September 13, 2023

CareDx, Inc.
Camilla Lu
Associate Director, Regulatory Affairs
3260 Bayshore Boulevard
Brisbane, CA 94005

Re: K221640
Trade/Device Name: AlloMap Heart Molecular Expression Testing
Regulation Number: 21 CFR 862.1163
Regulation Name: Cardiac Allograft Gene Expression Profiling Test System
Regulatory Class: Class II
Product Code: OJQ
Dated: April 7, 2023
Received: April 10, 2023

Dear Camilla Lu:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR

803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Paula V. Caposino -S

Paula Caposino, Ph.D.
Acting Deputy Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K221640

Device Name

AlloMap Heart Molecular Expression Testing

Indications for Use *(Describe)*

AlloMap Heart Molecular Expression Testing is an In Vitro Diagnostic Multivariate Index assay (IVDMIA) test service, performed in a single laboratory, assessing the gene expression profile of RNA isolated from peripheral blood mononuclear cells (PBMC). AlloMap Heart Testing is intended to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection (ACR) at the time of testing in conjunction with standard clinical assessment.

Indicated for use in heart transplant recipients:
- 15 years of age or older
- At least 2 months (≥55 days) post-transplant

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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TRADITIONAL 510(K) SUMMARY

(Complying with 21 CFR 807.92)

I. SUBMITTER

CareDx, Inc.
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Contact Person: Camilla Lu, PhD.
Date Prepared: September 6, 2023

II. DEVICE

Trade Name:	AlloMap Heart Molecular Expression Testing
Common Name:	AlloMap Heart Testing
Classification Name:	Cardiac Allograft Gene Expression Profiling Test System
Regulatory Class:	Class II
Regulation Number:	21 CFR 862.1163
Product Code:	OJQ
510(K) Number:	K221640

III. PREDICATE DEVICE

Trade Name:	AlloMap Molecular Expression Testing
Common Name:	AlloMap Testing
De Novo Number:	DEN080007
510(K) Number:	K073482

IV DEVICE DESCRIPTION

Principle of Operation: AlloMap Heart Molecular Expression Testing is an In Vitro Diagnostic Multivariate Index Assay (IVDMIA) test service performed in a single laboratory, assessing the gene expression profile of RNA isolated from peripheral blood mononuclear cells (PBMC). AlloMap Heart Testing is a non-invasive blood test that uses genomic technologies to identify the absence of cardiac rejection. When used in conjunction with standard clinical assessments, AlloMap Heart Testing may help identify patients with stable allograft function who have a low probability of moderate to severe acute cellular rejection (ACR) at the time of Testing.

AlloMap is a panel of 20 gene assays, 11 informative and 9 used for normalization and quality control, which produces gene expression data used to calculate a reported AlloMap test score – an integer ranging from 0 to 40. The Score is an algorithmic composite of the expression of 11

differentially weighted informative genes that have a role in rejection and reflect host immune response, representing multiple diverse molecular pathways. The clinician uses the AlloMap Score and other standard clinical assessments to evaluate the patient's probability of rejection and the need for additional diagnostic evaluations. Compared with patients in the same post-transplant period, the lower the Score, the lower the probability of acute cellular rejection at the time of Testing. AlloMap Heart Testing is only performed at the CLIA-certified and CAP-accredited clinical laboratory at CareDx in Brisbane, California.

AlloMap Heart Test Process: A whole blood sample is collected at the patient site in a BD Vacutainer® CPT™ Cell Preparation tube with sodium citrate anticoagulant. Peripheral blood mononuclear cells (PBMC) are isolated and lysed, and frozen PBMC lysate is shipped using kits provided by CareDx to the CareDx laboratory in Brisbane, CA. RNA is purified from the PBMC lysate and converted to cDNA using commercial reagents on a thermal cycler following a standard reverse transcriptase protocol. Using liquid handling instruments, the cDNA is diluted and dispensed into a 384-well AlloMap HTx assay plate. The AlloMap plate comprises pre-aliquoted qRT-PCR reagents created by CareDx Manufacturing, containing sequence-specific primers, probes, and Universal Master Mix to measure each of the 20 genes in triplicate. Gene expression levels are determined via the qRT-PCR instrument for each of the eleven (11) informative and nine (9) control genes. The Analyzer Software, SAX, converts sample data generated from the qRT-PCR plate to an AlloMap Test Score. Multiple automated QC checks are performed on every sample. AlloMap scores are calculated for all samples that pass QC. The AlloMap Test Score is then reported to the physician.

AlloMap Heart Test System: The test system consists of assay reagents, instruments, and system software. The test components have not changed from the predicate device **K073482**, including sample processing, reagents, formulations, and assay plates containing qRT-PCR reagents created by CareDx Manufacturing to measure each of the 20 genes. The difference between the subject device, the AlloMap Heart Testing, and the predicate device is the replacement of the current eight ABI 7900HT thermal cyclers in the CareDx laboratory with eight Roche LightCycler 480 II (LC480) thermal cyclers. Consequently, the Software Analyzer has been modified to support the LC480 Instrument and achieve AlloMap Heart test equivalency with the current ABI 7900HT instrument.

V. INTENDED USE

1. **Intended Use (s):**

   The Intended Use of the subject device is the same as the predicate device, **K073482.** See Indications for Use below.

2. **Indications (s) for Use:**

   AlloMap Heart Molecular Expression Testing is an In Vitro Diagnostic Multivariate Index assay (IVDMIA) test service, performed in a single laboratory, assessing the gene expression profile of RNA isolated from peripheral blood mononuclear cells (PBMC). AlloMap Heart Testing is intended to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection (ACR) at the time of testing in conjunction with standard clinical assessment.

Indicated for use in heart transplant recipients:

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

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

Similarities

First, the subject device has the same Intended Use as the predicate device. Second, the subject device has the same technological characteristics as the predicate device. At a high level, the subject and predicate devices are based on the following same technological elements:

- Sample processing
- Reagents used for RNA purification and cDNA synthesis
- The panel of 20 genes (11 informative and 9 control genes)
- AlloMap HTx assay plates containing qRT-PCR reagents created by CareDx Manufacturing measure the expression of each of the 20 genes
- Instruments used for RNA Quantification, cDNA Synthesis Pipetting, and cDNA Synthesis Reaction
- The Assay Quality Control rules and criteria for the AlloMap Heart test: rules of Data quality, PCR success, Genomic DNA contamination, Sample quality & quantity, Efficiency, Assay Ranges, Normalization genes, and Process control expected values
- The LIS software used to manage the workflow for the processing, reporting AlloMap Heart tests, and the AlloMap Score has not changed
- AlloMap Heart Report software has not changed
- Analyzer Software, SAX, which converts sample data to an AlloMap score, has not changed its primary functions, and the four modules, including the Algorithm to calculate the AlloMap Score, remain unchanged

Differences

The following assay component differences exist between the subject and predicate devices:

- The predicate device uses eight ABI 7900 HT thermal cyclers, which are replaced with eight Roche LightCycler 480 II (LC480) for the subject device in the CareDx laboratory.
- The Software Analyzer, SAX, has been modified in the subject device to support the LC480 Instrument and achieve AlloMap test equivalency with the current ABI 7900HT instrument. SAX changed its version from SAX v1.0 (for predicate device) to v1.2 (for subject device) to update existing modules:
  - SAX v1.2 extracts the raw fluorescence data generated by the LC480 from the resulting XML Summary output files
  - SAX v1.2 applies two gene-level correction factors - additive correction coefficient (δga) and multiplicative correction coefficient (δgb) – to map the LC480 response to the ABI 7900 HT response.

VII. PERFORMANCE DATA

The following performance data were provided in support of the substantial equivalence determination.

Software Verification and Validation Testing

Verification of the Software Analyzer, SAX v1.2, was conducted per Verification Plan against its requirement specification, followed by a software validation test at CareDx in Brisbane, CA, including Analyzer QC Criteria, Analyzer Algorithm Score, and Integration with LIMS. As demonstrated by the V&V reports in this submission, the modified Software Analyzer v1.2 and its specifications (design outputs) meet the product requirements (design inputs) and conform to the user's needs and Intended Use.

Analytical Performance Validation Testing

Four analytical validation studies were performed to evaluate the performance characteristics of the Roche LC480 Instrument (subject device) compared to the current ABI7900HT Instrument (predicate device). Previously tested heart transplant patient samples with clinical relevance to AlloMap score were used after deidentification in the studies. The modified Analyzer Software SAX v1.2 was used for the LC480 workflow during the validation, and SAX v1.0 was used for the data analysis of 7900HT.

1. Precision – Between Instrument Variability

Variance component analysis was performed to estimate the between-instrument variability for the AlloMap scores. This study demonstrated the between-instrument variability for LC480 to be comparable to that of the predicate device, the 7900HT instrument. The variance ratios of LC480 over 7900HT are less than (<) 1 for the Low, Medium, and High AlloMap score sample pools, respectively, and passed the pre-specified acceptance criteria. Hence, between-instrument variability for LC480 is acceptable.

2. Precision – Reproducibility

The study evaluated the within-lab precision of the LC480 versus the 7900HT using variance component analysis. The reproducibility for the AlloMap Heart test process was established for the following individual variance components: day-to-day, operator-to-operator, instrument-to-instrument, plate lot-to-lot, plate-to-plate nested within plate lot, and plate section-to-section nested within the instrument. The variability of AlloMap scores was analyzed separately in three sample pools representing low, medium, and high AlloMap scores. The within-lab precision of LC480 is summarized in **Table 2-1**, Overall Statistics of LC480 Data for the 3 sample pools.

Table 2-1: *Overall Statistics of LC480 Within-Lab Precision for the 3 Samples Pools, Representing Low, Medium, and High AlloMap Scores, tested using 2 Operators, 3 Instruments, and 3 Plate lots over 5 Non-Consecutive Days.*

Sample Pool	Number of Samples	Mean AlloMap Score	SD	CV (%)
Low	88	16.2	2.210	13.613
Medium	90	28.5	1.110	3.887
High	90	37.5	0.353	0.941

When comparing the precision between the LC480, and the 7900HT, the total variance ratio is less than (<) 1, i.e., the total variances were smaller for the LC480 in each of the AlloMap score sample pools, and the F-test p-value is less than 0.05. Thus, LC480 met the acceptance criteria and demonstrated acceptable precision. See **Table 2-2**, F-Test Results for the Total Variance Ratio between LC480 and ABI 7900HT.

Table 2-2. *F-Test Results for the Total Variance Ratio between LC480 and ABI 7900HT*

Sample Pool	Variance Ratio[1] ($\hat{\sigma}^2_{LC480}/\hat{\sigma}^2_{7900HT}$)	F Value[2]	P Value	Acceptance Criteria Variance Ratio ($\hat{\sigma}^2_{LC480}/\hat{\sigma}^2_{7900HT}$) is less than (<) 1 or not statistically significant
Low	0.459	0.446	<0.05	PASS[3]
Medium	0.316	0.326	<0.05	PASS[3]
High	0.132	0.154	<0.05	PASS[3]

[1] The ratio of the total variances is generated from the Variance Component Analysis using the Restricted Maximum Likelihood (REML) method.
[2] The F value is calculated as the ratio of the total variances as the mean squared of the error.
[3] $\hat{\sigma}^2_{LC480}$ is significantly smaller than $\hat{\sigma}^2_{7900HT}$. Hence, the variance ratio less than (<1) with significant p value.

3. Linearity

Six different amounts of the same patient RNA sample, representing a high AlloMap score (≥ 34), were used as input into the cDNA synthesis ranging from 150 to 500 ng (regular 300 ng). The linear regression method was used to analyze the mean of the normalized factors vs. the input $\log_{10}$RNA concentration. The result obtained an R^2 value of 0.989. The variability of the raw scores at each RNA input level was examined using the acceptance criteria established for the predicate device. The result showed that the LC480 met the acceptance criteria and is comparable to the predicate device, 7900HT. The study demonstrated that linearity of the AlloMap Heart test across various RNA inputs is maintained using the Roche LC480 qRT-PCR instruments especially around the current RNA input of 300ng per patient sample.

4. Method Comparison - Accuracy

The study validated that AlloMap results measured by the Roche LC480 are equivalent across the reportable range to the results measured by the ABI 7900HT in the CareDx Laboratory. The analysis included a total of 163 patient RNA samples processed by 2 LC480 instruments and a single 7900HT. The paired scores were analyzed using Passing-Bablok regression models. All pre-specified passing criteria were met at a two-sided significance level of 0.05 for the paired score differences and the regression parameters, i.e., intercept and slope. See **Table 4-1**.

Table 4-1: *Passing-Bablok Regression Results*

Parameters	Estimate and 95% CI		Acceptance Criteria 95% CI of the slope includes 1.0 and 95% CI of the intercept includes 0.0
	LC480_7296 vs 7900HT	LC480_7371 vs 7900HT	
Intercept	0 (-0.53, 0.53)	0 (-0.34, 0.34)	PASS
Slope	1 (0.99, 1.01)	1 (0.99, 1.01)	PASS

Following FDA Guidance for Assay Migration Studies, allowable total difference (ATD) zone were constructed separately for low, medium and high score ranges using reproducibility results for the 7900HT to define the limits for 95% of the differences between repeated measures by the 7900HT. For each LC480, over 95% of the samples across the entire clinical range fell within the ATD zones with a lower limit of 95% one-sided CI > 90%. Thus, the measurements by LC480 are within the analytical variation of the 7900HT and can be considered equivalent to the predicate. (see **Table 4-2**).

Table 4-2: *Percentages of the Samples within ATD Zones*

AlloMap Score Ranges (7900HT)		**Low <=20 (N=25, *15.3%*)**	**Medium 21-33 (N=86, 52.8%)**	**High 34-40 (N=52, 31.9%)**	**All Samples (n=163)**	**Acceptance Criteria 95% All Samples within ATD zones AND Lower 95% CI for All Samples 90%**
LC480_7296	n (%)	25 (100%)	85 (98.8%)	51 (100%)	162 (99.4%)	PASS
	Lower 95% CI	-	-	-	96.6%	
LC480_7371	n (%)	25 (100%)	83 (96.5%)	52 (100%)	160 (98.2%)	PASS
	Lower 95% CI	-	-	-	94.7%	

Using the 300 samples in the original clinical validation study from the predicate device, k073482, we calculated projected NPV and PPV for each Roche LC480 as the mean of the 500 simulated NPV and PPV from the simulated AlloMap scores for the LC480 by adding random errors (estimated from Accuracy study or Reproducibility study) to the 7900HT scores. The projected NPV and PPV for the Roche LC480 were then compared with the NPV and PPV for the 7900HT. The absolute differences between the two system platforms were within 1% or 95% CI including 0 for NPV, and within 2% or 95% CI including 0 for PPV, for both 2-6 months (55-182 days) and >6 months (>182 days) post-transplant periods. These results demonstrated that the Roche LC480 produces AlloMap results that are statistically and clinically equivalent across the whole clinical range to those produced by the ABI 7900HT qRT-PCR thermal cycler in the CareDx Laboratory.

Compliance with FDA Recognized Standards and Guidelines

- CLSI EP09-A3 *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*; Approved Guideline – Third Edition. Section 6 Measurement Procedure Comparison Studies; Section 7, Considerations for Clinical Laboratories; Section 10.2, Other Comparisons. Clinical and Laboratory Standards Institute; 2013
- CLSI EP31-A-IR *Verification of Comparability of Patient Results Within One Health Care System*; Approved Guideline (Interim Revision). Section 6.1, Commutability; Section 7.1, Evaluation of Comparability Based on Clinical Outcomes; Section 8.2, Statistical Analysis of Comparability Data. Clinical and Laboratory Standards Institute; 2012
- CLSI-EP06-A, *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. Sections 5.3, Determination of the Linear Range and 5.3.1, Summary of Methods – The Polynomial Evaluation of Linearity Wayne, PA: Clinical and Laboratory Standards Institute; 2003.
- CLSI EP05-A3, *Evaluation of Precision Performance of Quantitative Measurement Procedures*: *Approved Guideline* Chapter 3, Single-Site Precision Evaluation Study, Wayne, PA: Clinical and Laboratory Standards Institute, 2014.

- FDA Guidance: Assay Migration Studies for In Vitro Diagnostic Devices. Document issued on April 25, 2013
- FDA Guidance: Cardiac Allograft Gene Expression Profiling Test Systems – Class II Special Controls Guidance for Industry and FDA Staff. Document issued on October 21, 2009

VIII. CONCLUSIONS

CareDx has demonstrated through its evaluation and performance testing that the AlloMap Heart Testing using the Roche LC480 instruments is substantially equivalent to the legally marketed AlloMap Testing using the ABI 7900HT. Same as the predicate device, AlloMap Heart Testing is safe and effective for its intended Use.