510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k141575

B. Purpose for Submission:
New device

C. Measurand:
Lipoprotein-associated phospholipase A_2 (Lp-PLA_2)

D. Type of Test:
Quantitative enzyme assay

E. Applicant:
diaDexus, Inc.

F. Proprietary and Established Names:
PLAC Test for Lp-PLA_2 Activity
Lp-PLA_2 Activity Test Calibrators
Lp-PLA_2 Activity Test Controls

G. Regulatory Information:

1. Regulation section:
   21 CFR § 866.5600 Low-density lipoprotein immunological test system
   21 CFR § 862.1150 Calibrator
   21 CFR § 862.1660 Quality control material (assayed and unassayed)

2. Classification:
   Class II for 866.5600 and 862.1150
   Class I reserved for 862.1660

3. Product code:
   NOE Test, system, immunoassay, lipoprotein-associated phospholipase A_2
   JIT Calibrator, secondary
   JJX Single (specified) analyte controls (assayed and unassayed)

4. Panel:
   Immunology (82) for 866.5600
   Clinical Chemistry (75) for 862.1150 and 862.1660

H. Intended Use:

1. Intended use(s):
   See indication(s) for use

2. Indication(s) for use:
The PLAC Test for Lp-PLA_2 Activity is an enzyme assay for the in vitro quantitative determination of Lp-PLA_2 (lipoprotein-associated phospholipase A_2) activity in EDTA plasma and serum on automated clinical chemistry analyzers. Lp-PLA_2 activity is to be used in conjunction with clinical evaluation and patient risk assessment as an aid in predicting risk of coronary heart disease (CHD) in patients with no prior history of cardiovascular events.

The Lp-PLA_2 Activity Test Calibrators are intended to establish points of reference that are used in the determination of values in the measurement of Lp-PLA_2 activity by the PLAC Test for Lp-PLA_2 Activity.

The Lp-PLA_2 Activity Test Controls are intended for use as a quality control tool to monitor the performance within the clinical range of the PLAC Test for Lp-PLA_2 Activity, an enzyme assay for the quantitative determination of Lp-PLA_2 activity.

3. Special conditions for use statement(s):
For prescription use, for *in vitro* diagnostic use

The sponsor included the following boxed warnings in the "Warnings and Precautions" section of the package insert:

- Dilution of samples will create erroneous results. Samples cannot be diluted at any Lp-PLA2 activity level. Samples with Lp-PLA2 values above the measuring range should be reported as >382 nmol/min/mL. In the Clinical Validation Study, > 99% of subjects reported values ≤ 382 nmol/min/mL.
- Hemolyzed samples interfere with the assay and should not be tested. Samples that are visibly hemolyzed should be redrawn. Testing hemolyzed samples with >1.0 mg/mL hemoglobin may cause erroneous results.
- Reversing the positions of the reagents on the analyzer will lead to erroneous results. Make sure to load the R1 reagent in the right location on the analyzer, R1 position, and the R2 reagent in the instrument's R2 position.

4. Special instrument requirements:
All data for this submission was generated using Beckman Coulter AU400 Clinical Chemistry Analyzers.

I. Device Description:

The components of the PLAC Test for Lp-PLA_2 Activity are ready for use and include:

PLAC Test for Lp-PLA_2 Activity Reagent which consists of:

- R1: Buffer
- R2: Lp-PLA_2 Substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) Phosphatidylcholine

Lp-PLA_2 Activity Test Calibrators which consist of a set of 5 calibrators made with recombinant Lp-PLA_2 protein

Lp-PLA_2 Activity Test Controls which consist of two levels of Lp-PLA_2 controls made with recombinant Lp-PLA_2 protein

PLAC Test for Lp-PLA_2 Activity is available in multiple kit configurations. Kit configurations include device components (reagents, calibrators and controls) sufficient for 50 to 3000 tests.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   diaDexus PLAC Test

2. Predicate K number(s):
   k030477

3. Comparison with predicate:

Item	Device PLAC Test for Lp-PLA_2 Activity	Predicate (k030477)
Similarities		
Intended use and indications for use	For the quantitative determination of Lp-PLA_2 (lipoprotein-associated phospholipase A_2) to be used in conjunction with clinical evaluation and patient risk assessment as an aid in predicting risk of coronary heart disease	same
Measured analyte	Lp-PLA_2	same
Calibrators	A set of calibrators made with recombinant Lp-PLA_2 protein in a protein matrix	same
Control materials	2 levels made with recombinant Lp-PLA_2 protein in a stabilizing diluent	same
Differences		
Test principle	Enzyme kinetics activity rate of change using a standard curve	Enzyme immunoassay (ELISA)
Specimen type	Serum and EDTA plasma	EDTA plasma
Measurement units	Activity, nmol/min/mL	Concentration, ng/mL
Instrument	Automated clinical chemistry analyzer	Microtiter plate reader
Measuring Range	10-382.2 nmol/min/mL	1.2–2000 ng/mL

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

- Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline (EP5-A2)
- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (EP6-A)
- Interference Testing in Clinical Chemistry; Approved Guideline (EP7-A2),
- Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline (EP17-A2)
- Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline (EP25-A)
- Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline (C28-A3c)
- Medical devices - Symbols to be used with medical device labels, labelling, and information to be supplied - Part 1: General requirements (ISO 15223-1 Second Edition 2012-07-01)

L. Test Principle:

In the PLAC Test for Lp-PLA_2 Activity, Lp-PLA_2 in plasma or serum, hydrolyzes the sn-2 position of the substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine, producing 4-nitrophenyl succinate. The latter is immediately further hydrolyzed to produce the colored reaction product, 4-nitrophenol. The rate of formation of 4-nitrophenol is measured spectrophotometrically at 410 nm and the Lp-PLA_2 activity is calculated from the rate of change in absorbance. A set of five Lp-PLA_2 calibrators is used to generate a standard curve fit of change in absorbance versus Lp-PLA_2 activity level in nmol/min/mL from which the sample Lp-PLA_2 activity is derived.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>
   a. Precision/Reproducibility:

   CLSI EP5-A2 was used in developing the precision protocol. Testing was performed using 1 Beckman Coulter AU400 Clinical Chemistry Analyzer and 3 kit lots. Four (4) native plasma samples, kit control low (Control L), and kit control high (Control H) were tested with 2 replicates per run, 2 runs per day for 20 days. Within-lab precision includes within-run (repeatability), between-run, and between-day variance components. The results are summarized below where percent coefficient of variation is %CV, standard deviation is SD and the means and SDs are reported as nmol/min/mL:

Precision results for all lots combined-plasma

		Within-Run		Between-Run		Between-Day		Between-Lot		Within-Lab	
Sample	Mean n=240	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Control L	120.0	1.81	1.5%	2.16	1.8%	<0.05	0.0%	1.90	1.6%	3.40	2.8%
Control H	302.8	4.31	1.4%	5.03	1.7%	1.70	0.6%	4.60	1.5%	8.24	2.7%
Sample 1	113.0	1.41	1.2%	1.74	1.5%	0.65	0.6%	3.55	3.1%	4.24	3.8%
Sample 2	208.1	2.97	1.4%	2.63	1.3%	2.74	1.3%	4.99	2.4%	6.94	3.3%
Sample 3	244.4	3.67	1.5%	3.61	1.5%	3.54	1.5%	5.92	2.4%	8.61	3.5%
Sample 4	314.6	3.86	1.2%	4.34	1.4%	1.80	0.6%	7.68	2.4%	9.79	3.1%

The sponsor also performed precision studies using serum samples. Testing was performed using 1 Beckman Coulter AU400 Clinical Chemistry Analyzer and 3 kit lots. Four (4) native serum samples, kit control low (Control L), and kit control high (Control H) were tested with 2 replicates per run, 2 runs per day for 20 days. Within-lab precision includes within-run (repeatability), between-run, and between-day variance components. The results are summarized below where percent coefficient of variation is %CV, standard deviation is SD and the means and SDs are reported as nmol/min/mL:

Precision results for all lots combined-serum

		Within-Run		Between-Run		Between-Day		Between-Lot		Within-Lab	
Sample	Mean n=240	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Control L	122.2	2.27	1.9%	1.74	1.4%	<0.05	0.0%	3.29	2.7%	4.36	3.6%
Control H	296.7	5.00	1.7%	4.76	1.6%	0.55	0.2%	1.64	0.6%	7.12	2.4%
Sample 1	99.0	1.27	1.3%	1.74	1.8%	0.45	0.5%	4.78	4.8%	5.26	5.3%
Sample 2	204.4	2.69	1.3%	3.80	1.9%	1.12	0.5%	8.55	4.2%	9.80	4.8%
Sample 3	259.9	3.27	1.3%	5.35	2.1%	<0.05	0.0%	8.60	3.3%	10.64	4.1%
Sample 4	330.1	4.30	1.3%	6.23	1.9%	0.77	0.2%	13.30	4.0%	15.32	4.6%

b. Linearity/assay reportable range:

CLSI EP6-A was used in developing the linearity protocol. Testing was performed using 1 Beckman Coulter AU400 Clinical Chemistry Analyzer and 3 kit lots. High

native plasma samples were spiked with recombinant Lp-PLA_2 to create the high plasma samples and low plasma samples were prepared with animal plasma. Both were combined for the interval between the two extreme levels. Three sets of high/low plasma samples combined at 13 levels (total 39 samples) ranging in Lp-PLA_2 activity from 6 to 382 nmol/min/mL were tested in triplicate (117 test points) using each of 3 lots. Replicate values of each sample were averaged to determine the mean measured value of the sample. All actual results were plotted and the slope, intercept, and R^2 value were calculated using a least squares weighted regression. The degree of nonlinearity was assessed by analysis of second and third order polynomial regression.

Results of the linearity analyses demonstrated that from 6 to 382.2 nmol/min/mL there was a maximum deviation from linearity of ≤ 7%.

The sponsor claims that the measuring range of the assay is 10 to 382.2 nmol/min/mL.

The sponsor also evaluated the linearity of the device using serum samples. The results of those studies support the sponsor's reportable range claim.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):* Calibrators are traceable to internal master calibrators. Purified recombinant Lp-PLA_2 protein is utilized as the analyte in the proposed calibrators and controls. Kit calibrators are value assigned using the master calibrators and are assayed in multiple replicates and multiple runs. The grand mean for each kit calibrator is calculated. The kit calibrators must meet specifications compared to the activity values of the master calibrators. The value assignment process was reviewed and found acceptable.

Controls are prepared using purified recombinant Lp-PLA_2 protein. They are value assigned using multiple replicates, multiple runs, multiple calibrations and multiple days. The grand mean is calculated and must fall within a specified range. The control range is determined by calculating a ± 15% range from the overall mean. The resulting lot specific means and ranges for each control are supplied in the certificate of analysis provided with each kit lot. The value assignment process was reviewed and found acceptable.

Shelf life and open vial stability protocols for the controls and calibrators were reviewed and found acceptable. The sponsor claims that the controls and calibrators are stable for 12 months stored at 2 to 8°C. Once opened, the controls and calibrators are stable for 3 months when stored at 2 to 8°C.

Sample stability: The sponsor performed studies to demonstrate the stability of EDTA plasma samples and serum samples prior to centrifugation and after centrifugation. Based on the results of the studies, the sponsor recommends the following storage conditions:

Prior to centrifugation samples can be kept up to 4 hours at 20-22°C or up to 30 hours at 2-8°C prior to separation.

Following centrifugation EDTA plasma samples and serum samples can be tested immediately or stored prior to testing under the following conditions:

- 24 hours at 20-26°C
- Up to 2 weeks at 2-8°C
- Up to 18 months at -20°C
- Up to 2 years at -70°C

Plasma and serum samples can be freeze-thawed up to 5 times after freezing at either -70°C or -20°C.

Detection limit:
Detection limits were determined using the principles in CLSI EP17-A2 with 3 kit lots on 1 Beckman Coulter AU400 Clinical Chemistry Analyzer.

The limit of blank (LoB) was estimated using 60 replicates of the 0 level calibrator run over 3 separate runs for each kit lot. The LoB was calculated by the classical approach using the nonparametric option described in EP17-A2.

The limit of detection (LoD) was estimated using 5 plasma samples with Lp-PLA_2 activity levels at approximately 0.6 to 3.2 nmol/min/mL. Each sample was tested in 5 replicates over 5 days (n=25 for each sample) using 3 kit lots. The data measurements for each lot showed relatively consistent variability across the low level samples and were analyzed by the classical approach using the parametric analysis described in EP17-A2.

The limit of quantitation (LoQ) was estimated using the precision profile approach described in CLSI EP17-A2. Four native plasma samples were prepared to produce 8 total samples with low Lp-PLA_2 activity levels ranging from 0.6 to 5.9 nmol/min/mL. The samples were tested in 5 replicates over 5 days (n=25 for each sample) using each of the 3 kit lots. For each kit lot, a relationship was determined between the %CV and the Lp-PLA_2 activity for the set of low samples using a power function model. For each kit lot, this equation was then used to determine the Lp-PLA_2 activity at which the coefficient of variation (CV) was equal to 20%.

The LoB was estimated to be 0.40 nmol/min/mL.
The LoD was estimated to be 0.74 nmol/min/mL.
The LoQ was estimated to be 1.30 nmol/min/mL.

In the package insert the sponsor claims that the analytical sensitivity (limit of quantitation) of the assay is 10 nmol/min/mL.

The sponsor also estimated the LoB, LoD and LoQ using serum samples. The results of these studies support the label claim for the LoQ.

e. Analytical specificity:

Endogenous Interference: CLSI EP7-A2 was used in developing the studies to determine potential interference from endogenous substances. Testing was performed using 1 Beckman Coulter AU400 Clinical Chemistry Analyzer and one kit lot with 4 native plasma samples with Lp-PLA_2 activity ranging from 85 to 315 nmol/min/mL. The endogenous substances were titrated into samples with known levels of each endogenous substance and 5 replicates were tested for each level. The results from samples spiked with endogenous substances were averaged, compared to matching control sample results and % recoveries were calculated. Samples spiked with the following substances at the listed concentrations demonstrated percent recoveries of 90–110% in the measured Lp-PLA_2 activity level, calculated based on the expected (control) value.

Substance	Concentration
Albumin	60 g/L
Conjugated Bilirubin	12 mg/dL
Unconjugated Bilirubin	20 mg/dL
Cholesterol	300 mg/dL
Triglycerides	400 mg/dL
Hemoglobin	100 mg/dL (1 mg/mL)

In the package insert the sponsor states that no appreciable interference was observed for the substances at the concentrations listed in the table above.

In the "Warnings and Precautions" section of the package insert the sponsor included the following prominent warning: "Hemolyzed samples interfere with the assay and should not be tested. Samples that are visibly hemolyzed should be redrawn. Testing hemolyzed samples with >1.0 mg/mL hemoglobin may cause erroneous results."

The sponsor also evaluated potential endogenous interfering substances using serum samples with Lp-PLA_2 activity ranging from 80 to 315 nmol/min/mL. The results of these studies support the claims in the package insert.

Exogenous Interference: CLSI EP7-A2 was used in developing the studies to determine potential interference from exogenous substances. Testing was performed using 1 Beckman Coulter AU400 Clinical Chemistry Analyzer on 4 native plasma specimens and 1 kit lot. Native plasma samples with Lp-PLA_2 activity ranging from 101 to 315 nmol/min/mL were spiked with two levels of the potential interferent and tested in duplicate. The interferent sample results were averaged and compared to matching control sample results, and % recoveries were calculated. Samples spiked with the following substances at the listed concentrations demonstrated percent recoveries of 90–110% in the measured Lp-PLA_2 activity level, calculated based on the expected (control) value.

Potential Interferent	Test Concentration Low	Test Concentration High
Acetaminophen, µmol/L	33	1324
Aspirin, µmol/L	720	3600
Atorvastatin, µmol/L	2	20
Diphenhydramine, µmol/L	2	20
Fenofibrate, µmol/L	42	125
Lisinopril, µmol/L	0.25	0.74
Niacin, µmol/L	480	4800
Tolbutamide, µmol/L	400	2300
Warfarin, µmol/L	10	33
Metformin, µmol/L	31	310
Clopidogrel bisulfate, µmol/L	10	100
Vitamin C, µmol/L	14	342

In the package insert the sponsor states that no appreciable interference was observed for the substances at the concentrations listed in the table above.

The sponsor also evaluated potential exogenous interfering substances using serum samples with Lp-PLA_2 activity ranging from 100 to 250 nmol/min/mL. The results of these studies support the claims in the package insert.

Cross Reactivity:
The following members of the PLA_2 enzymes superfamily (sPLA_2 (IIA), sPLA_2 (V), sPLA_2 (III) and cPLA_2/sPLA_2 (IIA)), known to be widely expressed in various human tissues, were titrated in buffer solutions and were tested directly in singlicate with the proposed device using 1 one Beckman Coulter AU400 Clinical Chemistry Analyzer. The concentrations tested were far above that expected in normal samples. The results were all ≤ 5 nmol/min/mL activity. The sponsor concluded that no significant activity was measured for any of the tested enzymes at the concentrations or activity levels listed in the table below.

Enzyme	Estimated Enzyme Concentration or Activity
cPLA_2/sPLA_2 (IIA)	16666.7 ng/mL
sPLA_2 (III)	6270 nmol/min/mL
sPLA_2 (V)	8 fold dilution of supplied stock
sPLA_2 (IIA)	1.667 ng/mL

f. Assay cut-off:
See clinical cut-off

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

   b. *Matrix comparison:*
      The sponsor performed 2 studies to support the use of different sample matrices. In one study, native (unaltered) blood samples from 131 donors collected into serum and K_2 EDTA plasma tubes (both without separators) were evaluated. Samples were tested in singlicate for both matrices and compared using Deming regression with equal measurement error for the independent and dependent variable (orthogonal regression). The results of the study are summarized below:

      Serum and K_2 EDTA plasma matrix comparison results

Lp-PLA_2 activity range	n	Slope (95% CI)	Intercept (95% CI)	r- value
56-357 nmol/min/mL	131	1.00 (0.97-1.03)	0.05 (-1.28-1.38)	0.988

      In a separate study, native (unaltered) blood samples from 21 donors collected into K_2 EDTA tubes with and without separator, K_3 EDTA tubes without separator, and serum collection tubes with and without separator were evaluated. The Lp-PLA_2 activity in the tested samples ranged from 94 to 323 nmol/min/mL. Samples were tested in singlicate for all matrices and in triplicate for the comparator matrix (i.e., K_2 EDTA Plasma without separator). Results were compared using Deming regression with equal measurement error for the independent and dependent variable (orthogonal regression). The results of the study are summarized below:

      Tube type matrix comparison results

Tube type compared to K_2 EDTA Plasma tubes without separator	Slope (95% CI)	Intercept (95% CI)	r- value
Serum Separating Tubes	1.05 (1.01 to 1.09)	-1.34 (-3.38 to 0.71)	0.997
K_2 EDTA Plasma Separating Tubes	1.06 (1.01 to 1.10)	-8.49 (-10.66 to -6.32)	0.996
K_3 EDTA Plasma without separator	1.00 (0.96 to 1.05)	0.74 (-1.56 to 3.03)	0.995
Serum without separator	1.01 (0.97 to 1.05)	-1.20 (-3.17 to 0.77)	0.997

      Based on the results of both studies, the sponsor concluded that K_3 EDTA plasma tubes without separator, K_2 EDTA plasma tubes with and without separator and serum collection tubes with and without separator can be used with the device.

3. Clinical studies:
   a. *Clinical Sensitivity:*
      Not applicable

b. Clinical specificity:
Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):
The clinical effectiveness of the proposed device in its intended use population was established in the PLAC Test for Lp-PLA_2 Activity Validation Study (Clinical Validation Study), a sub-study from the Reasons for Geographic And Racial Differences in Stroke (REGARDS) Study. REGARDS, a nationwide observational population study by the National Institutes of Health with almost 31,000 participants enrolled, is designed to follow an "all comers" population for potential racial and geographical differences in the risk of stroke and CHD events. REGARDS targeted a balanced enrollment of sex and race, and only enrolled black and white participants.

For the Clinical Validation Study, a subset of the REGARDS study was used. A total of 4,598 subjects (including a total of 933 cases and 3,665 controls) from the REGARDS study with no history of CHD were tested. The subjects ranged from 45 to 92 years of age and included 41.7% males and 58.3% females, and 41.5% blacks and 58.5% whites. Median follow-up time was 5.3 years (minimum of 5 days to maximum of 7.9 years). The primary endpoint used in the Clinical Validation Study was a composite of total CHD events, comprising 1) acute myocardial infarction, 2) coronary revascularization, and 3) CHD-related death.

The Clinical Validation Study used a case-cohort study design. Consistent with the intended use, subjects were randomly sampled from all REGARDS participants with no prior history of cardiovascular events at baseline enrollment (23,019 participants). This sampled population was then enriched with all remaining REGARDS CHD cases that were not included in the original random subset of the Clinical Validation Study. Descriptive statistics and statistical tests confirmed that the case-cohort was representative of the full REGARDS population. Efficacy analyses, Kaplan-Meier analyses and Cox proportional hazards models, were all weighted to adjust the Clinical Validation Study to the underlying prevalence of cases in the parent study population.

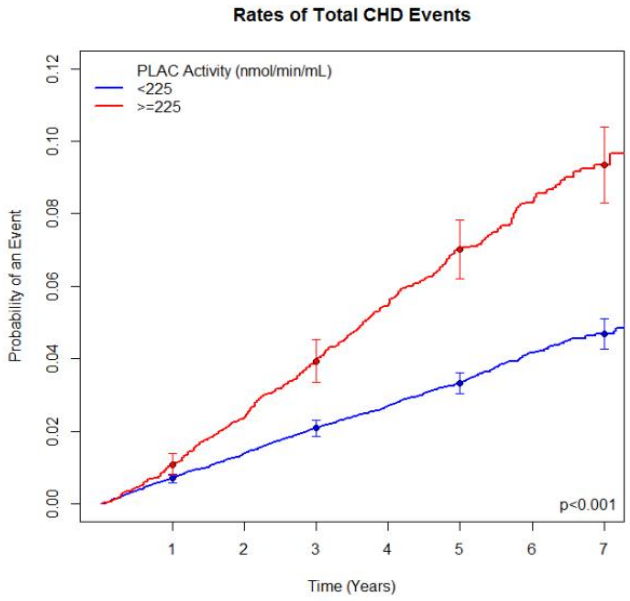
Frozen EDTA plasma specimens were tested with the PLAC Test for Lp-PLA_2 Activity following the recommended sample handling protocol and the observed values were analyzed. The sponsor provided data supporting the stability of the frozen EDTA plasma specimen used in the Clinical Validation Study for the length and condition of storage of the study specimens.

PLAC Activity Distribution: PLAC Test for Lp-PLA_2 Activity values in the sampled population ranged from a minimum of <10 nmol/min/mL to a maximum of >382 nmol/min/mL, with a median value of 178 nmol/min/mL (interquartile range (IQR) 145 – 216 nmol/min/mL).

Analysis Cut-point: The PLAC Test for Lp-PLA_2 Activity cut-point of 225 nmol/min/mL was pre-specified based on prior studies and publications using PLAC

activity in other independent cohorts. This analysis cut-point was used as a binary classifier in the study, dividing the study population into the low (below the cut-point) and high (at or above the cut-point) PLAC Activity groups.

Kaplan-Meier Analyses: In the Kaplan-Meier analysis of the Clinical Validation Study, absolute risk of CHD events was higher in the high PLAC Activity group (log-rank p-value < 0.001), as seen in the figure below. The absolute risk of CHD events of the two groups separated early and was consistently different after one year of follow-up and at all subsequent timepoints pre-specified for the analysis



The same analysis was also statistically significant within each sex and race analyzed independently (log-rank p-value < 0.001 for each analysis).

Absolute Rates of CHD by PLAC Activity Group: The absolute risk of CHD events at 5-year follow-up is presented for each PLAC Activity group of the Clinical Validation Study in the table below. The study indicated that the absolute risk of CHD events in the high PLAC Activity group is 2.1 times the absolute risk of CHD events in the low PLAC Activity group.

5-Year Risk of CHD Events (Kaplan-Meier Absolute Risk/Rate Analysis)

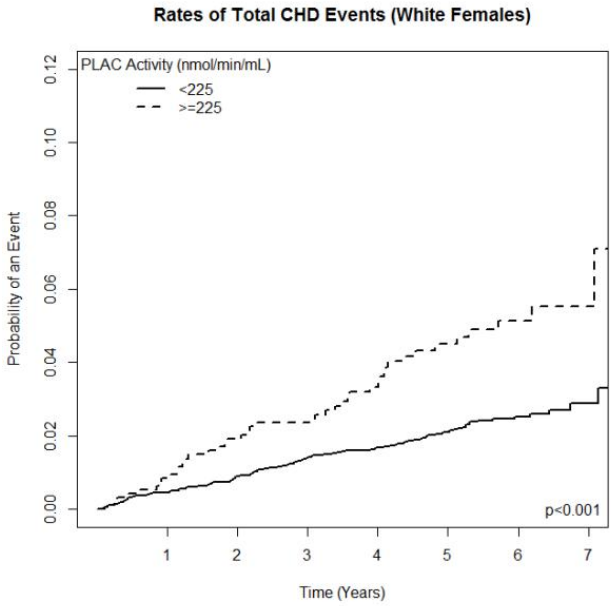
Population	Absolute Risk Pre-Test	Absolute Risk Post-Test Low Group (<225 nmol/min/mL)	Absolute Risk Post-Test High Group (≥225 nmol/min/mL)
All	4.1%	3.3%	7.0%
Females	2.7%	2.4%	5.3%
Males	6.1%	5.2%	7.7%
White	4.4%	3.5%	6.9%
Black	3.6%	3.2%	7.6%

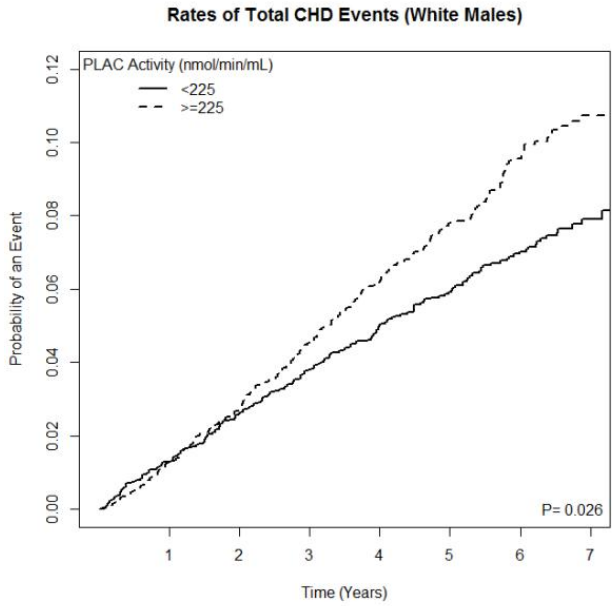
Race by Sex Subgroup Analyses: The predictive power of the PLAC Test for Lp-PLA_2 Activity was also assessed for each combination of race and sex of the Clinical Validation Study. The figure below shows the predictive power of PLAC Activity for each combination of race and sex using univariate Cox proportional hazards models [high versus low PLAC activity group], with 5-year Kaplan-Meier rate estimates provided for each PLAC Activity group. The PLAC Test for Lp-PLA_2 Activity was shown to be a statistically significant predictor of total CHD events in all subgroups, with a consistently higher HR estimate observed for women compared to that for men, regardless of race. Summary data is presented in the table below:

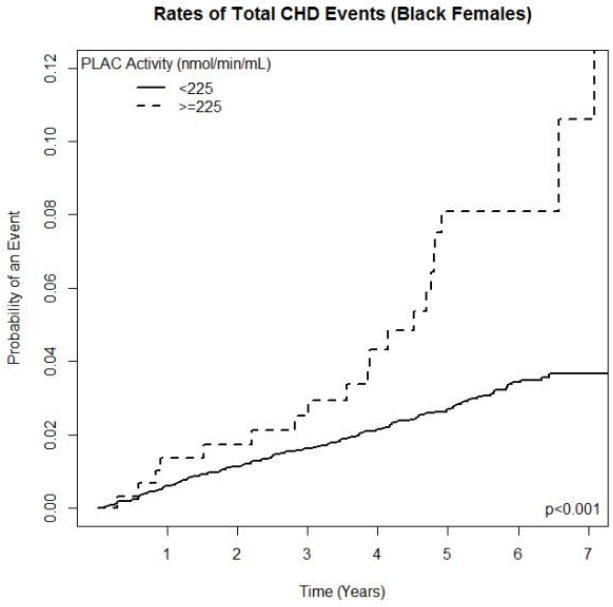
5-Year Absolute Risk of CHD Events and Cox Proportional Hazards Models (HR) for each combination of race and sex where the low group are subjects with Lp-PLA_2 activity <225 nmol/min/mL and the High group are subjects with Lp-PLA_2 activity ≥225 nmol/min/mL

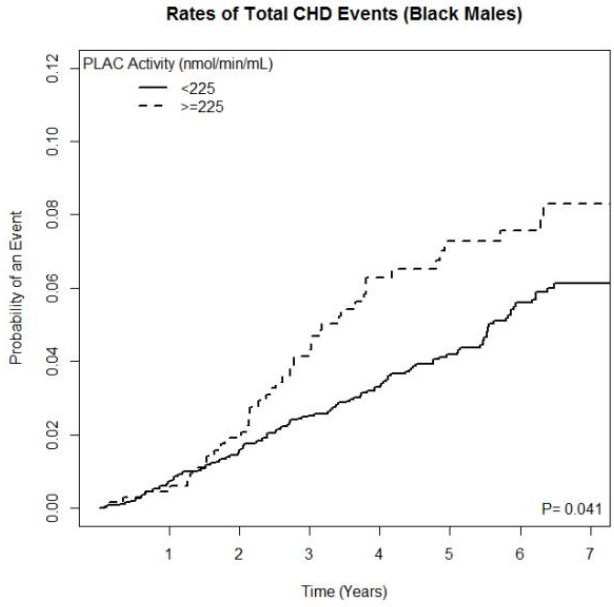
Population	HR (95%CI)	Absolute Risk low group (95% CI)	Absolute Risk high group (95% CI)	p value
All	2.04 (1.78-2.03)	3.3% (3.0-3.6%)	7.0% (6.2-7.8%)	<0.001
White Males	1.32 (1.09-1.60)	5.9% (5.1-6.8%)	7.8% (6.7-8.9%)	0.004
White Females	2.14 (1.52-3.02)	2.1% (1.7-2.5%)	4.5% (3.1-5.9)	<0.001
Black Males	1.49 (1.05-2.11)	4.2% (3.4-5.0%)	7.3% (5.0-9.4%)	0.024
Black Females	2.58(1.62-4.12)	2.7% (2.2-3.2%)	8.1% (4.4–11.7%)	<0.001

Kaplan-Meier Analyses in Each Race by Gender Subgroup:









Risk Adjusted Cox Proportional Hazards Model: Cox proportional hazards models showed the PLAC Test for Lp-PLA_2 Activity to be a statistically significant predictor

of total CHD events when comparing the high versus the low PLAC Activity groups of the Clinical Validation Study, with a fully-adjusted hazard ratio of 1.54 (1.31 – 1.82, $p < 0.001$) after adjustment for age, sex, race, diabetes, hypertension, smoking, LDL, and HDL.

4. Clinical cut-off:
   The PLAC Test for Lp-PLA_2 Activity cut-point of 225 nmol/min/mL was pre-specified based on prior studies and publications using PLAC activity in other independent cohorts. This analysis cut-point was used as a binary classifier in the study, dividing the study population into the low (below the cut-point) and high (at or above the cut-point) PLAC Activity groups.

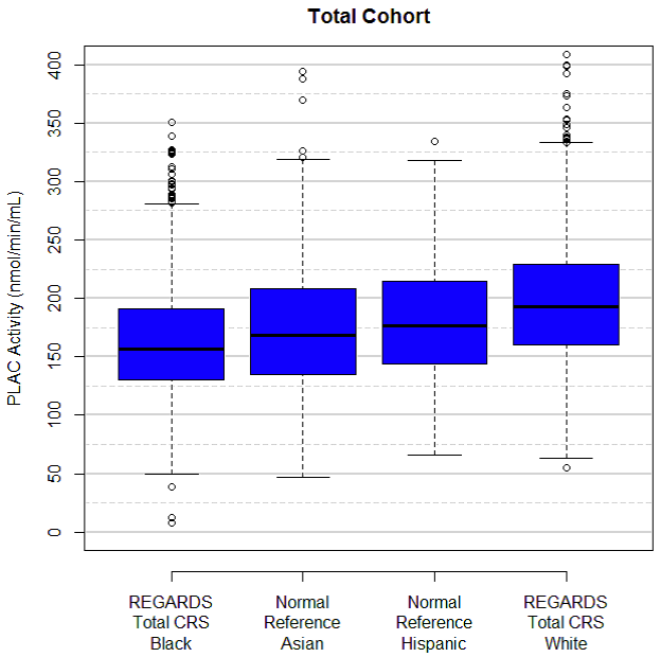
5. Expected values/Reference range:
   Fresh remnant native EDTA-plasma samples were obtained from an all-comers population of 300 subjects from a biobank and a clinical lab at 2 different geographical locations in the United States. The age range for inclusion was 35 to 75 years old (median age 57). The testing population included 154 males and 146 females of the clinically relevant age with a racial distribution of 38% White, 32% Black, 21% Hispanic and 9% Asian. Samples were tested with the PLAC Test for Lp-PLA_2 Activity in singlicate with 1 kit lot on 1 Beckman Coulter AU400 Clinical Chemistry Analyzer and the expected values were computed. The distribution of the samples tested is presented in the table below:

Percentile	All (N=300) Lp-PLA_2 (nmol/min/mL)	Female (N=146) Lp-PLA_2 (nmol/min/mL)	Male (N=154) Lp-PLA_2 (nmol/min/mL)
Min	50	50	70
2.5	84	74	92
5	94	86	102
25	137	130	149
33	148	139	155
50	167	154	176
67	196	179	204
75	211	200	219
95	276	264	295
97.5	303	300	329
99	369	339	>382
Max	>382	370	>382
Mean	176	166	186

In the package insert the sponsor indicates that the reference ranges are provided as guidelines only and are not intended to address “critical values” or medical decision limits since the test is intended to be used with the pre-determined cut-off. The sponsor

also states that each laboratory should establish its own reference intervals.

In order to address demographic groups not enrolled in the Clinical Validation Study the sponsor included the following information in the package insert: The distribution of PLAC Activity values observed in the Clinical Validation Study, which enrolled Blacks and Whites, encompasses those seen in other demographic groups (tested in a separate study) as shown in the figure below for Asians (n = 285) and Hispanics (n = 199).



N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.