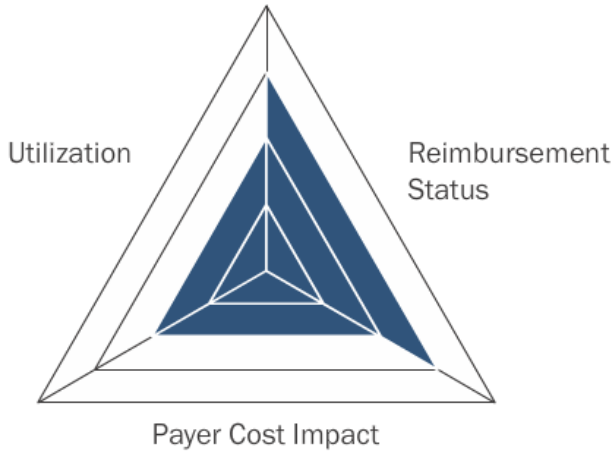
Gene Expression Test (AlloMap) for Monitoring Heart Transplant Rejection

HEALTH TECHNOLOGY ASSESSMENT INFORMATION SERVICE™

GENETIC TEST EVIDENCE REPORT

Executive Summary

AlloMap (CareDx, Inc.) is a noninvasive, blood-based gene-expression test to aid identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection at the time of testing. Clinicians use AlloMap in conjunction with standard clinical assessment to determine the need for further evaluation for acute cellular rejection using endomyocardial biopsy (EMB). The test is based on measurement of the expression level of multiple genes associated with acute cellular rejection. AlloMap is intended to reduce the number of EMBs required during post-transplant surveillance. Potential disadvantages of AlloMap include possible false test results. False-positive results could result in unnecessary EMBs and associated risks. False-negative results could delay an additional evaluation and treatment for heart transplant rejection.



Parameter Rating and Definition*	Rationale
Reimbursement Status: 3 Expanding: Medicare has no national coverage determination; 4 to 7 private payers have issued positive payment policies.	Our searches of 11 representative, private, third-party payers that provide online medical coverage policies found 6 payers with a policy describing coverage with conditions, 3 payers that deny coverage, and 2 payers with no specific policy. About two-thirds of the U.S. heart transplant population are covered for AlloMap. The American Medical Association has assigned a specific Current Procedural Terminology code to describe AlloMap effective January 1, 2016.
Cost Impact on Payers: 2 Small: Either >$500 to $2,000 per patient or limited utilization resulting in small aggregate cost to payers.	The list price for a single AlloMap test is $3,600. Because healthcare centers' rejection surveillance schedules vary, AlloMap testing for a single patient 6-months to 5-years post-transplant may cost from $25,200 to $57,600. In 2014, approximately 2,000 heart transplants were performed in the United States; therefore, the aggregate cost to payers is low because the patient population is small.
Utilization Status: 2 Low: Performed by only one or a small number of labs. A small number of clinicians are prescribing the test or the patient population currently using the test is small.	Since 2005, more than 75,000 AlloMap tests have been performed in the post-transplant care of more than 16,000 patients. According to CareDx, 110 of 130 U.S. transplant centers use AlloMap for monitoring some of their heart transplant patients. However, according to an online news source, AlloMap has captured 30% market share, and the overall patient population is small because the indication is relatively rare.

*Please see Appendix C for parameter definitions.

Evidence Summary of Selected Outcomes

Patients less than one-year post-transplant have a significantly higher risk of rejection than those who are more than one-year post transplant. For this reason, we report outcomes that may be affected by time post-transplant separately.

Key Outcomes Assessed	Evidence Base	Conclusions	GRADE-based Strength-of-evidence Rating*
Mortality	AlloMap vs. EMB: 1 RCT, patients 2- to 18-months post-transplant	Inconclusive: No deaths occurred in either group**	Very low
	AlloMap vs. EMB: 1 RCT, patients 1- to 5-years post-transplant	Inconclusive: Too few patients assessed†	Very low
Quality of life	AlloMap vs. EMB: 2 RCTs	Inconclusive: Inconsistent results	Very low
Sensitivity for detecting acute rejection (ISHLT Grade ≥2R)	1 diagnostic cohort study, patients <1-year post-transplant	Inconclusive: Insufficient data reported to determine sensitivity	Very low
	1 diagnostic cohort study, patients >1-year post-transplant	Inconclusive: Study has high risk of bias	Very low
Negative predictive value for the absence of acute rejection (ISHLT Grade <2R)	1 diagnostic cohort study, patients 2- to 6- months post-transplant	Inconclusive: Study has high risk of bias	Very low
	2 diagnostic cohort studies, patients >6-months post-transplant	The negative predictive value ranges from 98.9% (±0.4) to 100% using a cutoff threshold of 34	Low
Biopsy-related adverse events	AlloMap vs. EMB: 2 RCTs	Inconclusive: Too few biopsy-related adverse events assessed	Very low

*Note: We grade strength of evidence based on the concepts and methods proposed by the GRADE working group. Please see Appendix A for details.

**In this study, patients were enrolled an average of 50–53-days post-transplant, and outcomes were reported at 18-months post-transplant.

†In this study, the majority of patients (85%) enrolled >1 year post-transplant and outcomes were reported 2-years postrandomization.

EMB: Endomyocardial biopsy
ISHLT: International Society for Heart and Lung Transplantation
NA: Not applicable
RCT: Randomized controlled trial

Gene Expression Test (AlloMap) for Monitoring Heart Transplant Rejection

Table of Contents

Tables

Gene Expression Test (AlloMap) for Monitoring Heart Transplant Rejection

Overview

For the purpose of ECRI Institute Genetic Test Reports, we use the 2008 definition of genetic test developed by the U.S. Department of Health and Human Services Secretary's Advisory Committee on Genetics, Health, and Society. This definition states that a genetic or genomic test involves an analysis of human chromosomes, DNA, RNA, genes, and/or gene products (e.g., enzymes, other types of proteins), which are predominantly used to detect heritable or somatic mutations, genotypes, or phenotypes related to disease and health.

Background/Disease

Rejection After Heart Tranplantation

Cardiac allograft rejection occurs when the heart recipient's immune system recognizes the transplanted heart as foreign and mounts an immune response.[1] Rejection is classified as one of four types: hyperacute, acute cellular, antibody mediated, or chronic.[2] Hyperacute rejection can occur within minutes to hours after surgery and is mediated by preexisting antibodies to donor proteins.[3] Acute cellular rejection (ACR) is the most common form of allograph rejection (occurs in 30% to 50% of patients), and most cases occur in the first three to six months after transplantation.[2] ACR episodes are primarily mediated by recipients' T cells, which cause inflammation and damage heart cells.[1] Antibody-mediated rejection occurs when B lymphocytes produce antibodies directed against the transplanted heart. Antibody-mediated rejection occurs in 15% to 20% of heart transplant recipients.[2] Clinicians do not completely understand the cause of chronic rejection. Chronic rejection occurs years after transplantation and typically involves cardiac allograft vasculopathy (thickening and narrowing of the transplanted organ's arteries) and late graft failure.[2]

To prevent rejection, heart transplant recipients receive lifelong regimens of immunosuppressive drugs. Rejection surveillance and modification of the patient's drug regimen are critical to effective patient management. Physicians diagnose ACR on the basis of clinical data and the results of EMB and tissue histopathology and assessment. Although most patients with ACR are asymptomatic, patients can experience a range of symptoms, from arrhythmias to profound heart failure. Severity of clinical signs does not always correlate with a histologic rejection grade.

Histologic grading of a tissue specimen obtained by EMB is the gold standard for ACR diagnosis. A cardiologist performs EMB by placing a catheter via a neck or groin (femoral) vein, while the patient is under local anesthesia, to gain vascular access. The cardiologist then inserts a biopsy device through the right atrium and the tricuspid valve into the right ventricle. Several samples of right ventricular tissue are sequentially collected for histologic examination. The patient usually remains sedentary for one to four hours after the procedure and is observed for bleeding and other complications. Usually, the patient can resume normal activities the following day. Although surveillance protocols vary among transplant centers, patients typically undergo EMB as frequently as weekly during the first two-months post-transplant, as frequently as once or twice a month during the remainder of the first year, and then less frequently (e.g., annually). EMB is also required whenever clinical signs of rejection emerge.

Pathologically, ACR is recognized on the basis of predominantly lymphocytic infiltrates with varying degrees of cardiomyocyte degeneration in endomyocardial tissue. The International Society for Heart and Lung Transplantation (ISHLT) has established an acute rejection grading system as follows:[4]

- Grade 0 R: No rejection
- Grade 1 R: Mild. Interstitial and/or perivascular infiltrate with up to one focus of myocyte damage (previously Grades I A, I B and II)
- Grade 2 R: Moderate. Two or more foci of infiltrates with associated myocyte damage (previously Grade III A)
- Grade 3 R: Severe. Diffuse infiltrates with multifocal myocyte damage, with or without edema, hemorrhage and vasculitis (previously Grades III B and IV)

Clinicians typically modify doses and/or types of immunosuppressive agents for patients with grades 2R and 3R rejection.[5]

EMB is associated with rare but serious complications, such as perforation/pericardial tamponade, tricuspid valve damage, bleeding, pneumothorax, arrhythmias, coronary artery-right ventricular fistulas, and death;[4] thus, less invasive options are desired. Additionally, some heart transplant patients experience "biopsy negative rejection," in which patients have unremarkable EMB results. Therefore, EMB and histopathologic analysis does not identify all cases of transplant rejection.[6]

According to the Organ Procurement & Transplantation Network, 2,035 heart transplants were performed in the United States in 2012, the most recent year from which data are available.[7] Worldwide, approximately 5,935 heart transplants occurred in 2012.[8]

Genetic Test Description: AlloMap

The AlloMap test (CareDx, Inc., Brisbane, CA, USA) is intended as a noninvasive monitoring approach to aid identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe ACR at the time of testing. Clinicians use AlloMap in conjunction with standard clinical assessment.[9] The test is not intended for patients exhibiting clinical symptoms of rejection; symptomatic patients would undergo EMB.

Although clinical protocols vary, patients usually undergo gene-expression assessment in lieu of EMB as frequently as monthly during the first year post-transplant and then less frequently (e.g., annually). Before gene expression testing, transplant clinicians obtain a thorough history, perform a complete physical examination, and may use echocardiography to evaluate allograft function. A phlebotomist obtains the venous blood sample from the arm, and a technician then performs the preparation and lysis of peripheral blood mononuclear cells according to the specifications outlined in the CareDx Laboratory Services Guide.[9]

The AlloMap test employs a quantitative real-time reverse transcriptase polymerase chain reaction. CareDx laboratory personnel receive preparations from peripheral blood mononuclear cells, extract and purify messenger ribonucleic acid (mRNA), and perform reverse transcription and amplification. The test measures the expression levels of 20 genes; 11 are informative genes, and 9 genes are used for internal quality control and normalization. The assay includes internal quality controls and mRNA quality assessments. The final result is based on an algorithm composed of the weighted expression levels of analyzed genes and is reported as a single score.[10]

Results for the AlloMap test are usually available within one to two days of sample receipt by CareDx. Reported scores range from 0 to 40. Low AlloMap scores are associated with a greater probability that the patient is free from ACR. Each transplant center must select its own AlloMap threshold for recommending follow-up EMB based on clinical experience and desired negative predictive value (NPV).[11] Published clinical trials used the cutoff score of 30 for patients fewer than 6-months post-transplant and 30 or 34 for patients more than 6-months post-transplant.[12,13]

Intended Benefits and Potential Disadvantages

Because AlloMap replaces EMB for monitoring heart transplant ACR, patients whose results indicate low risk of ACR may reduce the number of EMBs required post-transplant.[9] Potential disadvantages of AlloMap include possible false test results. False-positive results could result in unnecessary EMBs and associated risks. False-negative results could delay an additional evaluation and treatment for heart transplant rejection. Additionally, compared with EMB that detects ACR and antibody-mediated rejection, AlloMap assesses only the risk of ACR.

Gene Expression Detected by AlloMap

AlloMap uses an algorithm that incorporates expression data from 20 genes, 11 that correlate with patient rejection status and 9 used for normalization and quality control.[14] The 11 genes that correlate with patient rejection were identified from patient samples from a cohort study of heart transplant patients (CARGO trials) and supplemented with genes implicated in transplant rejection in the clinical literature. The gene candidates were further selected and incorporated into a final algorithm using bioinformatics. The genes for which expression levels are determined by the AlloMap test and used to produce an AlloMap test score are found in Table 1. According to CareDx, "Many of the AlloMap test genes are associated with various biological pathways involved in the rejection process."[15]

Table 1. Genes Included in the AlloMap Test Panel

Role in ACR	Gene Name (Protein Name)	Proposed Protein Mechanism/Notes	Expression Change during Rejection
T cell priming	ITGA4 (Integrin alpha-4)	Produces protein used by T cells to infiltrate allograft heart tissue	Increased
	PDCD1 (Programmed cell death protein 1)	Expressed in circulating antigen-specific T cells during active immune response	Increased
Proliferation and mobilization of red blood cells	MARCH8 (Cellular mediator of immune response)	Expressed in immature red blood cells	Increased
	WDR40A (WD repeat domain 40A)	Expressed in immature red blood cells	Increased
Platelet activation	PF4 (Platelet factor 4)	Expressed in blood platelets	Decreased
	C6orf25 (G6b inhibitory receptor)	Expressed in blood platelets	Decreased
Steroid response	IL1R2 (Interleukin-1 receptor type II)	Steroid expression dependent inhibitor of cytokine signaling	Decreased
	ITGAM (Integrin alpha-M)	Involved in cell trafficking	Decreased
	FLT3 (FMS-like tyrosine kinase)	Expressed in monocytes, involved in signaling	Decreased
Unknown	SEMA7A (Semaphorin 7A)	Expressed by T cells, B cells, and immature granulocytes	Increased
	RHOU (Ras homolog gene family, member U)	Involved in the modulation of cytoskeleton organization	Increased

ACR: Acute cellular rejection

Costs

The list price for a single AlloMap test is $3,600.[16]

Cost-effectiveness and Considerations

The cost and cost-effectiveness of using AlloMap compared with EMB depend on each transplant center's surveillance strategy. ECRI Institute searches identified two cost-effectiveness studies. Evans et al. 2005 compared EMB and AlloMap testing costs at five cardiac transplant centers that participated in the CARGO study.[17] The authors assumed that AlloMap testing would replace routine EMB and patients would undergo eight tests in the first post-transplant year and three in years two to five. The comparison also accounted for follow-up biopsies. The authors reported per-patient savings to hospitals of $3,741 over five years of management, savings to Medicare of $4,193, and savings to private insurers of $6,511.

The second cost-effectiveness study, Heidenreich et al. 2010,[18] published an abstract of an economic impact analysis of AlloMap presented at the Heart Failure Society of America 2010 Scientific Meeting. We include this abstract because it is the only published cost-analysis of AlloMap using data from the IMAGE trial. The authors tracked the costs (including intensive care unit [ICU] and non-ICU hospital days, outpatient visits, emergency department visits, cardiac procedures, and medications) for the group monitored with AlloMap and the group monitored with EMB. The costs were determined using average U.S. payer prices or wholesale prices. The authors used bootstrap analysis to determine that the 95% confidence interval for the difference in cost between the EMB and AlloMap group was -$7,446 to $3,344. The authors concluded: "Cost of care is similar for post-transplant surveillance strategies using gene expression profiling or biopsy."

Reimbursement

ECRI Institute provides the following as reference and for information purposes only. Coding, coverage, and reimbursement information provided does not constitute legal advice and does not guarantee payment.

Coverage

The U.S. Centers for Medicare & Medicaid Services (CMS) has no national coverage determination for gene expression testing to monitor heart transplant rejection. Thus, coverage decisions are left to the discretion of local Medicare carriers. According to CareDx and confirmed by ECRI Institute, the following local Medicare carriers have specific policies describing coverage for AlloMap: Noridian in California, Montana, and Arizona, Palmetto in South Carolina, and CGS in Kentucky.[19-23]

Our searches of 11 representative, private, third-party payers that provide online medical coverage policies (Aetna, Anthem, Blue Cross/Blue Shield [BC/BS] of Alabama, BC/BS of Massachusetts, CIGNA, HealthPartners, Humana, Medica, Regence, United Healthcare, Wellmark) found 6 payers with a policy describing coverage with conditions, 3 payers that deny coverage, and two payers that have no specific policy. See Table 2 for details.

Table 2. Third-party Payer Policies

Payer	Policy Name	Date of Last Review	Coverage Policy
Aetna[24]	Heart Transplantation	8/7/2015	"Aetna considers the Allomap gene expression profile medically necessary for monitoring rejection in heart transplant recipients more than six months post-heart transplant. "Aetna considers the Allomap gene expression profile experimental and investigational for all other indications because its clinical value has not been established."
Anthem[25]	Laboratory Testing as an Aid in the Diagnosis of Heart Transplant Rejection	5/15/2014	"AlloMap molecular expression testing is considered medically necessary as a non-invasive method of determining the risk of rejection in heart transplant recipients between 1 and 5 years post-transplant. "AlloMap molecular expression testing is considered investigational and not medically necessary when the criteria above are not met."
Blue Cross Blue Shield of Alabama[26]	Heart Transplant Rejection Laboratory Testing	5/2015	"The use of peripheral blood genetic profiling tests in the management of patients post-heart transplantation, including, but not limited to the detection of acute heart transplant rejection or heart transplant graft dysfunction does not meet Blue Cross and Blue Shield of Alabama's medical criteria for coverage and is considered investigational."
Blue Cross Blue Shield of Massachusetts[27]	Laboratory Tests for Heart Transplant Rejection	7/2014	"The use of peripheral blood genetic profiling tests in the management of patients after heart transplantation, including but not limited to the detection of acute heart transplant rejection or heart transplant graft dysfunction, is investigational."
CIGNA[28]	Genetic Expression Profiles for Detection of Heart Transplantation Rejection (e.g., AlloMap)	2/15/2015	"Covers genetic expression profile (i.e., AlloMap) in lieu of endomyocardial biopsy as medically necessary when all of the following criteria are met: • result will be used to determine the need for subsequent endomyocardial biopsy to clarify rejection status • age 15 years or older • six months to five years post-heart transplantation • 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 acute cellular rejection as demonstrated by both of the following:

Payer	Policy Name	Date of Last Review	Coverage Policy
			• International Society for Heart and Lung Transplantation [ISHLT] rejection status Grade 0R or 1R on all previous endomyocardial biopsies • no history or evidence of antibody mediated rejection • no history of elevated genetic expression profile (i.e., AlloMap) that prompted subsequent endomyocardial biopsy to clarify rejection status • Cigna does not cover genetic expression profile (i.e., AlloMap) for any other indication because it is considered experimental, investigational, or unproven."
Humana[29]	Molecular Diagnostic Assay and Breath Testing for Transplant Rejection	2/26/2015	"Humana members may be eligible under the Plan for gene expression profiling (e.g., AlloMap) for heart transplant recipients who are between one and five years post-transplant."
Medica[30]	Laboratory Tests	1/1/2013	Medica covers laboratory tests when it has been reviewed by Medica and considered a published service, or when it meets Medica's definition of a standard laboratory test and has been ordered and submitted under the direction of a physician. AlloMap may satisfy these requirements and therefore, may be a required test.
Regence[31]	Laboratory Tests for Heart Transplant Rejection	5/2014	"The use of peripheral blood genetic profiling tests in the management of patients after heart transplantation, including but not limited to the detection of acute heart transplant rejection or heart transplant graft dysfunction, is considered investigational."
UnitedHealthcare[32]	Molecular Pathology/Molecular Diagnostics/Genetic Testing	1/28/2015	This reimbursement policy lists the AlloMap test.

Coding

The American Medical Association has assigned a specific Current Procedural Terminology (CPT) code to describe AlloMap effective January 1, 2016. Until that time, users may describe AlloMap using the following CPT codes:[33]

- Unlisted molecular pathology procedure
- Unlisted multianalyte assay procedure with algorithmic analysis
- Unlisted immunology procedure

Submission of a claim with an unlisted code typically requires a paper claim, a detailed procedure note, and a cover letter to the health plan/payer. The cover letter should contain an explanation of the procedure, the patient selection, the medical necessity, and the clinical benefits as well as identify a comparable procedure to assist the insurer in establishing a payment level. Third-party payers do not universally accept unlisted codes.

Payment

Pathology and laboratory procedures performed in the United States are reimbursed based on the Clinical Laboratory Fee Schedule. According to CMS, "the payment is the lesser of the amount billed, the local fee for a geographic area, or a national limit."[34]

The payment rate for this test has not yet been established.[35]

Regulatory Status

United States

In August 2008, the U.S. Food and Drug Administration (FDA) granted 510(k) clearance for the AlloMap Molecular Expression Test.[36]

Other Countries

In April 2011, AlloMap received the CE mark permitting distribution in the European Union under the In Vitro Diagnostics Directive.[37]

In 2013, CML Healthcare (Mississauga, Ontario, Canada) licensed the exclusive right to market AlloMap in Canada.[38]

In 2015, Diaxonhit, the exclusive distributor of AlloMap in Europe, "signed a service sublicensing agreement with the Strasbourg University Hospitals to perform all AlloMap testing at a dedicated facility in Strasbourg, France."[39]

Clinical Laboratory Improvements Amendments

Medicare regulates the XDx Reference Laboratory (owned and operated by CareDx), the laboratory that performs AlloMap, through the Clinical Laboratory Improvement Amendments (CLIA) of 1988. CLIA tests are classified into categories according to test complexity: waived, moderate, or high complexity. AlloMap is classified as a high-complexity test.

The XDx Reference Laboratory (CLIA number 05D1029609) has received a CLIA certificate of accreditation and is accredited by the College of American Pathologists. The XDx Reference Laboratory holds clinical laboratory licenses for the following states that require licensure: California, Florida, Maryland, New York, and Pennsylvania.[40]

Clinical Guidelines and Standards

ECRI Institute searches identified the following two relevant guidelines.

The Association for European Cardiovascular Pathology and the Society for Cardiovascular Pathology published a consensus statement on endomyocardial biopsy in 2011.[41] The guideline states,

> The use of gene expression profiling in which peripheral blood specimens are analyzed is the most recent challenge to the EMB for rejection surveillance. However, the recent Invasive Monitoring Attenuation through Gene Expression trial was limited to recipients more than 6 months post-transplant (when rejection frequency is lower) with an endpoint of no increased risk of serious clinical outcome (not the number of rejection episodes detected), confirming that, to date, the EMB remains the 'gold standard' for diagnosis of acute allograft rejection.

The International Society of Heart and Lung Transplantation (ISHLT) published a guideline titled *Guidelines for the Care of Heart Transplant Recipients Task Force 2: Immunosuppression and Rejection* in 2010. This guideline recommends the following, "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 6 months and 5 years after heart transplantation." ISHLT rated this recommendation class IIa (weight of evidence is in favor of this test), evidence level B (i.e., evidence is based on results of a randomized controlled trial [RCT]).[42]

Evidence Reports Published by Other Health Technology Assessment Organizations

Our searches identified two relevant evidence reports. Both of these reports were published before publication of the most recent study, the 2015 EIMAGE trial:

- California Technology Assessment Forum: *Gene Expression Profiling for the Diagnosis of Heart Transplant Rejection*. 2010. The authors concluded that the test had a high negative but a low positive predictive value (PPV). The Invasive Monitoring Attenuation through Gene Expression (IMAGE) trial supported noninferiority of the AlloMap test to EMB in the late postoperative period (i.e., one-year post-transplant). More clinical trials would be needed to explore AlloMap's utility during the early postoperative period when the majority of EMBs are performed and when most acute rejection episodes occur.[43]
- Blue Cross/Blue Shield Association Technology Evaluation Center: *Gene Expression Profiling as a Noninvasive Method for Cardiac Allograft Rejection*. 2011. This report concluded, "The evidence is insufficient to make conclusions regarding whether AlloMap testing either improves the net health outcome or is as beneficial as any established alternatives for the monitoring of heart transplant patients."[44]

Evidence Review

This report is intended to focus on literature addressing the clinical validity and utility of gene expression testing for monitoring heart transplant rejection. We use the following definitions:

- Clinical validity: a test's ability to accurately and reliably predict the clinically defined disorder or phenotype of interest.
- Clinical utility: a test's ability to improve measureable clinical outcomes and its usefulness and added value to patient management decision-making compared with current management without genetic testing.

This report focuses on heart transplant recipients 15 years of age or older who are at least 2-months post-transplant. For the current technology assessment, the appropriate comparator for AlloMap is EMB. The outcomes of interest for clinical validity are sensitivity, specificity, PPV, NPV, and area under the receiver operating characteristic curve (AUC ROC). The outcomes of interest for clinical utility are mortality (all-cause and cardiac related), rejection, allograft dysfunction, number of EMBs, rejection episode detection, adverse events (AEs), patient satisfaction, and quality of life.

Methods

In June 2015, we searched MEDLINE, EMBASE, the Cochrane Library, CINAHL, and PubMed to identify relevant studies. See *Search Strategy* section for keywords and subject headings used in this search. We further retrieved relevant information via review of bibliographies/reference lists from peer-reviewed and gray literature. Gray literature consists of reports, studies, articles, and monographs produced by government agencies, private organizations, educational facilities, consulting firms, and corporations.

Study Selection Criteria

ECRI Institute applied the following study-selection criteria to identify appropriate studies that assess the clinical validity and clinical utility of AlloMap.

- Study must be published in English.
- Study must be reported as a peer-reviewed, full-length article. We excluded abstracts and meeting presentations because they do not give complete results and sufficient detail about methodology to assess the risk of bias, and final results may differ from preliminary results.
- To avoid double counting of patient outcomes, if more than one article has been published to describe the same study, the article must be the latest published report or have the most complete report of an outcome.
- Comparative studies must assess at least 10 patients in each arm, and cohort studies must assess at least 100 patients. Smaller studies are at greater risk of patient-selection bias and often are not statistically reliable.
- To address clinical validity, we include diagnostic cohort studies.
- To address clinical utility, we include RCTs and nonrandomized comparative studies.

Included Studies

We identified three studies that assessed the clinical validity and clinical utility of AlloMap. See Table 3.

Table 3. Included Studies

Author/Year	Study Design/Objective Patient Population (n Per Group)	Key Outcomes and Follow-up Times	Addresses
Kobashigawa et al. 2015[12] EIMAGE study	Single-center RCT to compare AlloMap (n = 30) to EMB (n = 30) for rejection surveillance starting 55 days post-transplant	Death/retransplant, rejection with hemodynamic compromise, allograft dysfunction, rejection episode detection, number of EMBs biopsy-related AEs, quality of life, patient satisfaction 2, 3, 4, 5, 6, 8, 10, and 12 months	Clinical utility
Pham et al. 2010[13] IMAGE trial	Multicenter RCT comparing AlloMap (n = 297) to EMB (n = 305) for rejection surveillance. Greater than 85% of patients were at least 1-year post-transplant	Death/retransplant, rejection with hemodynamic compromise, allograft dysfunction; number of EMBs, rejection episode detection, biopsy-related AEs, quality of life, patient satisfaction Monitoring with EMB or AlloMap was performed at prespecified intervals determined by each of 13 centers	Clinical utility
FDA 510(k) Substantial Equivalence Determination Decision Summary[45] 2008 CARGO trial	Multicenter cohort study (n = 629) intended to identify candidate genes for a gene expression analysis test (AlloMap using microarray analysis, develop a PCR assay based on the candidate genes (including algorithm development), and validate the final algorithm Analysis included: 300 samples from 154 patients enrolled in CARGO trial that were not used to develop the test algorithm	Sensitivity, specificity, NPV, PPV, AUC ROC	Clinical validity
Starling et al.[46] 2006 Early-post CARGO	"Pooled clinical data from several transplant centers, follow-up date March 31, 2006." 211 samples from an unknown number of patients more than 12-months-post transplant	Sensitivity, specificity, NPV, PPV	Clinical validity

AEs Adverse events
AUC ROC: Area under the receiver operating characteristic curve
EMB: Endomyocardial biopsy
PPV: Positive predictive value
NPV: Negative predictive value
PCR: Polymerase chain reaction
RCT: Randomized controlled trial

Strength-of-evidence Assessment

We graded strength of evidence (SOE) for selected patient outcomes. Our grading approach is based on the concepts and methods proposed by the GRADE working group. We also incorporated the evidence assessment methods used by the Agency for Healthcare Research and Quality's Evidence-based Practice Centers. Our grading approach addressed risk of bias, consistency, directness, precision, magnitude of effect, dose-response gradient, and plausible confounders that would reduce a demonstrated effect. We assigned an evidence grade of "high," "moderate," "low," or "very low" for each selected outcome. The definitions of these evidence grades and more detailed description of the grading methods are provided in Appendix A.

Findings

Clinical Validity

We present the clinical validity results from the CARGO trial as reported in the FDA 510(k) Substantial Equivalence Determination Decision Summary[45] and Starling et al.[46] (early-post CARGO clinical data). Although the clinical validity of AlloMap from the CARGO trial has been reported in more than one published article,[10,46] the FDA decision summary provides the most recent analysis of the data. The CARGO results as presented in the FDA decision summary are also

presented in Pham et al. as context for the IMAGE trial.[13] A list of clinical validity outcomes (i.e., sensitivity, specificity, NPV, PPV, AUC ROC) from the CARGO trial not included here can be found in Appendix D.

The FDA decision summary[45] reports clinical validity from two cohorts of patients: one group two- to six-months post-transplant and one group at least six-months post-transplant. We present outcomes from these cohorts separately because the prevalence of rejection is higher before six-months post-transplant, which can affect predictive values, and because AlloMap score increases with time after transplant. Consequently, some clinicians choose a higher cutoff threshold for determining AlloMap test result in patients after six-months follow-up.

The FDA decision summary reports an NPV of 98.6% (standard error 0.4%) and PPV of 4.6% (standard error 1.6%) for patients 2- to 6-months post-transplant (n = 166), using a cutoff threshold value of 30. The reported AUC for this patient cohort was 0.71, and the authors calculated a 95% confidence interval using bootstrap methods to be 0.54 to 0.84. The FDA document does not report the raw patient sample results or AlloMap's sensitivity and specificity. ECRI Institute could not determine AlloMap's sensitivity and specificity for this patient cohort because the FDA decision summary did not contain enough information.

The FDA decision summary[45] (n = 134) and Starling et al.[46] (n = 211) report on AlloMap's clinical validity in patients more than 6-months post-transplant. See Table 4.

Table 4. Clinical Validity for Patients >6-Months Post-transplant

Author/Year Cutoff threshold	Sensitivity, %	Specificity, %	PPV, % (Standard error)	NPV, % (Standard error)
FDA 510(k) Decision Summary[45] 2008 30	NR	NR	2.1 (0.6)	98.7 (0.6)
FDA 510(k) Decision Summary[45] 2008 34	NR	NR	4.1 (1.7)	98.9 (0.4)
Starling et al.[46] 2006 34	100	71.4	7.8	100

NPV: Negative predictive value
NR: Not reported
PPV: Positive predictive value

Table 5 presents the sample outcomes from Starling et al.

Table 5. Test Results from Starling et al. for Patients, >12-Months Post-transplant, Threshold 34

	Endomyocardial Biopsy (ISHLT grade)	
AlloMap Result	**≥2R (acute rejection)**	**<2R (quiescence)**
Positive	5	59
Negative	0	147
Total	5	206

Clinical Utility

We identified two studies (Kobashigawa et al.[12] and Pham et al.[13]) that provided data to address AlloMap's clinical utility.

Death, Retransplant, Rejection with Hemodynamic Compromise, Graft Dysfunction

Kobashigawa et al.[12] and Pham et al.[13] reported a composite outcome, including death, retransplant, rejection with hemodynamic compromise, and graft dysfunction. Hemodynamic compromise was defined as "the presence of ≥1 of the following: an absolute echocardiographic LVEF ≤40% or a proportional drop in LVEF of ≥25% compared with the baseline

value at visit 1, a cardiac index <2L/min/m^2, plus the use of inotropic drugs to support circulation." The authors defined allograft dysfunction as "hemodynamic compromise without histologically confirmed rejection."

Kobashigawa et al.[12] reported the incidence of this composite outcome at 18-months post-transplant was not significantly different between the AlloMap group (3/30, 10%) and the EMB group (5/30, 17%; log-rank p = 0.44). See Table 6.

Table 6. Death, Rejection, and Graft Dysfunction from Kobashigawa et al.

Event	AlloMap Group n = 30 [# Events]	EMB Group n = 30 [# Events]
Death or retransplantation	0	0
Rejection with hemodynamic compromise as first event	2	1
Graft dysfunction as first event	1	4

EMB: Endomyocardial biopsy

Pham et al.[13] reported no statistically significant difference between the AlloMap and EMB groups in the composite outcome (log-rank p = 0.86) or any of the components of the composite outcome at two-year post-study enrollment. See Table 7 for details.

Table 7. Death, Rejection, and Graft Dysfunction from Pham et al.

Event	AlloMap Group (n = 297) # Events	EMB Group (n = 305) # Events	p value
Death at any time (all causes, first event, and subsequent event)	13	12	0.82
Death at any time (cardiovascular causes, first event, and subsequent event)	8	9	NR
Death as first event (all causes)	11	6	0.23
Death as first event (cardiovascular causes)	7	5	NR
Rejection with hemodynamic compromise as first event	11	13	>0.99
Graft dysfunction not caused by rejection as first event	11	14	0.68

NR: Not reported

Rejection Episode Detection

Kobashigawa et al.[12] reported no significant difference in the number of rejection episodes between the AlloMap (3/30, 10%) and EMB groups (1/30, 3.3%, log-rank p = 0.31). Of the three detected rejection episodes in the AlloMap group, 1/30 (3.3%) was detected as a consequence of an elevated AlloMap score, which prompted follow-up EMB. The authors do not report whether the single case of rejection in the EMB group was detected because of routine or clinically driven EMB.

Pham et al.[13] reported no statistically significant difference in the number of rejection episodes between the AlloMap group (34/297, 11.4%) and the EMB group (47/305, 15.4%). In the AlloMap group, 6/34 (17.6%) rejections were detected because of follow-up EMB after a high AlloMap score. In the EMB group, 22/47 (46.8%) rejections were detected because of surveillance EMB.

Number of Endomyocardial Biopsies

Kobashigawa et al.[12] reported 253 EMBs were performed in the EMB group (8.4 EMBs/patient) compared with 42 EMBs in the AlloMap group (1.4 EMBs/patient). Of the 42 EMBs performed in the AlloMap group, 29 (69.0%) were performed because of an elevated AlloMap score (≥30 for patients fewer than 6-months post-transplant and ≥34 for patients more than 6-months post-transplant).

Pham et al.[13] reported fewer EMBs were performed in the AlloMap group (409, median 0.5 EMBs/year [range 0 to 15.9]) compared with the EMB group (1,249, median 3.0 EMBs/year [range 0 to 22.1]), and the difference was statistically significant (p <0.0001). Of the 409 EMBs performed in the AlloMap group, 274 (67.0%) were performed because of an elevated AlloMap score.

Quality of Life/Patient Satisfaction

Study authors assessed quality of life using the Short-Form-12 Health Survey (SF-12). The SF-12 is a reliable and validated abbreviation of the Short-Form-36 Health Survey. The results from the SF-12 are a physical health composite score and a mental health composite score that range from 0 (the lowest level of health) to 100 (the highest level of health.)[47]

Kobashigawa et al.[12] reported no statistically significant difference between the AlloMap group and EMB group for the SF-12 mental health composite score (p = 0.75) or the physical health composite score (p = 0.564) at 1-year follow-up. Authors presented the SF-12 scores as a box plot but did not report the score means or standard deviations as numerical values.

Pham et al.[13] assessed quality of life using the SF-12 at one- and two-years post-enrollment. At 1-year postenrollment, authors reported no difference in the mental health summary score between the 2 groups (AlloMap = 50.3 ±10.8 versus EMB = 51.7 ±9.7; p = 0.23), but the physical health summary score of the AlloMap group was lower than that of the EMB group (44.7 ±11.4 versus 47.3 ±9.6), and the difference was statistically significant (p = 0.03). At 2-years postenrollment, authors reported no difference between the AlloMap group and EMB group for either the mental health summary score (AlloMap = 50.8 ±10.1 versus EMB = 50.7 ±9.8; p = 0.66) or the physical health summary score (AlloMap = 45.1 ±11.6 versus EMB = 46.2 ±10.9; p = 0.52).

Kobashigawa et al.[12] and Pham et al.[13] assessed patient satisfaction using an unvalidated patient satisfaction survey. Therefore, we do not report these findings because the interpretation of differences between and/or within groups is unclear. See the *Discussion* section for more information.

Biopsy-related Adverse Events and Complications

Kobashigawa et al.[12] reported no statistically significant difference in the rate of EMB-related complications in the EMB group (0/42, 0%) compared with the EMB group (2/253, 0.8%; p = 0.563).

Pham et al.[13] reported no statistically significant difference in the rate of EMB-related complications in the AlloMap group (1/409, 0.24%) compared with the EMB group (4/1,249, 0.32%; p = 0.81).

Ongoing Clinical Trials

ECRI Institute searches identified one relevant ongoing trial. The prospective cohort study, titled Outcomes AlloMap Registry Study: the Clinical Longterm Management and Outcomes of Heart Transplant Recipients with Regular Rejection Surveillance Including Use of AlloMap Gene-expression Profile testing (NCT01833195), is intended to "observe short and long term clinical outcomes in heart transplant recipient who receive regular AlloMap testing as part of allograft rejection surveillance." The study investigators plan to enroll 2,000 heart transplant patients. The primary endpoints include vital status, number of hospitalizations, and their causes. The study is being conducted at Allegheny General Hospital (Pittsburg, PA, USA) and Aurora St. Luke's Medical Center (Milwaukee, WI, USA) and will be completed in December 2018.

Discussion

Overall, the studies assessing the clinical validity of AlloMap (CARGO and early post-CARGO) do not provide sufficient detail about their patient populations, which prevents full assessment of study quality. Although publications of the CARGO study report PPVs and NPVs, we were unable to determine the sensitivity and specificity of AlloMap from the reported results because we could not account for patients' rejection status and test outcomes with the information in the study reports. However, AlloMap's reported NPV is high, and the sensitivity reported from the early post-CARGO (Starling et al.) data is high, despite this study's high risk of bias. Typically, clinicians consider tests with high sensitivity and NPV as good "rule-out" tests in the clinical setting.[48] However, some clinicians may consider AlloMap's AUC ROC to be low for use in clinical practice.[49]

Before the publication by Kobashigawa et al., clinicians expressed concern that the clinical utility outcomes in Pham et al., in which 85% of patients were more than one-year post-transplant, may not apply to patients less than one-year post-transplant.[50,51] This was a reasonable concern because the risk of acute cellular rejection is highest in the first three- to six-months post-transplant.[2] Although Kobashigawa et al. enrolled relatively few patients, the majority were less than one-year post-transplant, and the clinical utility results from the two studies are generally consistent. Ideally, researchers would confirm these results in a larger follow-up study.

A possible advantage of the AlloMap test compared with EMB that may be difficult to address in clinical studies is patient preference. Generally, patients with heart transplants find EMBs to be undesirable and anxiety producing, and some may even find them uncomfortable or painful.[50] Pham et al. and Kobashigawa et al. attempted to capture this outcome by asking patients, "How satisfied were you with the current method of detecting rejection?" Both studies found that patients monitored with AlloMap reported higher levels of satisfaction than those monitored with EMB, and the difference was statistically significant. However, because this method of determining patient satisfaction is unvalidated, its real-world implications are unclear.

An additional challenge in determining how AlloMap fits into the clinical context for monitoring rejection in heart transplant patients is the controversy over rejection surveillance protocols. As of August 2015, no published guideline establishes a recommended schedule for EMB or AlloMap testing post-heart-transplant, although guidelines do recommend follow-up monitoring with EMB.[41,52] As a result, post-transplant surveillance varies among heart transplant centers. Some clinicians have reported that the diagnostic yield for surveillance EMB has decreased since the routine use of mycophenolate mofetil for immunosuppression circa 2000.[53] This observation has led some heart transplant centers to discontinue surveillance EMBs for low-risk patients as early as 6 months after transplant, but more commonly at 12-months post-transplant.[51] This changing landscape may affect the generalizability of the outcomes from studies of AlloMap's clinical utility.

Genetic Test Significance: Evidence-base Conclusions and ECRI Institute Opinion

Typically, clinicians consider tests with high sensitivity and high NPV as good "rule-out" tests in the clinical setting. The evidence base assessing AlloMap suggests it has high NPV in patients more than six-months post-transplant, but deficiencies in the data prevent us from determining its NPV fewer than six-months post-transplant or its sensitivity at any time post-transplant.

We are unable to determine AlloMap's NPV for the absence of acute rejection (ISHLT Grade <2R) in patients two- to six-months post-transplant because the only published study assessing this outcome has a high risk of bias. Strength of evidence: Very low.

AlloMap's NPV for the absence of acute cellular rejection in patients more than six-months post-transplant ranges from 98.9% to 100%. Strength of evidence: Low.

We are unable to determine AlloMap's sensitivity for detecting acute cellular rejection (ISHLT Grade ≥2R) for patients less than one-year post-transplant because study authors reported insufficient data. Strength of evidence: Very low.

We are unable to determine AlloMap's sensitivity for detecting acute cellular rejection for patients more than one-year post-transplant because the only published study assessing this outcome has a high risk of bias. Strength of evidence: Very low.

We were unable to determine AlloMap's influence on mortality and biopsy-related AEs because the studies assessing these outcomes enrolled too few patients, resulting in few occurrences. Strength of evidence: Very low.

We were unable to compare the quality of life in patients monitored with AlloMap with those monitored with EMB because published studies reported inconsistent results. Strength of Evidence: Very low.

References

1. Maleszewski JJ. Heart transplant rejection pathology. In: Medscape Reference [internet]. New York (NY): WebMD, LLC; 2014 Feb 12 [accessed 2015 May 25]. [7 p]. Available: http://emedicine.medscape.com/article/1612493-overview.

2. Pham MX, Berry GJ, Hunt CA. Chapter 29. Cardiac transplantation. In: Fuster V, Walsh RA, Harrington RA, editors. Hurst's the heart. 13th ed. [internet]. New York (NY): McGraw-Hill; 2011 [accessed 2015 Mar 25]. p. 40 p. Available: http://accessmedicine.mhmedical.com/content.aspx?bookid=376&Sectionid=40279756.

3. Malhotra P, Malu S, Kapur S. Immunology of transplant rejection. In: Medscape Reference [internet]. New York (NY): WebMD, LLC; 2013 Apr 16 [accessed 2015 May 25]. [7 p]. Available: http://emedicine.medscape.com/article/432209-overview.

4. Kilo J, Laufer G, Antretter H. Endomyocardial biopsy â€“ jugular/subclavian vein approach. Multimed Man Cardiothorac Surg. 2006 Jan 1;2006(1110):1-6. Also available: http://mmcts.ctsnetjournals.org/cgi/reprint/2006/1110/mmcts.2005.001149.pdf.

5. Yamani MH, Taylor DO, Haire C, Smedira N, Starling RC. Post-transplant ischemic injury is associated with up-regulated AlloMap gene expression. Clin Transplant. 2007 Jul-Aug;21(4):523-5. PMID: 17645713

6. Bhalodolia R, Cortese C, Graham M, Hauptman PJ. Fulminant acute cellular rejection with negative findings on endomyocardial biopsy. J Heart Lung Transplant. 2006 Aug;25(8):989-92. PMID: 16890123

7. Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, Dobbels F, Goldfarb SB, Levvey BJ, Meiser B, Yusen RD, Stehlik J, International Society of Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report--2014; focus theme: retransplantation. J Heart Lung Transplant. 2014 Oct;33(10):996-1008. Also available: http://dx.doi.org/10.1016/j.healun.2014.08.003. PMID: 25242124

8. Recent facts & figures. In: Fifth Journalist Workshop on Organ Donation and Transplantation; 2014 Nov 26; Brussels (Belgium). p. 9.

9. XDx laboratory services guide: AlloMap HTx molecular expression testing. Brisbane (CA): XDx, Inc.; 2008. 24 p.

10. Deng MC, Eisen HJ, Mehra MR, Billingham M, Marboe CC, Berry G, Kobashigawa J, Johnson FL, Starling RC, Murali S, Pauly DF, Baron H, Wohlgemuth JG, Woodward RN, Klingler TM, Walther D, Lal PG, Rosenberg S, Hunt S, CARGO Investigators. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. Am J Transplant. 2006 Jan;6(1):150-60.

11. AlloMap testing overview. [internet]. Brisbane (CA): XDx, Inc.; 2009 Jan 01 [accessed 2011 Mar 24]. [1 p]. Available: http://allomap.com/healthcare-providers/testing-overview/.

12. Kobashigawa J, Patel J, Azarbal B, Kittleson M, Chang D, Czer L, Daun T, Luu M, Trento A, Cheng R, Esmailian F. Randomized pilot trial of gene expression profiling versus heart biopsy in the first year after heart transplant: early invasive monitoring attenuation through gene expression trial. Circ Heart Fail. 2015 May;8(3):557-64. Also available: http://dx.doi.org/10.1161/CIRCHEARTFAILURE.114.001658. PMID: 25697852

13. Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, Deng MC, Cappola TP, Kao A, Anderson AS, Cotts WG, Ewald GA, Baran DA, Bogaev RC, Elashoff B, Baron H, Yee J, Valantine HA. Gene-expression profiling for rejection surveillance after cardiac transplantation. New Eng J Med. 2010 May 20;362(20):1890-900. Also available: http://dx.doi.org/10.1056/NEJMoa0912965. PMID: 20413602

14. Understanding test results - AlloMap. [internet]. Brisbane (CA): CareDx, Inc.; 2014 [accessed 2015 May 24]. [3 p]. Available: http://www.allomap.com/patients-and-caregivers/understanding-test-results/.

15. The standard of care for the management of stable heart transplant patients: overview for health care professionals. Brisbane (CA): CareDx, Inc.; 2014 May. 20 p.

16. CAREDX, Inc. Quarterly Report (SEC form 10-Q). Brisbane (CA): CAREDX, Inc.; 2014 Nov 14. Item 2: management's discussion and analysis of financial condition and results of operations.

17. Evans RW, Williams GE, Baron HM, Deng MC, Eisen HJ, Hunt SA, Khan MM, Kobashigawa JA, Marton EN, Mehra MR, Mital SR. The economic implications of noninvasive molecular testing for cardiac allograft rejection. Am J Transplant. 2005 Jun;5(6):1553-8. PMID: 15888068

18. Heidenreich PA, Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, Deng MC, Cappola TP, Kao A, Anderson AS, Cotts WG, Ewald GA, Baran DA, Bogaev RC, Elashoff B, Baron H, Yee J, Valantine HA. Economic impact of monitoring heart transplant recipients with gene expression profiling to detect rejection: an IMAGE analysis. J Card Fail. 2010 Nov;16(11):911-2.

19. Local coverage article for MolDX: AlloMap billing and coding guidelines (A54363). [internet]. Baltimore (MD): Centers for Medicare & Medicaid Services (CMS); 2015 Oct 1 [accessed 2015 Oct 19]. [3 p]. Available: https://www.cms.gov/medicare-coverage-database/details/article-details.aspx?articleId=54363&ver=4&ContrId=360&ContrVer=1&Cntrctr=140&name=CGS+Administrators%2c+LLC+(18003%2c+DME+MAC)&DocStatus=Retired&CntrctrSelected=140*2&LCntrctr=140*2&bc=AgCAAAAAAAAAA%3d%3d&.

20. Local coverage article for MolDX: AlloMap billing and coding guidelines (A54365). [internet]. Baltimore (MD): Centers for Medicare & Medicaid Services (CMS); 2015 Oct 1 [accessed 2015 Oct 19]. [3 p]. Available: https://www.cms.gov/medicare-coverage-database/details/article-details.aspx?articleId=54365&ver=5&ContrId=340&ContrVer=1&Cntrctr=140&name=CGS+Administrators%2c+LLC+(18003%2c+DME+MAC)&DocStatus=Retired&CntrctrSelected=140*2&LCntrctr=140*2&bc=AgCAAAAAAAAAA%3d%3d&.

21. Local coverage article for MolDX: AlloMap billing and coding guidelines (A54366). [internet]. Baltimore (MD): Centers for Medicare & Medicaid Services (CMS); 2015 Oct 1 [accessed 2015 Oct 19]. [3 p]. Available: https://www.cms.gov/medicare-coverage-database/details/article-details.aspx?articleId=54366&ver=5&ContrId=345&ContrVer=1&Cntrctr=140&name=CGS+Administrators%2c+LLC+(18003%2c+DME+MAC)&DocStatus=Retired&CntrctrSelected=140*2&LCntrctr=140*2&bc=AgCAAAAAAAAAA%3d%3d&.

22. Local coverage article for MolDX: AlloMap billing and coding guidelines update (A53099). [internet]. Baltimore (MD): Centers for Medicare & Medicaid Services (CMS); 2015 Oct 1 [accessed 2015 Oct 19]. [4 p]. Available: https://www.cms.gov/medicare-coverage-database/details/article-details.aspx?articleId=53099&ver=9&ContrId=378&ContrVer=1&Cntrctr=140&name=CGS+Administrators%2c+LLC+(18003%2c+DME+MAC)&DocStatus=Retired&CntrctrSelected=140*2&LCntrctr=140*2&bc=AgCAAAAAAAAAA%3d%3d&.

23. Local coverage article for MolDX: AlloMap billing and coding guidelines update (A54186). [internet]. Baltimore (MD): Centers for Medicare & Medicaid Services (CMS); 2015 Oct 1 [accessed 2015 Oct 19]. [4 p]. Available: https://www.cms.gov/medicare-coverage-database/details/article-details.aspx?articleId=54186&ver=8&ContrId=228&ContrVer=2&Cntrctr=140&name=CGS+Administrators%2c+LLC+(18003%2c+DME+MAC)&DocStatus=Retired&CntrctrSelected=140*2&LCntrctr=140*2&bc=AgCAAAAAAAAAA%3d%3d&.

24. Aetna, Inc. Clinical policy bulletin: heart transplantation. Number: 0586. [internet]. Hartford (CT): Aetna, Inc.; 2015 Aug 7 [accessed 2015 Oct 19]. [43 p]. Available: http://www.aetna.com/cpb/medical/data/500_599/0586.html.

25. Anthem Blue Cross and Blue Shield. Laboratory testing as an aid in the diagnosis of heart transplant rejection. Policy #: TRANS.00025. [internet]. Indianapolis (IN): Anthem Blue Cross and Blue Shield; 2014 Jul 15 [accessed 2015 May 23]. [7 p]. Available: http://www.anthem.com/medicalpolicies/policies/mp_pw_a046259.htm.

26. BlueCross BlueShield of Alabama. Heart transplant rejection laboratory testing. Policy #: 212. East Birmingham (AL): BlueCross BlueShield of Alabama; 2015 May. 12 p. Also available: https://www.bcbsal.org/providers/policies/final/212.pdf.

27. Blue Cross Blue Shield of Massachusetts. Laboratory tests for heart transplant rejection. Policy number: 530. Boston (MA): Blue Cross Blue Shield of Massachusetts; 2014 Jul. 4 p. Also available: https://www.bluecrossma.com/.

28. CIGNA Corporation. Genetic expression profiles for detection of heart transplantation rejection (e.g., AlloMap). Coverage policy number 0465. Bloomfield (CT): CIGNA Corporation; 2015 Feb 15. 9 p. Also available: https://cignaforhcp.cigna.com/public/content/pdf/coveragePolicies/medical/mm_0465_coveragepositioncriteria_blood_tests_heart_transplantation_rejection.pdf.

29. Humana, Inc. Molecular diagnostic assays and breath testing for transplant rejection. Policy number: CLPD-0313-008. Louisville (KY): Humana, Inc.; 2015 Feb 26. 8 p. Also available: https://www.humana.com.

30. Medica. Laboratory tests. Minnetonka (MN): Medica; 2013 Jan 1. 4 p. Also available: https://www.medica.com/.

31. Regence Group. Laboratory tests for heart transplant rejection. Policy No: 51. Portland (OR): Regence Group; 2014 Aug 1. 8 p. Also available: https://www.regence.com/.

32. UnitedHealthcare, Inc. Molecular pathology/molecular diagnostics/genetic testing. Policy number GEN01252012RP. Atlanta (GA): UnitedHealthcare, Inc.; 2015 Jan 28. 17 p. Also available: https://www.unitedhealthcareonline.com/ccmcontent/ProviderII/UHC/en-US/Main%20Menu/Tools%20&%20Resources/Policies%20and%20Protocols/Medicare%20Advantage%20Reimbursement%20Policies/M/MolecularGeneticTest_01252013.pdf.

33. HCPCS Expert.com [online book]. Salt Lake City (UT): Optum360, LLC; 2015. Optum 2015 HCPCS Level II book. Available: https://www.medicalcodeexpert.com/expert/.

34. Centers for Medicare and Medicaid Services (CMS). Clinical laboratory fee schedule: overview. [internet]. Baltimore (MD): Centers for Medicare and Medicaid Services (CMS); [accessed 2011 Apr 05]. [2 p]. Available: https://www.cms.gov/ClinicalLabFeeSched/.

35. Mediregs Coding and Revenue Research Center [database online]. [accessed 2014 Mar 25]. Available: http://www.mediregs.com.

36. FDA clears test to help doctors manage heart transplant patients. [internet]. Rockville (MD): U.S. Food and Drug Administration (FDA); 2008 Aug 27 [accessed 2011 Mar 24]. [2 p]. Available: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2008/ucm116939.htm.

37. XDx announces that AlloMap has been CE marked in the European Union. [internet]. Brisbane (CA): XDx, Inc.; 2011 Apr 13 [accessed 2011 Apr 20]. [2 p]. Available: http://www.businesswire.com/news/home/20110413005772/en/XDx-Announces-AlloMap%C2%AE-CE-Marked-European-Union#.U_NYqXl0yLM.

38. CML HealthCare licenses rights to XDx's AlloMap. [internet]. New York (NY): Genomeweb LLC; 2013 Jun 20 [accessed 2015 May 24]. [2 p]. Available: https://www.genomeweb.com/clinical-genomics/cml-healthcare-licenses-rights-xdxs-allomap.

39. Strasbourg U inks deal to process CareDx AlloMap tests in Europe. [internet]. New York (NY): Genomeweb LLC; 2015 Jan 12 [accessed 2015 May 24]. [2 p]. Available: https://www.genomeweb.com/business-news/strasbourg-u-inks-deal-process-caredx-allomap-tests-europe.

40. Clinical laboratory - AlloMap. [internet]. Brisbane (CA): CareDx, Inc.; 2014 [accessed 2015 May 24]. [2 p]. Available: http://www.allomap.com/healthcare-providers/clinical-laboratory/.

41. Leone O, Veinot JP, Angelini A, Baandrup UT, Basso C, Berry G, Bruneval P, Burke M, Butany J, Calabrese F, dAmati G, Edwards WD, Fallon JT, Fishbein MC, Gallagher PJ, Halushka MK, McManus B, Pucci A, Rodriguez ER, Saffitz JE, Sheppard MN, Steenbergen C, Stone JR, Tan C, Thiene G, van der Wal AC, Winters GL. 2011 consensus statement on endomyocardial biopsy from the Association for European Cardiovascular Pathology and the Society for Cardiovascular Pathology. Cardiovasc. Pathol.. 2012 Jul-Aug;21(4):245-74. Also available: http://dx.doi.org/10.1016/j.carpath.2011.10.001. PMID: 22137237

42. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, Fedson S, Fisher P, Gonzales-Stawinski G, Martinelli L, McGiffin D, Smith J, Taylor D, Meiser B, Webber S, Baran D, Carboni M, Dengler T, Feldman D, Frigerio M, Kfoury A, Kim D, Kobashigawa J, Shullo M, Stehlik J, Teuteberg J, Uber P, Zuckermann A, Hunt S, Burch M, Bhat G, Canter C, Chinnock R, Crespo-Leiro M, Delgado R, Dobbels F, Grady K, Kao W, Lamour J, Parry G, Patel J, Pini D, Towbin J, Wolfel G, Delgado D, Eisen H, Goldberg L, Hosenpud J, Johnson M, Keogh A, Lewis C, O'Connell J, Rogers J, Ross H, Russell S, Vanhaecke J. The International Society of Heart and Lung Transplantation guidelines for the care of heart transplant recipients. J Heart Lung Transplant. 2010 Aug;29(8):914-56. PMID: 20643330

43. Tice JA. Gene expression profiling for the diagnosis of heart transplant rejection. San Francisco (CA): California Technology Assessment Forum; 2010 Oct 13. 24 p. Also available: http://ctaf.org/sites/default/files/assessments/1208_file_AlloMap_2010_W.pdf.

44. Gene expression profiling as a noninvasive method to monitor for cardiac allograft rejection. Technol Eval Cent Asses Program. 2011 Nov;26(8):1-18. Also available: http://www.bcbs.com/blueresources/tec/vols/26/26_08.pdf.

45. 510(k) substantial equivalence determination decision summary, assay and instrument combination template for AlloMap molecular expression testing. K073482. Rockville (MD): U.S. Food and Drug Administration (FDA); 16 p.

46. Starling RC, Pham M, Valantine H, Miller L, Eisen H, Rodriguez ER, Taylor DO, Yamani MH, Kobashigawa J, McCurry K, Marboe C, Mehra MR, Zuckerman A, Deng MC, Working Group on Molecular Testing in Cardiac Transplantation. Molecular testing in the management of cardiac transplant recipients: initial clinical experience. J Heart Lung Transplant. 2006 Dec;25(12):1389-95. PMID: 17178330

47. McDowell I. Measuring health: a guide to rating scales and questionnaires. 3rd ed. New York (NY): Oxford University Press, Inc.; 2006. 748 p.

48. Akobeng AK. Understanding diagnostic tests 1: sensitivity, specificity and predictive values. Acta Paediatr. 2007 Mar;96(3):338-41. Also available: http://dx.doi.org/10.1111/j.1651-2227.2006.00180.x. PMID: 17407452

49. Fan J, Upadhye S, Worster A. Understanding receiver operating characteristic (ROC) curves. CJEM. 2006 Jan;8(1):19-20. PMID: 17175625

50. Jarcho JA. Fear of rejection--monitoring the heart-transplant recipient. N Engl J Med. 2010 May 20;362(20):1932-3. Also available: http://dx.doi.org/10.1056/NEJMe1004355. PMID: 20413601

51. Mehra MR, Parameshwar J. Gene expression profiling and cardiac allograft rejection monitoring: is IMAGE just a mirage? J Heart Lung Transplant. 2010 Jun;29(6):599-602. Also available: http://dx.doi.org/10.1016/j.healun.2010.04.010. PMID: 20497885

52. Taylor D, Meiser B, Webber S, Baran D, Carboni M, Dengler T, Feldman D, Frigerio M, Kfoury A, Kim D, Kobashigawa J, Shullo M, Stehlik J, Teuteberg J, Uber P, Zuckermann A. Task Force 2: immunosuppression and rejection. In: the International Society of Heart and Lung Transplantation guidelines for the care of heart transplant recipients. J Heart Lung Transplant. 2010 Aug;29(8):926-33.

53. Shah KB, Flattery MP, Smallfield MC, Merinar G, Tang DG, Sheldon EH, Thacker LR, Kasirajan V, Cooke RH, Hess ML. Surveillance endomyocardial biopsy in the modern era produces low diagnostic yield for cardiac allograft rejection. Transplantation. 2015 Feb 20;99:E75-80. Also available: http://dx.doi.org/10.1097/TP.0000000000000615. PMID: 25706277

54. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol. 2003 Nov 10;3(1):25. Also available: http://www.biomedcentral.com/content/pdf/1471-2288-3-25.pdf. PMID: 14606960

55. Lijmer JG, Mol BW, Heisterkamp S, Bonsel GJ, Prins MH, van der Meulen JH, Bossuyt PM. Empirical evidence of design-related bias in studies of diagnostic tests. JAMA. 1999 Sep 15;282(11):1061-6. PMID: 10493205

56. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011 Oct 18;155(8):529-36. PMID: 22007046

Classifications

Technology Class

Biotechnology

Clinical Category

Decision Support, Screening

Clinical Specialty

Cardiovascular Medicine, Clinical Laboratory, Surgery, Surgery-Cardiothoracic Surgery, Transplantation

UMDNS

Molecular Microarrays, Expression Analysis [20-817].

MeSH

Gene Expression Profiling; Polymerase Chain Reaction; Heart Transplantation; Graft Rejection; Risk Assessment; Biopsy

ICD-9-CM

Heart transplantation [37.51]; Complications of transplanted heart [996.83]; Biopsy of heart [37.25]

FDA SPN

CARIAC ALLOGRAFT GENE EXPRESSION PROFILING TEST SYSTEM [OJQ]

SNOMED CT

Polymerase chain reaction [258066000]; Transplantation of heart [32413006]; Graft rejection [72627004]; Acute cellular graft rejection [40442005]; Cardiac transplant rejection [233933006]; Risk assessment [225338004]; Endomyocardial biopsy [387829002]; Polymerase chain reaction [258066000]

Publication History

Date	Action	Comments
9/12/2007	Published	Initial Publication
9/11/2008	Updated	Reported Patient Indications/Contraindications [New FDA labeled indication]; Regulatory Status [FDA 510(k) clearance received]
4/27/2009	Reviewed	Reviewed Hotline Response; Conclusion unchanged; Added Selected Sources
7/1/2011	Updated	Updated Complete Report
12/30/2011	Updated	Evidence Report: Published report added
8/20/2014	Reviewed	Reviewed Product Brief; Added Editor's Note
11/23/2015	Updated	Updated Complete Report

Search Strategy

EMBASE syntax (EMBASE and MEDLINE were searched together):

1. 'heart transplantation'/exp OR 'heart transplantation'/syn or ('organ transplantation'/de AND heart) OR ((cardiac OR heart) NEAR/2 (transplant* OR allograft*))
2. 'graft rejection'/exp OR (reject* OR monitor* OR surveillance):de,ab,ti
3. 'gene expression profiling'/exp OR 'gene expression'/exp OR 'biological marker'/exp OR 'classifier'/exp OR 'proteomics'/exp OR 'molecular diagnostics'/de OR 'peripheral blood':de,ab,ti OR classifier:de,ab,ti OR 'gene expression':de,ab,ti OR 'biological marker':de,ti,ab
4. Allomap:dn,ab,ti OR CareDx:df,ab,ti OR Xdx:df,ti,ab
5. #1 and (#2 or #3 or #4)
6. #5 NOT ('conference paper'/exp OR 'case report'/de OR 'book'/de OR 'editorial'/de OR 'erratum'/de OR 'letter'/de OR 'note'/de OR 'short survey'/de OR book:it OR conference:it OR editorial:it OR erratum:it OR letter:it OR note:it OR 'short survey':it OR book:pt OR 'conference proceeding':pt)
7. #6 AND [humans]/lim AND [english]/lim AND [2008-2015]/py

This search may be executed in PubMed using the following strategy:

1. "Heart Transplantation"[mh] OR "heart transplantation"[tiab] OR "cardiac allograft"[tiab] OR "cardiac transplant"[tiab] OR "heart transplant"[tiab] OR "heart allograft"[tiab] OR "heart graft"[tiab]
2. "Graft Rejection"[mh] OR (("graft" OR "allograft" OR "transplant") AND ("rejection" OR "rejected" OR "rejecting" OR "rejects" OR "monitor" OR "monitoring" OR "monitored" OR "surveillance"))
3. "Gene Expression"[mh] OR "Gene Expression Profiling"[mh] OR "gene expression" OR "genetic expression" OR "genes" OR "gene signatures" OR "gene assay" OR "biological markers" OR "RNA" OR "peripheral blood"
4. "Allomap" OR "CareDx" OR "Xdx"
5. (#1 AND #2 AND #3) OR #4
6. #5 NOT (editorial [pt] OR comment [pt] OR letter [pt] OR news[pt] OR “case reports”[pt])
7. #6 Sort by: PublicationDate Filters: Publication date from 2008/01/01; English

Appendix A. Strength-of-evidence Assessment Methods

We grade strength of evidence (SOE) for selected patient outcomes in this report. Our grading approach is based on the concepts and methods proposed by the GRADE working group. Our approach also incorporates the evidence assessment methods adopted by the Agency for Healthcare Research and Quality's Evidence-based Practice Centers. Detailed descriptions of the GRADE and EPC methods are accessible using the links we provided above. To grade evidence in this report, we consider seven domains that may affect strength of evidence: risk of bias, consistency, directness, precision, magnitude of effect, dose-response gradient, and plausible confounders that would reduce a demonstrated effect. For each selected outcome, we assign a grade of "high," "moderate," "low," or "very low." The definitions of the grades are provided in Table A-1.

Table A-1. Strength-of-evidence Grade Definitions

Grade	Definition
High	We have high confidence in the findings for this outcome. The body of evidence has few or no deficiencies. We believe that the findings are stable (i.e., another study would not change the conclusions).
Moderate	We have moderate confidence in the findings for this outcome. The body of evidence has some deficiencies. We believe that the findings are likely to be stable, but some doubt remains.
Low	We have limited confidence in the findings for this outcome. The body of evidence has major or numerous deficiencies. We believe that additional evidence is needed before concluding either that the findings are stable or that the estimate of effect is close to the true effect.
Very low	We have no confidence in the findings for this outcome. No conclusion is appropriate, either because no evidence is available, or the existing evidence has unacceptable deficiencies.

Risk-of-bias Assesment for Test Performance Studies

For studies of test performance, we use an internal validity rating scale for diagnostic studies to assess the risk of bias of each individual study (see Table A-2). Our list of items is based on a modification of the QUADAS instrument with reference to empirical studies of design-related bias in diagnostic test studies.[54-56] Each item addresses an aspect of study design or conduct that can help protect against bias. Each item is answered "yes," "no," or "not reported," and an answer of "yes" indicates that the study reported a protection against bias on that aspect. We assessed each study of test performance as having low, medium, or high risk of bias using the items in Table A-2.

Table A-2. Items Used for Risk-of-bias Assessment for Test Performance Studies

Item		Comment
1.	Did the study enroll all, consecutive, or a random sample of patients?	—
2.	Were more than 85% of the approached/eligible patients enrolled?	—
3.	Were the patient inclusion and exclusion criteria applied consistently to all patients?	—
4.	Was the study affected by obvious spectrum bias?	—
5.	Did the study account for inter-reader differences?	—
6.	Were readers of the diagnostic test of interest blinded to the results of the reference standard?	—
7.	Were readers of the reference standard blinded to the results of the diagnostic test of interest?	—
8.	Were readers of the diagnostic test of interest blinded to all other clinical information?	—
9.	Were readers of the reference standard blinded to all other clinical information?	—
10.	Were patients assessed by a reference standard regardless of the test's results?	—
11.	Were all patients assessed by the same reference standard regardless of the test's results?	—
12.	If the study reported data for a single diagnostic threshold, was the threshold chosen a priori?	—
13.	Were the study results unaffected by intervening treatments or disease progression/regression?	—
14.	Were at least 85% of the enrolled patients accounted for?	—

We assess the risk of bias (ROB) of each included comparative study using the items specified in Table A-3.

Table A-3. Items Used for Risk-of-bias Assessment for Comparative Studies

Item	Comment
Were patients randomly or pseudorandomly (e.g., using instrumental variable analysis) assigned to the study groups?	Instrumental variable analysis can account for both measured and unmeasured confounders as long as the chosen variables have a strong association with treatment choice but no association with health outcomes. Studies using this method received a "yes" for this item. Studies using propensity scoring or multivariate regression received a "no."
Was there concealment of group allocation?	—
Were data analyzed based on the intention-to-treat-principle?	—
Were the patients blinded to the group assigned?	—
Were those who treated the patient blinded to the group to which the patients were assigned?	—
Were those who assessed the patient outcomes blinded to the group to which the patients were assigned?	—
Was the outcome measure of interest objective, and was it objectively measured?	The following were considered objective outcomes: mortality, number of endomyocardial biopsies, adverse events. The following were considered subjective outcomes: quality of life, patient satisfaction.
Was there a 15% or less difference in the length of follow-up for the 2 groups?	—
Did 85% or more of enrolled patients provide data at the time point of interest?	—
Was there fidelity to the protocol?	—

Appendix B. Results of Risk-of-bias and Strength-of-evidence Assessment

Table B-1. Results of Risk-of-bias Assessment Test Performance Studies

Study Author/Year	Q1. Did the study enroll all, consecutive, or a random sample of patients?	Q2. Were >85% of the approached, eligible patients enrolled?	Q3. Were the patient inclusion and exclusion criterial applied consistently?	Q4. Was the study affected by obvious spectrum bias?	Q5. Did the study account for inter-reader differences?	Q6. Were readers of the diagnostic test of interest blinded to the results of the reference standard?	Q7. Were the readers of the reference standard blinded to the results of the diagnostic test of interest?	Q8. Were the readers of the diagnostic test of interest blinded to all other clinical information?	Q9. Were the readers of the reference standard blinded to all other clinical information?	Q10. Were patients assessed by the reference standard regardless of the test results?	Q11. Were all patients assessed by the same reference standard regardless of the test results?	Q12. If the study reported data for a single diagnostic threshold, was the threshold chosen a priori?	Q13. Were the study results unaffected by intervening treatments or disease progression/regression?	Q14. Were at least 85% of the enrolled patients accounted for?	Risk-of-bias Category
FDA 510(k) Substantial Equivalence Determi-nation Decision Summary[45] CARGO	NR	NR	NR	NR	Yes	NR	NR	NR	No	Yes	Yes	NR	Yes	No	High
Starling et al.[46] Early-post CARGO	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Yes	Yes	NR	High

FDA: U.S. Food and Drug Administration
NR: Not reported

Table B-2. Results of Risk-of-Bias Assessment for Comparative Studies

Study Author/Year	Outcome(s)	Q1. Were patients randomly assigned to treatment groups?	Q2. Was there concealment of group allocation?	Q3. Were data analyzed based on the intention-to-treat-principle?	Q4. Were the patients blinded to the group assigned?	Q5. Were those who treated the patient blinded to the group to which the patients were assigned?	Q6. Were those who assessed the patient outcomes blinded to the group to which the patients were assigned?	Q7. Was the outcome measure of interest objective, and was it objectively measured?	Q8. Was there a 15% or less difference in the length of follow-up for the 2 groups?	Q9. Did 85% or more of enrolled patients provide data at the time point of interest?	Q10. Was there fidelity to protocol?	Risk-of-bias Category
Kobashigawa et al.[12]	Mortality	Yes	NR	Yes	No	No	NR	Yes	Yes	Yes	Yes	Medium
	Biopsy-related complications	Yes	NR	Yes	No	No	NR	Yes	Yes	Yes	Yes	Medium
	Quality of life	Yes	NR	Yes	No	No	No	No	Yes	Yes	Yes	High
Pham et al. 2010[13] IMAGE trial	Mortality	Yes	NR	Yes	No	No	No	Yes	Yes	Yes	No	Medium
	Biopsy-related complications	Yes	NR	Yes	No	No	No	Yes	Yes	Yes	No	Medium
	Quality of life	Yes	NR	No	No	No	No	No	Yes	No	No	High

NR: Not reported

Table B-3. Results of Strength-of-evidence Assessment

Comparison/ Reference	Outcome	Risk of Bias	Consistency	Directness	Precision	Evidence Favors	SOE Grade
AlloMap vs. EMB Kobashigawa et al. [12] Pham et al. 2010[13] Patients 2- to 18-months post-transplant	Mortality	Medium	Consistent	Direct	Imprecise	Neither	Very low
AlloMap vs. EMB Kobashigawa et al.[12] Pham et al. 2010[13]	Quality of life*	High	Inconsistent	Direct	Precise	Neither	Very low
AlloMap vs. EMB Kobashigawa et al.[12] Pham et al. 2010[13]	Biopsy-related complications	Medium	Consistent	Direct	Imprecise	Neither	Very low
Starling et al.[46]	Sensitivity, >12-months post-transplant, 34 threshold	High	Unknown	Direct	Imprecise	NA	Very low

Comparison/ Reference	Outcome	Risk of Bias	Consistency	Directness	Precision	Evidence Favors	SOE Grade
FDA 510(k) Substantial Equivalence Determination Decision Summary[45] CARGO	NPV 2- to 6-months post-transplant, 30 threshold	High	Unknown	Direct	Precise	NA	Very low
FDA 510(k) Substantial Equivalence Determination Decision Summary,[45] CARGO Starling et al.[46]	NPV >6- months post-transplant, 34 threshold	High	Consistent	Direct	Precise	NA	Low

*We evaluate quality of life based on outcomes for the longest time frame presented in the article. For Pham et al., we used the quality of life outcome 2-years postenrollment.

NPV: Negative predictive value
SOE: Strength of evidence

Appendix C. Impact Ratings Definitions

Genetic Test Report Ratings: three-dimension triangle with four divisions per slice

Reimbursement Status

Definition: The extent to which third-party payer coverage and coding are in effect to enable insured patients' access to the genetic test.

(4) Wide coverage: Medicare has a positive national coverage determination and/or ≥8 private payers provide coverage.

(3) Expanding coverage: Medicare has no national coverage determination; some local Medicare carriers provide coverage; 4 to 7 major private payers provide coverage; others deny coverage, have no published policy in place, or decide coverage on a case-by-case basis

(2) Limited coverage: Medicare has no national coverage determination or provides coverage only in the context of a clinical trial (i.e., coverage with evidence development); 1 to 3 major private payers provide coverage

(1) No coverage: Medicare has a national coverage determination that denies coverage or most private third-party payers explicitly state that they do not cover the technology because they consider the technology or intervention to be "investigational" or "experimental" or consider the evidence insufficient

Cost Impact

Definition: The cost to perform the test and its effect on further evaluation and treatment. Considerations also include the size of the patient population expected to use it.

(4) Substantial per patient cost (>$5,000), a substantial number of patients are expected to undergo this test, or this test may result in a significant amount of additional follow-up (testing, procedures) and associated costs.

(3) Moderate per patient cost (>$2,000 to $5,000), a moderate number of patients are expected to undergo this test, or this test results in a moderate amount of additional follow-up (testing, procedures) and associated costs.

(2) Small per patient costs (>$500 to $2,000), a small number of patients is expected to undergo this test, or this test results in a limited amount of additional follow-up (testing, procedures) and associated costs.

(1) Negligible per patient costs (<$500), a negligible number of patients expected to undergo this test, incurs negligible additional testing and associated costs, or it may prevent the need for a more costly test.

Utilization Status

Definition: The extent to which the genetic test is in use at this time. Considerations include the number of labs performing and distributing the test, the number of clinicians who have prescribed the test, and the potential patient population.

(4) Substantial: Performed by a substantial number of labs or performed by a small number of labs and widely distributed. Nonspecialist clinicians prescribe the test and the potential patient population is large because the indication is common.

(3) Moderate: Performed by a moderate number of labs or performed by a small number of labs and moderately distributed. Nonspecialist clinicians mostly prescribe the test, and the potential patient population is moderate in size because the indication is relatively common.

(2) Low: Performed by a small number of labs with limited distribution. A limited number of mostly specialist clinicians prescribe the test and the potential patient population is small because the indication is relatively rare.

(1) Negligible: Use is limited to clinical trials or a small number of labs who distribute the test to a restricted number of healthcare facilities. Only specialist clinicians at select healthcare facilities prescribe the test and the potential patient population is very small because the indication is extremely rare.

Appendix D. Clinical Validity Data Published Before FDA Approval

Table D-1. Reported Results from the CARGO Trial

Publication Author/Year	Patient Population (n Per Group)	Reported Diagnostic Accuracy	Notes
FDA 510(k) Substantial Equivalence Determination Decision Summary[45]	Subgroup analysis of 166 samples from an unknown number of patients between 55- and 182-days post-transplant	AUC = 0.71 [95% CI: 0.56–0.84] Accuracy threshold: 30 PPV: 4.6% NPV: 98.6%	The selection of these patients from the full CARGO study group is not described.
	Subgroup analysis of 134 samples from an unknown number of patients who were >182-days post-transplant	AUC = 0.67 [95% CI: 0.5–0.88] Accuracy threshold: 30 PPV: 2.1% NPV: 98.7% Accuracy threshold: 34 PPV: 4.1% NPV: 98.9%	The selection of these patients from the full CARGO study group is not described.
Deng et al. 2006[10]	Training set 145 samples from 107 patients	Accuracy threshold: 20 Sensitivity: 80% Specificity: 59%	Excluded because samples were used in algorithm development and threshold not clinically relevant.
	Primary validation set 63 samples (31 rejection and 32 quiescent) from 63 patients	AUC = 0.72 ±0.06 Accuracy threshold: 20 Sensitivity = 84% (95% CI: 66%–94%) Specificity = 38% (95% CI: 22%–56%)	Excluded because n <100 and threshold not clinically relevant.
	Primary validation set subgroup, including unknown number of samples from 26 patients >6-months post-transplant	Accuracy threshold: 28 Sensitivity: 83.3% Specificity: 71.4%	Excluded because n <100 and threshold not clinically relevant.
	Primary validation set subgroup, including unknown number of samples from 13 patients >12-months post-transplant	Accuracy threshold: 30 Sensitivity: 100% Specificity: 57.1%	Excluded because n <100 and threshold not clinically relevant.
	Secondary validation set 184 samples (62 rejection, 122 quiescent) from 124 patients, including the 63 primary validation set patients	Accuracy threshold: 20 Sensitivity = 76% (95% CI: 63%–85%) Specificity = 41% (95% CI: 32%–50%)	Excluded because included samples were used to develop the algorithm and threshold not clinically relevant.
	Secondary validation set subgroup, including unknown number of samples from 57 patients >6-months post-transplant	AUC = 0.8 ±0.114 Accuracy threshold: 28 Sensitivity = 71% Specificity = 79%	Excluded because included samples were used to develop the algorithm, n <100, and threshold not clinically relevant.
	Secondary validation set subgroup, including unknown number of samples from 25 patients >12-months post-transplant.	AUC = 0.86 ±0.09 Accuracy threshold: 30 Sensitivity = 80% Specificity = 77.8%	Excluded because included samples were used in test development and n <100.

Publication Author/Year	Patient Population (n Per Group)	Reported Diagnostic Accuracy	Notes
	Prevalent population set: 218 samples from 166 patients ≥1-year post-transplant with defined patient rejection composition (i.e., nested case-control study design).	Accuracy threshold: 30 PPV: 6.8% NPV: 99.6%	Excluded because of study design.
Starling et al.[46]	Post hoc subgroup analysis of CARGO patients (unclear exactly which patients and samples are included from CARGO) 440 samples from unknown number of patients >2-months and <6-months post-transplant	Accuracy threshold: 30 PPV: 6.2% NPV: 97.5%	Patient population not well described.
	Post hoc subgroup analysis of CARGO patients (unclear exactly which patients and samples are included from CARGO) 239 samples from unknown number of patients 6- to 12-months post-transplant	Accuracy threshold: 30 PPV: 3.2% NPV: 98.4% Accuracy threshold: 34 PPV: 4.7% NPV: 98.5%	Patient population not well described.
	Post hoc subgroup analysis of CARGO patients (unclear exactly which patients and samples are included from CARGO). 111 samples from unknown number of patients more than 12- months post-transplant	Accuracy threshold: 30 PPV: 4.0% NPV: 99.6% Accuracy threshold: 34 PPV: 5.6% NPV: 99.2%	Patient population not well described.

AUC: Area under the curve
CI: Confidence interval
NPV: Negative predictive value
PPV: Positive predictive value

Policy Statement

Genetic Test Evidence Reports present profiles and literature reviews of new and emerging genetic tests. Each Genetic Test Evidence Report is designed to provide a snapshot of the current status, efficacy, and use of that technology. The information contained in Genetic Test Evidence Reports is derived primarily from the currently available, published, peer-reviewed scientific literature, trade publications, and World Wide Web sites. Publications referenced are generally limited to the English language. Often, there is a relative paucity of published clinical data on new and emerging technologies; therefore, information from health technology resources on the Internet and elsewhere may be included. The conclusions and recommendations in any Genetic Test Evidence Report must be interpreted cautiously and judiciously. The data on which they are based are often insufficient to permit unequivocal resolution of the scientific and clinical issues most relevant to patient care. ECRI Institute implies no warranty and assumes no liability for the information, conclusions, and recommendations contained in Genetic Test Evidence Reports.

The conclusions and recommendations of each Genetic Test Evidence Report and the studies on which they are based are highly perishable and reflect the state of the technology at the time at which the report was compiled. Genetic Test Evidence Reports are produced and updated by a multidisciplinary staff of scientists, clinicians, information specialists, medical writers, and other health professionals. For quality assurance, all reports are subject to review by experts within ECRI Institute and one or more selected external experts. Genetic Test Evidence Reports reflects the views of ECRI and not necessarily those of outside reviewers. Neither ECRI Institute nor its employees accept gifts, grants, or contributions from, or consult for medical device or pharmaceutical manufacturers.

The Health Technology Assessment Information Service (HTAIS) provides Genetic Test Evidence Reports and many other forms of information support to help governments, hospitals, health systems, managed care organizations, health insurers, health professionals, and the public meet the challenge of evaluating healthcare technology objectively and rationally.

HTAIS is a service of ECRI Institute, a nonprofit health services research agency. ECRI Institute has been designated an Evidence-based Practice Center by the U.S. Agency for Healthcare Research and Quality. ECRI Institute's mission is to provide information and technical assistance to the healthcare community worldwide to support safe and cost-effective patient care. The results of ECRI Institute's research and experience are available through its publications, information systems, databases, technical assistance programs, laboratory services, seminars, and fellowships.