510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:
k073482

B. Purpose for Submission:
New device

C. Measurand:
RNA expression of 20 genes

D. Type of Test:
Quantitative RT-PCR analysis of 20 genes (11 informative; 9 control); IVDMIA Test service performed in a single laboratory in XDx's Brisbane, CA facility

E. Applicant:
XDx

F. Proprietary and Established Names:
AlloMap® Molecular Expression Testing

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.1163
2. Classification:
   Class II
3. Product code:
   OJC Cardiac allograft gene expression profiling test system
4. Panel:
   Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See Indications for use below.
2. Indication(s) for use:
   AlloMap 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 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

3. Special conditions for use statement(s):
   For prescription use only.

4. Special instrument requirements:

Liquid Handler Instruments:

System Identifying Number	Manufacturer	Model	Serial Number
969050	Beckman Coulter	Biomek FXp (8 single)	A318430104
504671	Beckman Coulter	Biomek FXp (8 single)	A318430102
504672	Beckman Coulter	Biomek FXp (8 single)	A318430103

PCR Instruments: All RT-PCR instruments are from Applied Biosystems, Model 7900HT. The System Identifying Number and Serial Number are the same. Serial numbers of the instruments are: 200866, 279001667, 279001146, 200899, 201747, 201308, 279000980, 279000917.

Note: The liquid handling instruments and RT-PCR instruments are components of this assay and are cleared only for this assay and not for any other application. In addition, clearance is only limited to the liquid handling and PCR instruments with the serial numbers as specified above.

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I. Device Description:

AlloMap Molecular Expression Testing is performed in a single laboratory (XDx Laboratories). Blood is drawn from the patient and subsequently processed, packaged and shipped frozen to the XDx Laboratory. At the XDx Laboratory, gene expression levels are determined via real time PCR for each of the test and control genes and converted to the AlloMap Test Score. The AlloMap Test Score is then reported to the physician.

A sample collection kit is sent to ordering laboratories/physicians. The collection kits contain the following components:

CPT™ Cell Preparation Tube with Sodium Citrate, 8.0 ml, 60/box
(only tubes supplied by XDx should be used)

AlloMap Sample Processing Reagents

- 10 centrifuge tubes with 5.0 mL phosphate buffered saline
- 10 tubes with 1.8 mL of LyseDx™ lysing reagent
- 10 disposable transfer pipettes
- Sample preparation instructions

XDx Laboratory Frozen Shipper Pack

- Aqui-Pak 6-Bay absorbent pouch
- 95 kPa Specimen Transport Bag
- Dry ice label
- List of Contents card
- Styrofoam™ cooler
- Outer shipping box
- FedEx Shipping Packets (customized airbills and labels)

- 9 x 12 plastic zip bag
- Requisitions, 2-part NCR
- Aqui-Pak 6-Bay absorbent pouch, each
- 95 kPa specimen transport bags, each

J. Substantial Equivalence Information:

1. Predicate device name(s):
   None.
2. Predicate K number(s):
   Not applicable.
3. Comparison with predicate:
   Not applicable.

K. Standard/Guidance Document Referenced (if applicable):

• Draft Guidance for Industry, Clinical Laboratories, and FDA Staff - In Vitro Diagnostic Multivariate Index Assays

• Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Class II Special Controls Guidance Document: Gene Expression Profiling Test System for Breast Cancer Prognosis

• *Interference Testing in Clinical Chemistry;* Approved Guideline (EP7-A2)

L. Test Principle:

Blood specimen collection at the referring facility is initiated by a physician order. Whole blood is collected in an 8.0 mL Vacutainer CPT Cell Preparation Tube with Sodium Citrate (CPT tube). The PBMCs are separated from the whole blood sample via centrifugation of the CPT tube. The PBMCs are washed, pelleted and lysed using reagents provided to customers by XDx. This PBMC lysate material is frozen and shipped to XDx. Upon arrival at XDx, RNA from the PBMC lysate is purified and quantified using spectrophotometric methods. Upon meeting acceptance criteria, cDNA synthesis proceeds using a liquid handling instrument to dispense samples and reagents to the 96-well reaction plates.

Purified RNA is converted to cDNA. The cDNA provides the template for the qRT-PCR. cDNA synthesis steps are monitored for run to run variability by including pooled human RNA prepared using the same procedures utilized for patient testing. The cDNA is added to the AlloMap reagent plates and the plates are sealed and loaded into the qRT-PCR instruments.

The XDx-developed Analyzer software converts sample data generated from the qRT-PCR plate to an AlloMap test score using the proprietary XDx AlloMap algorithm for multivariate analysis. A report is generated that provides the physician with the AlloMap test score. Longitudinal results, negative predictive values (NPV) for two time post-transplant periods and patient demographics are also provided. The physician interprets the results along with other standard clinical assessments of cardiac allograft status to determine probability of ACR.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*
Precision was evaluated using two types of samples, XDx donor samples and patient samples. Patient samples were obtained from 6 individual cardiac transplant recipients from two CARGO centers (3 patients per center). 3 samples per patient were provided from one center, and 4 samples per patient were provided from the second center.

Patient Information for Samples used in Precision Study

Patient	Sex	Race	Days Since Tx	Age at visit	ISHLT Grade
P1	Male	Hispanic	294	31	Grade 1A
P2	Male	Asian	653	32	Grade 1B
P3	Male	Black	431	59	Grade 1A
P4	Male	Caucasian	232	60	Grade 0
P5	Male	Asian	279	44	Grade 1B
P6	Female	Caucasian	274	34	Grade 1B

The AlloMap scores for healthy donors ranged from 28 to 30. The AlloMap scores for patients ranged from 24 to 34 (no biopsy information available).
There are three arms for each type of sample: Arms A, B, and C.
For Arms A and B, three sources of variability, run-to-run, operator-to-operator (inter-operator) and within operator (intra-operator), were estimated at different points of the process. Arm A provides variability estimates from RNA→cDNA→Assay, while Arm B provides variability estimates from cDNA→Assay. Arm C estimates variability of lot-to-lot, plate-to-plate within a lot, and section-to-section within a plate and a lot at the Assay level.
Arm A: For each operator in this study, testing was conducted on four separate days. All four operators performed 4 separate runs with samples from each of the 4 donors. For each operator, each run was initiated on a separate day and included 1 sample from each of the four donors. One lot of reagents was used in testing.
Arm B: For each operator in this study, testing was conducted on two separate days. One lot of reagents was used in testing.
Arm C: All testing was performed by a single operator on a single day; run-to-run precision was not a component of this arm. This arm used 3 lots of reagents for the analytical phase of testing to measure lot-to-lot, plate-to-plate and section-to-section precision.

The precision of the AlloMap Test process is summarized below. Summary of maximum CV's are:

- Overall CV's (Tables A1, A2, A7 and A8):
  - 4 Donor Samples (Arm A): ≤6.3%

    - 6 Patient Samples (Arm A): ≤10.4%
    - Donor Sample (Arm C): 3.8%
    - Pooled Patient Sample (Arm C): 4.4%
- Run-to-run CV's (Tables A3, A4)
    - Donor Samples: ≤9.2%
    - Patient Samples: ≤11.9%
- Operator-to-operator CV's (Tables A5, A6)
    - Donor Samples: ≤8.3%
    - Patient Samples: ≤11.7%
- Lot-to-lot CV's (Tables A9, A10)
    - Donor Samples: ≤4.7%
    - Patient Samples: ≤4.5%
- Plate-to-plate CV's (Tables A11, A12)
    - Donor Samples: ≤5.1%
    - Patient Samples: ≤4.5%
- Section-to-section CV's (Tables A13, A14)
    - Donor Samples: ≤5.7%
    - Patient Samples: ≤5.8%

Table A1: Overall statistics for 4 healthy donor samples (D1-D4), 4 runs per sample, 4 operators (Op1-Op4) per run and 2 reagent plates per operator.

Donor	n	Mean	SD	CV(%)
D1	28	30.047	1.654	5.5%
D2	28	30.678	1.105	3.6%
D3	30	28.365	1.771	6.2%
D4	30	30.139	1.897	6.3%

Table A2: Overall statistics for 6 heart transplant patient samples (Stanford1-Stanford3 and UCLA1-UCLA3), 2 runs per sample, 2 operators (Op1 and Op2) per run and 2 reagent plates per operator. NOTE: no run 2/operator 2 data obtained for UCLA samples.

Patient	n	Mean	SD	CV(%)
Stanford1	8	27.456	1.642	6.0%
Stanford2	8	34.481	0.598	1.7%
Stanford3	8	31.656	2.014	6.4%
UCLA1	6	33.720	0.295	0.9%
UCLA2	4	34.858	1.262	3.6%
UCLA3	6	24.263	2.534	10.4%

Table A3: Descriptive statistics for run to run assessment for healthy donor samples.

Run:	1				2				3				4			
Donor	**n**	**Mean**	**SD**	**CV(%)**	**n**	**Mean**	**SD**	**CV(%)**	**n**	**Mean**	**SD**	**CV%**	**n**	**Mean**	**SD**	**CV(%)**
D1	8	30.915	0.773	2.5%	8	29.959	1.406	4.7%	4	29.175	1.752	6.0%	8	29.702	2.284	7.7%
D2	8	30.763	0.996	3.2%	8	30.143	0.867	2.9%	4	32.306	0.648	2.0%	8	30.314	0.887	2.9%
D3	8	27.847	1.974	7.1%	8	28.695	1.259	4.4%	6	28.071	2.594	9.2%	8	28.74	1.418	4.9%
D4	8	30.016	1.332	4.4%	8	29.992	1.457	4.9%	6	29.105	2.607	9.0%	8	31.185	1.986	6.4%

Table A4: Descriptive statistics for run to run assessment for patient samples.

Run:	1				2			
Patient	**n**	**Mean**	**SD**	**CV(%)**	**n**	**Mean**	**SD**	**CV(%)**
Stanford1	4	27.911	1.878	6.7%	4	27.001	1.487	5.5%
Stanford2	4	34.748	0.442	1.3%	4	34.214	0.670	2.0%
Stanford3	4	32.868	1.410	4.3%	4	30.444	1.887	6.2%
UCLA1	4	33.730	0.354	1.0%	2	33.700	0.241	0.7%
UCLA2	2	34.320	0.319	0.9%	2	35.396	1.875	5.3%
UCLA3	4	23.556	2.804	11.9%	2	25.679	1.585	6.2%

Table A5: Descriptive statistics for operator to operator assessment for healthy donor samples.

Operator:	Op1				Op2				Op3				Op4			
Donor	**n**	**Mean**	**SD**	**CV(%)**	**n**	**Mean**	**SD**	**CV(%)**	**n**	**Mean**	**SD**	**CV(%)**	**n**	**Mean**	**SD**	**CV(%)**
D1	6	30.631	1.896	6.2%	6	30.947	1.294	4.2%	8	30.258	0.829	2.7%	8	28.723	1.748	6.1%
D2	6	29.957	0.971	3.2%	6	31.198	0.437	1.4%	8	30.932	1.507	4.9%	8	30.574	0.931	3.0%
D3	6	28.649	1.241	4.3%	8	27.557	2.110	7.7%	8	28.759	1.437	5.0%	8	28.567	2.082	7.3%
D4	6	30.072	2.499	8.3%	8	29.671	2.399	8.1%	8	31.342	0.838	2.7%	8	29.453	1.240	4.2%

Table A6: Descriptive statistics for operator to operator assessment for patient samples.

Operator:	Op1				Op2			
Patient	**n**	**Mean**	**SD**	**CV(%)**	**n**	**Mean**	**SD**	**CV(%)**
Stanford1	4	26.843	1.552	5.8%	4	28.069	1.697	6.0%
Stanford2	4	34.459	0.851	2.5%	4	34.502	0.328	1.0%
Stanford3	4	31.722	1.396	4.4%	4	31.590	2.740	8.7%
UCLA1	4	33.663	0.162	0.5%	2	33.835	0.562	1.7%
UCLA2	4	34.858	1.262	3.6%	0	—	—	—
UCLA3	4	23.644	2.771	11.7%	2	25.503	2.114	8.3%

Table A7: Overall statistics for 1 healthy donor sample using 3 reagent lots (made from 3 distinct lots of raw materials), 4 plates per lot and 4 sections per plate.

N	Mean	SD	CV(%)
48	31.630	1.214	3.8%

Table A8: Overall statistics for 1 pooled patient sample using 3 reagent lots (made from 3 distinct lots of raw materials), 4 plates per lot and 4 sections per plate.

N	Mean	SD	CV(%)
48	28.875	1.273	4.4%

Table A9: Descriptive statistics for reagent lot assessment for healthy donor samples.

Lot	n	Mean	SD	CV(%)
1	16	31.279	1.470	4.7%
2	16	31.258	0.920	2.9%
3	16	32.354	0.878	2.7%

Table A10: Descriptive statistics for reagent lot assessment for patient samples.

Lot	n	Mean	SD	CV(%)
1	16	28.611	1.299	4.5%
2	16	28.582	1.284	4.5%
3	16	29.431	1.114	3.8%

Table A11: Descriptive statistics for plate to plate assessment for healthy donor samples.

Plate	n	Mean	SD	CV(%)
1	12	31.428	1.594	5.1%
2	12	31.864	0.960	3.0%
3	12	31.467	1.010	3.2%
4	12	31.762	1.289	4.1%

Table A12: Descriptive statistics for plate to plate assessment for patient samples.

Plate	n	Mean	SD	CV(%)
1	12	28.834	1.116	3.9%
2	12	29.359	1.323	4.5%
3	12	28.958	1.304	4.5%
4	12	28.349	1.287	4.5%

Table A13: Descriptive statistics for plate section to section assessment for healthy donor samples (Note: UL=upper left quadrant of plate; LL=lower left quadrant of plate; UR=upper right quadrant of plate; LR=lower right quadrant of plate).

Sections	n	Mean	SD	CV(%)
UL	12	31.771	1.035	3.3%
LL	12	31.072	1.764	5.7%
UR	12	31.735	1.026	3.2%
LR	12	31.942	0.759	2.4%

Table A14: Descriptive statistics for plate section to section assessment for patient samples (Note: UL=upper left quadrant of plate; LL=lower left quadrant of plate; UR=upper right quadrant of plate; LR=lower right quadrant of plate).

Sections	n	Mean	SD	CV(%)
UL	12	29.244	0.997	3.4%
LL	12	28.558	1.645	5.8%
UR	12	28.896	1.036	3.6%
LR	12	28.801	1.373	4.8%

b. *Linearity/assay reportable range:*
Linearity is not applicable for this type of assay. The AlloMap Test provides clinicians with an AlloMap Score between 0 and 40.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Quality Control Materials: The RNA control pools are generated from patient and/or donor samples. cDNA controls are generated from the RNA control pools. Established values are set by testing the materials (RNA or cDNA) on plates from at least two AlloMap reagent plate lots and on multiple qRT-PCR machines (all that are in use at the time of testing). The mean AlloMap raw score and standard deviation are generated by this testing. The AlloMap raw score of the RNA control is evaluated for each test run (run pass/fail) and plotted weekly on 3-month rolling graphs (to monitor the

accuracy and consistency of the test system over time). The RNA control evaluates the performance of the test system from the cDNA synthesis step through the qRT-PCR phase of testing. The cDNA control is used as a process control only and is plotted weekly like the RNA control for monitoring of the test system over time. The cDNA control evaluates the test system starting from the addition of cDNA to the AlloMap reagent plates through the qRT-PCR phase of testing. Evaluation of both the RNA and cDNA controls separately and in combination allow for monitoring of reagent lot-to-lot and instrument-to-instrument variability or issues.

RNA Stability:
The sponsor provided study data to establish the length of time over which RNA, stored at ≤ -65ºC, is stable and suitable for AlloMap testing. This evaluation demonstrated the stability of the RNA for 8 months. The expiration date of RNA used for AlloMap testing is claimed as 6 months from date of purification from the PBMC lysates.

cDNA Stability:
The sponsor provided study data to establish the length of time over which cDNA is stable and suitable for AlloMap testing. This evaluation demonstrated the stability of the cDNA for 8 months. The expiration date of cDNA used for AlloMap testing is claimed as 6 months from date of preparation from purified RNA.

d. Detection limit:
A minimum RNA yield of 400 ng is required to run the assay. XDx requires that each of the gene assays provide a C_T at or below 38. As the amount of input RNA decreases, low abundance genes become increasingly likely to have a C_T greater than 38. RNA from samples with CT values greater than 38 is considered to be undetectable for the purposes of calculating AlloMap scores. All informative gene assays must be detectable to generate an AlloMap score.

RNA Yield:
The average yield of RNA from a CPT tube in XDx Reference Laboratory samples has been over 5.3 μg, with more than 97.8% of all submitted samples providing at least 400 ng of RNA. Therefore, the CPT blood draw provides sufficient RNA to run the AlloMap test on nearly 98% of patient samples.

RNA Purity:
The claimed range of RNA purity for the XDx AlloMap test is 1.5 - 3.5. The generally accepted A_{260}/A_{280} ratio range for most applications is 1.7 – 2.3. The sponsor provided data demonstrating that samples with a broader range of A_{260}/A_{280} ratios yield acceptable results. A subset of RNA samples collected from patients in the CARGO study and a separate sample set obtained from a sample archive (non-CARGO). These sample sets were chosen for their wide

range of A_{260}/A_{280} ratios. A total of 398 samples with A_{260}/A_{280} ratios ranging from 1.5 to 11.8 were evaluated. 373 samples had an A_{260}/A_{280} ratio between 1.5 and 3.5. The results of the 18S rRNA gene assay are a measure of RNA template input as well as a measure of the ability to amplify the template. Additional QC parameters that assess sample quality, including presence of genomic DNA contamination, high normalization marker variation, missing or out of range gene assay values and high signal variation were applied to samples that passed the primary endpoint. These additional QC parameters also include a test for reaction efficiency (low abundance vs. high abundance transcripts).
95% of the samples with an A_{260}/A_{280} ratio between 1.5 and 3.5 met the 18s acceptance criterion and 90% of samples met all testing QC criteria. Only 24% of the samples with an A_{260}/A_{280} ratio between 3.6 and 11.8 met the 18 acceptance criterion and only 12% met all testing QC criteria. Data separated into 4 A_{260}/A_{280} ratio range bins and total within acceptable range (A_{260}/A_{280} = 1.5 to 3.5) are shown in the table below.

A_{260}/A_{280} ratio range	**# Samples**	**Met 18s criterion (C_T ≤15)**		**Did not meet 18s criterion (C_T >15)**		**Met all testing QC criteria**	
		#	%	#	%	#	%
1.5 to 2.1	235	226	96	9	4	217	92
2.2 to 2.8	128	120	94	8	6	111	87
2.9 to 3.5	10	7	70	3	30	6	60
3.6 to 11.8	25	6	24	19	76	3	12
1.5 to 3.5	**373**	**353**	**95**	**20**	**5%**	**334**	**90**

The acceptable A_{260}/A_{280} ratio range for AlloMap testing is 1.5 to 3.5.

d. Analytical specificity:

Immunosuppression therapy:

Serum drug levels for cyclosporine A, tacrolimus and sirolimus, were examined in the XDx CARGO database to assess the potential of immunosuppressant interference on the AlloMap test. These drugs were chosen based on common usage and on the inclusion of more than one mechanism of action.
Drug levels were extracted for the 700 CARGO samples used in the XDx Population study. The distribution of the three immunosuppressants ranged across and above the therapeutic levels typical of cardiac transplant patients. The level of concordance between AlloMap scores and biopsy grades were compared in samples with therapeutic levels versus those samples with drug levels greater than the therapeutic range.

Serum levels of immunosuppressants (cyclosporine A, tacrolimus and sirolimus) for the samples from the Population Study data set were obtained.

The distribution of all samples, and samples broken down into rejection (R) and no rejection (NR) subsets are shown for below, within and above the respective therapeutic ranges. Class definitions are: R is local biopsy grades ≥3A and NR is local biopsy grade <3A. Therapeutic ranges for cyclosporine A, tacrolimus and sirolimus are 150-300 ng/mL, 6-15 ng/mL and 5-15 ng/mL, respectively. Mean AlloMap scores are calculated for R and NR samples within and above therapeutic ranges.

Serum levels of cyclosporine A

Cyclosporine A level (ng/mL)	All	# R	% R	# NR	% NR	Mean AlloMap Score R	Mean AlloMap Score NR
None	354	16	44%	338	51%		
<150	31	1	3%	30	5%		
150-300	183	12	33%	171	26%	31.1	26.7
≥300	132	7	19%	125	19%	33.8	25.8

Serum levels of tacrolimus

Tacrolimus level (ng/mL)	All	# R	% R	# NR	% NR	Mean AlloMap Score R	Mean AlloMap Score NR
None	369	22	61%	347	52%		
<6	46	3	8%	43	6%		
6-15	235	11	31%	224	34%	30.7	26.7
≥15	50	0	0%	50	8%	—	26.7

Serum levels of sirolimus

Sirolimus level (ng/mL)	All	# R	% R	# NR	% NR	Mean AlloMap Score R	Mean AlloMap Score NR
None	619	31	86%	588	89%		
<5	20	3	8%	17	3%		
5-15	52	2	6%	50	8%	33.1	27.8
≥15	9	0	0%	9	1%	—	26.2

Conclusions: The distribution of cyclosporine A, tacrolimus and sirolimus levels are similar between R and NR sample classes. There were no rejection samples the above therapeutic range category for tacrolimus and sirolimus Where data was available to calculate, the mean AlloMap score for within and above therapeutic ranges was calculated. The mean AlloMap score for

rejection samples ranged from 30.7 to 33.8, and the mean AlloMap score for no rejection samples was 25.8 to 27.8. There was no detectable affect of drug level.
Therefore, there appears to be no interference of cyclosporine A, tacrolimus or sirolimus with AlloMap molecular expression testing.

CMV interference:
Since CMV infection is the most significant infection affecting transplant patients, AlloMap test scores were compared between CMV viremic patients and CMV non-viremic patients. 61 samples from CARGO; 11 CMV+ (3 R and 8 NR) and 50 CMV- (9 R and 31 NR) were analyzed. 10 CMV- samples were classified as mild rejection (MR). Plasma CMV viremia was established with the Roche AMPLICOR® quantitative DNA PCR assay. Student's t-test was used to compare average raw scores between the CMV+ and CMV- infection groups for all samples examined and for the R and NR sub-groups. There was no significant difference in raw scores between the CMV+ and CMV– patient samples. There was no significant difference within R and NR sub-groups tested. Overt CMV viremia does not impact AlloMap test performance results.

Potential interference by compounds/drugs:

Hemoglobin: To determine if hemoglobin (Hgb) interferes with the performance of the AlloMap test, a human hemoglobin (hHgb) solution was prepared at a concentration of 20.0 mg/ml in PBS. 50 µL, 100 µL and 200µL of this solution were added to separate lysate samples resulting in the addition of 0.5mg/mL, 1.0mg/mL and 2.0mg/mL, respectively. RNA yield, purity and assay performance for each set of samples was compared to controls without added hemoglobin. Except for the addition of hHgb to the lysates, the blood samples were processed, and frozen according to standard operating procedures (SOP). RNA and cDNA sample preparation, AlloMap testing, and quality control were performed according to established SOPs.

Heparin: To determine if heparin in the peripheral blood sample collected interferes with the performance of the AlloMap test, CPT tubes containing heparin anticoagulant were used to draw peripheral blood samples from volunteer XDx donors. Blood samples were drawn in CPT tubes containing citrate as the anticoagulant from the same donors as controls. The heparin present in the blood samples collected in the CPT tubes with heparin anticoagulant was 13.3 U/mL. Except for the anticoagulant in the blood collection tube (heparin vs citrate), the blood samples were processed, and frozen according to standard operating procedures (SOP). RNA and cDNA sample preparation, AlloMap testing, and quality control were performed according to established SOPs.

Acetaminophen, acetylsalicylic acid, triglycerides or bilirubin: To

determine if a high level of acetaminophen, acetylsalicylic acid, triglycerides or bilirubin present in peripheral blood samples collected for AlloMap testing interferes with the performance of the test, the following study was performed. Samples for AlloMap testing were collected in CPT blood collection tubes. Once collected, the blood is centrifuged to separate the peripheral blood mononuclear cells (PBMC) and plasma. The separated PBMC and plasma were mixed together and poured into 5.0 ml phosphate buffered saline (PBS), mixed again and centrifuged to pellet the PBMC. The PBS-plasma supernatant was decanted and the tube was drained to achieve a "dry" pellet of cells prior to addition of the lysing reagent used to release and preserve the PBMC RNA. Any circulating interferent present in the blood sample when collected will be present in the separated plasma-PBMC fraction following centrifugation of the CPT tube. In this study, two CPT tubes were drawn from each of 5 donors. The plasma-PBMC fraction from each of the two tubes per donor was pooled to ensure a common starting material, and then split into a Control sample and a Test (spike-in) sample.

Compound	Conc. tested
Acetaminophen	1324 μmol/L
Acetylsalicylic acid	3.62 mmol/L
Bilirubin	20 mg/dL
Triglycerides (triolein)	337 mmol/L

All subsequent sample preparation steps were performed according to established AlloMap testing SOPs. The samples were frozen a minimum of overnight. AlloMap testing was performed and sample quality control was assessed following standard procedures. Each sample pair (sample + compound and sample + diluent only) was tested in the same testing batch using the same reagents and instrumentation.

Results demonstrate that heparin, hemoglobin, acetylsalicylic acid, acetaminophen, triglyceride or bilirubin present in a CPT peripheral blood sample does not interfere with performance of the AlloMap test.

Genomic DNA interference:
The sponsor provided data to demonstrate the amount of genomic DNA contamination in purified mononuclear cell RNA that can be present without interfering with the AlloMap test. Genomic DNA contamination up to 25% of the lowest abundance housekeeping gene does not interfere with the AlloMap test. The difference between the raw AlloMap score in the presence of up to 25% gDNA and the AlloMap score for the same sample after reducing gDNA to less than 12.5% is within the 99% CI for the AlloMap test. The AlloMap

sample QC requires the gDNA be less than or equal to 12.5% of the lowest abundance housekeeping gene to pass, but even double that amount has no influence on the AlloMap score. Therefore, genomic DNA as controlled by the AlloMap testing procedures does not interfere with the AlloMap test.

f. Assay cut-off:
Not Applicable.

2. Comparison studies:
   a. Method comparison with predicate device:
   Not applicable.
   b. Matrix comparison:
   Not applicable.

3. Clinical studies:
   a. Clinical Sensitivity:
   See c below.
   b. Clinical specificity:
   See c below.
   c. Other clinical supportive data (when a. and b. are not applicable):
   Clinical validation was performed to evaluate the performance characteristics of the AlloMap Test in cardiac transplant patients. Samples used for the study were derived from the prospective, observational multi-center Cardiac Allograft Rejection Gene Expression Observational (CARGO) study[1]. The objective of the clinical validation study was to estimate predictive values for the AlloMap Test for acute cellular rejection in the intended clinical population.
   The study used the following definition for rejection: a local biopsy grade ≥3A that was also assigned grade ≥3A by at least one of the three panel pathologists ("confirmed rejection"). All local ≥3A biopsies were graded by the central pathologists. The no rejection class included all samples that did not qualify as rejection.

   A total of 300 samples, from 154 patients enrolled in CARGO not used to develop the AlloMap Test algorithm, were assayed with the AlloMap Test. These samples conformed to the following inclusion criteria:
   - At least 55 days since transplantation, and
   - More than 30 days after administration of immunosuppressive therapy for treating rejection.

   The AUC calculated from the 300 samples from 154 patients using the method of Emir *et al.* (1998) was 0.67 with 95% confidence interval from 0.56 to 0.78 calculated by bootstrap.

   The AUC for the 55-182 days post-transplant period was 0.71 with 95% confidence interval from 0.56 to 0.84. The AUC for the ≥183 days post-

transplant period was 0.67 with 95% confidence interval from 0.50 to 0.88.

The table below provides the clinical performance characteristics in two time periods post-transplant. (PPV = positive predictive value, NPV = negative predictive value)

AlloMap Score	>2 – 6 months (n=166 samples) % Pts Below	PPV ≥3A(2R)	PPV Std. Err.	NPV <3A(2R)	NPV Std. Err.	>6 months (n=134 samples) % Pts Below	PPV ≥3A(2R)	PPV Std. Err.	NPV <3A(2R)	NPV Std. Err.	AlloMap Score
≤19	<22.4	≤2.7%	≤0.1%	100.0%	0.0%	≤5.4	≤1.8%	0.0%	100.0%	0.0%	≤19
20	24.3%	2.8%	0.2%	100.0%	0.0%	8.1%	1.8%	0.1%	100.0%	0.0%	20
21	33.6%	2.5%	0.4%	99.8%	0.8%	9.8%	1.9%	0.1%	100.0%	0.0%	21
22	38.8%	2.7%	0.5%	99.9%	0.7%	11.0%	1.9%	0.1%	100.0%	0.0%	22
23	41.8%	2.9%	0.5%	99.0%	0.6%	14.1%	2.0%	0.1%	100.0%	0.0%	23
24	47.5%	3.2%	0.6%	99.1%	0.6%	18.4%	2.1%	0.1%	100.0%	0.0%	24
25	56.0%	3.8%	0.7%	99.2%	0.5%	22.1%	2.2%	0.1%	100.0%	0.0%	25
26	61.4%	3.8%	0.9%	99.0%	0.5%	26.8%	2.3%	0.1%	100.0%	0.0%	26
27	63.6%	3.4%	1.0%	98.7%	0.5%	31.6%	1.9%	0.4%	98.7%	0.9%	27
28	68.3%	3.3%	1.1%	98.8%	0.5%	39.1%	2.1%	0.5%	98.8%	0.7%	28
29	73.7%	4.0%	1.3%	98.6%	0.4%	40.8%	2.1%	0.5%	99.0%	0.7%	29
30	77.2%	4.6%	1.6%	98.6%	0.4%	50.6%	2.1%	0.6%	98.7%	0.6%	30
31	81.0%	3.3%	1.6%	98.2%	0.4%	54.1%	2.3%	0.7%	98.8%	0.6%	31
32	85.6%	2.9%	2.0%	98.0%	0.3%	63.1%	2.9%	0.9%	99.0%	0.5%	32
33	89.4%	4.0%	2.7%	98.1%	0.3%	72.4%	3.8%	1.3%	99.1%	0.4%	33
34	91.7%	5.0%	3.5%	98.2%	0.3%	79.1%	4.1%	1.7%	98.9%	0.4%	34
35	94.5%	5.7%	4.8%	98.1%	0.2%	84.1%	4.0%	2.2%	98.7%	0.4%	35
36	97.3%	7.6%	13.8%	98.1%	0.2%	90.2%	5.4%	3.2%	98.7%	0.3%	36
37	97.8%	9.5%	21.1%	98.1%	0.2%	91.7%	–	-	98.4%	0.2%	37
38	100.0%	–	-	97.9%	0.0%	96.5%	–	-	98.2%	0.0%	38
39	100.0%	–	-	97.9%	0.0%	97.7%	–	-	98.2%	0.0%	39

[1] Deng, M.C., et. al. "Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling," *American Journal of Transplantation,* 6(1):150-60 (2006).

4. Clinical cut-off**:**
   See above for clinical performance characteristics.

5. Expected values/Reference range:
   The AlloMap Test service provides clinicians with an AlloMap Score between 0 and 40. The data and clinical performance characteristics for each numerical score are provided on a Report that provides the score.

N. Instrument Name: AlloMap HTx Specific Software and the XDx LIMS Software

O. System Descriptions:

1. Modes of Operation:
   Automated
2. Software:
   The Allomap HTx Specific Software parses the raw C_T values, calculates data sets, performs the QC check and calculates the AlloMap score. The XDx LIMS

Software guides and analyzes, for purposes of quality control, steps of sample preparation and is also the user interface (e.g., produces the test report).

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
Yes ___X____ or No _____

The sponsor's submitted software documentation demonstrated that the software design meets the stated requirements for this device and were verified and validated.

3. Specimen Identification:
   Sample classification is assigned by an identification code on the sample tube sent from the location where the test was ordered.
4. Specimen Sampling and Handling:
   The LIMS software guides the batch processing of multiple samples through the following steps: RNA purification and quantification, cDNA synthesis and preparation for the qRT-PCR assay.
5. Calibration:
   No user calibration required.
6. Quality Control:
   The LIMS software evaluates the results of each individual sample after each step of the sample preparation and qRT-PCR process and compares the results to pre-set acceptance criteria for each step. If a sample fails QC, the LIMS determines what steps to repeat based on what criteria was not achieved. The LIMS software also evaluates the results of a batch and flags the user if two or more samples per batch fail a particular step. The user will investigate the possible causes and perform re-testing if that's deemed acceptable.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:
None.

Q. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:
The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 862.1163 with special controls. The special control guidance document "Class II Special Controls Guidance Document: Cardiac Allograft Gene Expression Profiling Test Systems" will be published soon.