor-Derived Cell-Free DNA)

Allosure® (CareDx, Brisbane, CA) is a targeted next-generation sequencing test that evaluates single nucleotide polymorphisms (SNP) in cell-free DNA samples. It is hypothesized that transplant patients experiencing organ injury associated with acute rejection will have higher levels of donor-derived cell free DNA (dd-cfDNA) which is thought to be due to cell-free DNA being an indicator of dying cells. In recent years, dd-cfDNA tests have become clinically available to quantify the amount of dd-cfDNA in kidney and heart transplant recipients. AlloSure Kidney is proposed for use in renal transplant patients who are 18 years or older and a minimum of 14 days post-transplant. AlloSure Heart is proposed for use in conjunction with AlloMap in heart transplant patients who are 15 years or older and at least 55 days post-transplant (CareDx, 2019).

These tests are intended to assess the probability of allograft rejection in a particular patient. The technology does not rely on previous genotyping of either the patient or the donor, which is a benefit over previous methods that have been used to measure dd-cfDNA. Additionally, these tests do not require invasive tissue biopsy, which is necessary for the standard methods of histopathological interpretation that are used to diagnose allograft rejection. However, biopsy is still necessary to confirm and establish the type of active rejection in affected patients (Jordan et al., 2018).It has been proposed that these tests be used for serial monitoring in order to detect new onset injury or rejection prior to clinical symptoms, however the optimal time interval has yet to be established (Bloom et al., 2017). The use of dd-cfDNA to evaluate transplant rejection is a new development in the field of transplant medicine, however the clinical utility of this technology has yet to be established.

Literature Review-Heart
There is insufficient evidence to support the accuracy and clinical utility of donor-derived cell free DNA for assessing and monitoring the probability of allograft rejection in heart transplant patients. Studies are primarily in the form of an author manuscript, a conference abstract, retrospective reviews and registry data (Khush, et al., 2019; Crespo-Leiro et al., 2015; De Vlaminck, et al., 2014; Hidestrand, et al., 2014).

Literature Review-Kidney
Zhang et al. (2020) conducted a prospective single center observational study to evaluate the diagnostic performance of donor-derived plasma cell-free DNA (cfDNA) in discriminating antibody-mediated rejection (ABMR) or de novo donor-specific antibodies (DSA) without histological lesions in kidney allograft recipients. The plasma cfDNA fraction was measured using a targeted, single nucleotide polymorphism (SNP)-based assay. Pathological diagnosis was made according to the 2015 Banff Kidney Rejection Classification. The area under

the ROC curve (AUC-ROC) was determined using the bootstrapping method to estimate median and 95% confidence interval (95% CI). The sensitivity, specificity and Youden index, positive predictive value (PPV), and negative predictive value (NPV) were calculated for specific cfDNA fractions. The study included 37 consecutive patients who received kidney allografts, including 18 recipients in the ABMR group and 19 recipients in the stable allograft group (7 DSA-positive and 12 DSA-negative). All patients in the ABMR group were DSA positive and 7 patients in the stable group were DSA positive but had no pathologically proven ABMR. The median donor-derived plasma cfDNA fraction was 2.4% (Q1 1.52% -Q3 3.70%) in the ABMR group, and was significantly higher than that of the stable group (0.65%, Q1 0.57% -Q3 0.97%; P < 0.001), but comparable with that of the DSA-positive patients in the stable allograft group (P = 0.074). The AUC-ROC of cfDNA was 0.90 (95% CI, 0.79-0.98). When a cfDNA threshold of 1% was chosen, it had a sensitivity of 88.9% and a specificity of 73.7%. The PPV was 76.2% and the NPV was 87.5%. The authors concluded that donor-derived plasma cfDNA fraction increased in kidney allograft recipients with ABMR. The study is limited by the small number of participants.

Bloom et al. (2017) conducted a prospective observational study (DART study). Blood specimens were collected from 102 patients at scheduled intervals and at the time of clinically indicated biopsies. Plasma levels of donor-derived cell-free DNA (dd-cfDNA) were measured and correlated with allograft rejection status ascertained by histology in 107 biopsy specimens. The dd-cfDNA level discriminated between biopsy specimens showing any rejection (T cell-mediated rejection or antibody-mediated rejection [ABMR]) and controls (no rejection histologically), P<0.001 (receiver operating characteristic area under the curve [AUC], 0.74; 95% confidence interval [95% CI], 0.61 to 0.86). Positive and negative predictive values for active rejection at a cutoff of 1.0% dd-cfDNA were 61% and 84%, respectively. The AUC for discriminating ABMR from samples without ABMR was 0.87 (95% CI, 0.75 to 0.97). Positive and negative predictive values for ABMR at a cutoff of 1.0% dd-cfDNA were 44% and 96%, respectively. Median dd-cfDNA was 2.9% (ABMR), 1.2% (T cell-mediated types ≥IB), 0.2% (T cell-mediated type IA), and 0.3% in controls (P=0.05 for T cell-mediated rejection types ≥IB versus controls).

Jordan et al. (2018) conducted a study with a cohort from above DART study to assess the combined use of donor-derived cell-free DNA (dd-cfDNA) and Donor-specific antibodies (DSA) testing to diagnose active antibody-mediated rejection (ABMR). Donor-derived cell-free DNA was assayed in 90 blood samples with paired DSA and clinically indicated biopsies from 87 kidney transplant patients. Sixteen cases met criteria for active ABMR. Performance characteristics of dd-cfDNA for diagnosis of active ABMR were determined for samples with prior or current positive DSA (DSA+, n = 33). The median level of dd-cfDNA (2.9%) in DSA+ patients with active ABMR was significantly higher than the median level (0.34%) in DSA+ patients without ABMR (P<0.001). The median level of dd-cfDNA in DSA- patients was 0.29%. The positive predictive value of dd-cfDNA (at 1%) to detect active ABMR in DSA+ patients was 81%, whereas the negative predictive value was 83%. The positive predictive value for DSA+ alone was 48%.

Broomberg et al. (2017) conducted an observational study of a cohort of the above DART study to establish biological variation and clinical reference intervals of dd-cfDNA in renal transplant recipients by using an analytically validated assay that has a CV of 6.8%. Venous blood was sampled at patient surveillance visits (typically at posttransplant months 1–4, 6, 9, and 12). Patients with stable renal allograft function spanning ≥3 serial visits were selected. AlloSure was used to measure dd-cfDNA in the plasma and computed the intraindividual CV (CVI) and interindividual CV (CVG), the index of individuality (II), and reference change value (RCV). The study included 93 patients with 61% men, 56% Caucasian, mean ages 49 years, and 63% were deceased donor kidney recipients. Of the 380 blood samples, the dd-cfDNA median value was 0.21% (interquartile range 0.12%–0.39%) and the 97.5th percentile was 1.20%. In 18 patients with an average of 4.1 tests, the CVI was 21%, CVG was 37%, II was 0.57, and RCV was 61%. The authors concluded that in a renal transplant recipient, a dd-cfDNA level above 1.2% is out of range and potentially abnormal. A serial increase of up to 61% in level of dd-cfDNA in a patient may be attributable to biological variation.

Technology Assessments

BlueCross BlueShield Association (BCBSA) Technology Evaluation Center (TEC): BCBSA (2011) conducted a systematic review of the literature to determine if AlloMap testing improved health outcomes compared to other methods used for monitoring rejection following heart transplantation. Validation studies that only included patients with no rejection or class 3A rejection reported a sensitivity of 76–84% and specificity of 38–41% at a cutoff score of 20. Post hoc analyses of subgroups (n< 30 patients) who were > 6 months or > 12 months post-transplant with higher cutoff scores reported sensitivities of 71.4–80% and specificities of 77.8%–

78.7%. Depending on the cutoff score used to denote a positive test, other studies reported a positive predictive value (PPV) typically < 7% and a negative predictive value (NPV) > 98%. According to BCBSA, the data used for these values was not available and the results were not consistent with results from actual patient samples. One study reported a 7.8% PPV and a 100% NPV using a cutoff score of 34 but out of the total 243 samples, only five were rejection samples. The authors reported that higher AlloMap scores were associated with a greater likelihood of rejection in class 3A or higher patients but the "diagnostic characteristics of AlloMap testing were uncertain", study methods were unclear, study samples were not completely described, number of cases of rejection were small and cutoff scores appeared "to be determined post hoc." According to BCBSA, the "sensitively of the test for detecting rejection is uncertain." The available evidence was insufficient to permit conclusions regarding the effects of AlloMap on net health outcomes, or if the test is as beneficial as any established alternatives for monitoring heart transplant patients.

Professional Societies/Organizations
In their 2010 guidelines on the care of heart transplant (HT) recipients, the International Society for Heart and Lung Transplantation stated "gene expression profiling (AlloMap) can be used to rule out the presence of ACR [acute cardiac rejection] of grade 2R [i.e., an infiltrate plus the presence of multifocal myocyte damage] or greater in appropriate low-risk patients, between 6 months and 5 years after HT". This recommendation is based on data from the CARGO and IMAGE clinical trials.

The evaluation of donor-derived cell free DNA has not yet been addressed by professional societies including the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline for care of kidney transplant recipients.

Use Outside of the US
Diaxonhit (Paris: ALEHT), a French provider of specialty diagnostic solutions, has entered an agreement with CareDx™ to perform AlloMap for European heart transplant recipients. For the European market, the AlloMap test will be performed in the Strasbourg University Hospital Central Immunology Laboratory (CareDx, 2016).

Medicare Coverage Determinations

	Contractor	Policy Name/Number	Revision Effective Date
NCD		No National Coverage Determination found	
LCD	CGS Administrators, LLC	MolDX: Allosure® Donor-Derived Cell-Free DNA Test (L37362)	10/31/2019
	Noridian Healthcare Solutions, LLC	MolDX: Allosure® Donor-Derived Cell-Free DNA Test (L37303)	11/01/2019
	Palmetto GBA	MolDX: Allosure® Donor-Derived Cell-Free DNA Test (L37266)	10/31/2019
	Wisconsin Physicians Service Insurance Corporation	MolDX: Allosure® Donor-Derived Cell-Free DNA Test (L37665)	11/1/2019

Note: Please review the current Medicare Policy for the most up-to-date information.

Coding/Billing Information

Note: 1) This list of codes may not be all-inclusive.
2) Deleted codes and codes which are not effective at the time the service is rendered may not be eligible for reimbursement.

Considered Medically Necessary when criteria in the applicable policy statements listed above are met:

CPT®* Codes	Description
81595	Cardiology (heart transplant), mRNA, gene expression profiling by real-time quantitative PCR of 20 genes (11 content and 9 housekeeping), utilizing subfraction of peripheral blood, algorithm reported as a rejection risk score.

Considered Experimental/Investigational/Unproven when used to report AlloSure®-Kidney and AlloSure®-Heart testing:

CPT®* Codes	Description
81479	Unlisted molecular pathology procedure

***Current Procedural Terminology (CPT®) ©2019 American Medical Association: Chicago, IL.**

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