

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

A. 510(k) Number:

k101853

B. Purpose for Submission:

Modified formulation of previously cleared device k072599

C. Measurand:

Lp-PLA₂ (lipoprotein-associated phospholipase A₂)

D. Type of Test:

Quantitative turbidimetric immunoassay

E. Applicant:

diaDexus

F. Proprietary and Established Names:

PLAC® Test Reagent Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
NOE	Class II	21 CFR 866.5600	82 Immunology

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The PLAC® Test Reagent Kit is a turbidimetric immunoassay for the quantitative determination of Lp-PLA₂ (lipoprotein-associated phospholipase A₂) in human serum or plasma on automated clinical chemistry analyzers, to be used in

conjunction with clinical evaluation and patient risk assessment as an aid in predicting risk for coronary heart disease, and ischemic stroke associated with atherosclerosis.

3. Special conditions for use statement(s):

For prescription use

4. Special instrument requirements:

The PLAC® Test was validated on the Hitachi® 917.

I. Device Description:

The diaDexus PLAC® Test assay consists of separately packaged reagents, calibrators and controls for the measurement of Lp-PLA₂ in serum or plasma. The PLAC® Test Kit consists of two reagents. R1 is a buffer solution with protein stabilizers and R2 is a suspension of polymeric microparticles coated with mouse monoclonal antibodies specific to Lp-PLA₂ (2C10 and 4B4).

J. Substantial Equivalence Information:

1. Predicate device name(s):

diaDexus, PLAC Test ELISA Kit

2. Predicate K number(s):

k062234

3. Comparison with predicate:

Similarities and Differences to the Predicate

Similarities

Item	Predicate – k062234 PLAC Test ELISA Kit	Modified - PLAC Test Reagent Kit
Intended Use/ Indications for use	For the quantitative determination of Lp-PLA ₂ (lipoprotein-associated phospholipase A ₂) in human plasma and serum, to be used in conjunction with clinical evaluation and patient risk assessment as an aid in predicting risk for coronary heart disease, and ischemic stroke associated with atherosclerosis.	Same
Key Reagent Components	Anti-PLA ₂ (2C10) and anti-PLA ₂ (4B4)	Same
Calibrators Matrix	Recombinant Lp-PLA ₂ antigen in a protein stabilizing diluent	Same
Control Matrix	Recombinant Lp-PLA ₂ antigen in a protein stabilizing diluent	Same
Controls Levels	2	Same

Differences

Item	Predicate – k062234 PLAC ELISA Kit	Modified - PLAC Test Reagent Kit
Measuring range	Up to 1000 ng/mL	25 to 500 ng/mL
Methodology	Dual monoclonal antibody sandwich ELISA read at 450 nm on a microwell plate reader	Immuno- Turbidimetric assay read at 570 nm on clinical chemistry analyzers
Reagent Configuration	Anti-Lp-PLA ₂ mAb (2C10) coated stripwells Wash buffer Enzyme conjugate anti-Lp-PLA ₂ mAb (4B4)-HRP TMB substrate Stop solution	Two reagent system R1- Buffer solution with protein stabilizers R2- antibody coated microparticles (mAbs 2C10 and 4B4)
Kit Configuration	Reagent, calibrators and controls all in one kit	Reagent, calibrators and controls in separate kits.
Calibrator Levels	6 levels - 0,50,100, 250, 500, 1000 ng/mL	5 levels - 0, 50, 100, 250, 500 ng/mL
Samples Types	Serum, EDTA, Heparin	Serum, K ₂ EDTA

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

CLSI Documents:

How to Define Reference Ranges; Approved Guideline (C28 A3).

Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline (EP5-A2)

Evaluation of the Linearity of Quantitative Measurement of Procedures; Approved Guideline (EP6-A)

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

Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP9 – A2)

Protocols for Determination of LOQ and LOD; Approved Guideline (EP17-A)

Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline (EP25A)

L. Test Principle:

The PLAC Test Reagent Kit is a turbidimetric immunoassay (TIA) using two specific monoclonal antibodies (2C10 and 4B4) coated to polymeric microparticles in suspension. As the concentration of Lp-PLA₂ increases, the turbidity of the suspension also increases, and this change in absorbance is read at 570 nm on a clinical chemistry analyzer. The change in absorbance is proportional to the amount of Lp-PLA₂ in the patient sample. A set of Lp-PLA₂ calibrators is used to plot a standard curve of absorbance (y-axis) versus Lp-PLA₂ concentration in ng/mL (x-

axis) from which the Lp-PLA₂ sample concentration can be determined by interpolation from the standard curve.

M. Performance Characteristics (if/when applicable):

The PLAC Test was validated on the Hitachi® 917

1. Analytical performance:

a. Precision/Reproducibility:

Intra-assay, inter-assay and total precision was assessed according to CLSI EP5 – A2 with three reagent lots. Two buffered controls and five serum samples (values ranging from 51 to 449 ng/mL) were assayed in duplicates in 2 separate runs on the Hitachi 917 analyzer for 20 days. The results are shown in the table below:

Reagent Lot	Sample	Mean Lp-PLA ₂ Conc. (ng/mL)	Intra-assay %CV n=40	Inter-assay %CV n=40	Total %CV n=80
1	Serum Sample 1	56.2	2.50%	4.60%	5.30%
	Serum Sample 2	108.9	1.30%	3.00%	3.30%
	Low Buffer Control	191.2	0.70%	0.90%	1.40%
	Serum Sample 3	250.1	1.00%	2.70%	2.90%
	Serum Sample 4	275.6	0.90%	2.60%	2.70%
	High Buffer Control	370.8	1.00%	0.70%	1.60%
	Serum Sample 5	439.1	1.70%	2.90%	3.30%
2	Serum Sample 1	55.4	2.10%	5.40%	5.80%
	Serum Sample 2	108.2	1.60%	2.50%	3.00%
	Low Buffer Control	189.2	0.70%	0.80%	1.50%
	Serum Sample 3	235.4	1.40%	1.90%	2.30%
	Serum Sample 4	275	1.00%	1.50%	1.80%
	High Buffer Control	367.8	0.90%	1.20%	1.50%

	Serum Sample 5	449.1	1.40%	2.00%	2.40%
3	Serum Sample 1	51.1	2.10%	5.30%	5.70%
	Serum Sample 2	101.3	1.40%	3.50%	3.70%
	Low Buffer Control	190.8	0.90%	1.00%	1.40%
	Serum Sample 3	227.6	0.80%	2.50%	2.80%
	Serum Sample 4	256.8	0.90%	2.50%	2.80%
	High Buffer Control	372.1	0.80%	1.10%	1.60%
	Serum Sample 5	416	1.70%	2.90%	3.40%

b. Linearity/assay reportable range:

The recovery of the reportable range was assessed according to CLSI EP6-A. Ten sample sets were prepared using 3 sets of low and high serum samples and 7 sets prepared using a high serum sample and bovine calf serum (negative serum source). The samples were assayed in duplicates with 3 lots on a single analyzer. Percent recovery was determined and linear regression analysis was conducted. The results are presented in the table below. The linear range was determined as 25-500 ng/mL.

Lot 1 Results:

Serum Samples Used	High Sample (ng/mL)	Low Sample (ng/mL)	Mean % Recovery	Linear Regression Parameters		
				Slope	Intercept	R ²
Pair 1 Human High, Human Low	509	63	102%	0.994	6.3988	0.9992
Pair 2 Human High, Human Low	527	63	99%	0.997	-1.5402	0.9988
Pair 3 Human High, Human Low	493	61	104%	1.0027	8.1817	0.9986
Pair 4 Human High, BCS Low; 50 ng	504	58	103%	0.9979	5.0396	0.9994
Pair 5 Human High, BCS Low; 50 ng	503	48	102%	0.9764	9.4004	0.9987
Pair 6 Human High, BCS Low; 50 ng	459	47	102%	0.988	5.8214	0.9996
Pair 7 Human	545	54	100%	0.9759	4.2625	0.999

Serum Samples Used	High Sample (ng/mL)	Low Sample (ng/mL)	Mean % Recovery	Linear Regression Parameters		
				Slope	Intercept	R ²
High, BCS Low; 50 ng						
Pair 8 Human High, BCS Low; 25 ng	503	21	103%	0.9852	6.9042	0.999
Pair 9 Human High, BCS Low; 25 ng	459	22	102%	0.9932	4.9752	0.9996
Pair 10 Human High, BCS Low; 25 ng	545	24	101%	0.9779	4.6619	0.9991

Lot 2 Results:

Serum Samples Used	High Sample (ng/mL)	Low Sample (ng/mL)	Mean % Recovery	Linear Regression Parameters		
				Slope	Intercept	R ²
Pair 1 Human High, Human Low	517	63	102%	0.9785	6.2799	0.9978
Pair 2 Human High, Human Low	523	64	100%	0.9942	12.823	0.9979
Pair 3 Human High, Human Low	515	62	102%	1.0176	4.0659	0.9977
Pair 4 Human High, BCS Low; 50 ng	519	58	102%	0.9898	6.528	0.9992
Pair 5 Human High, BCS Low; 50 ng	507	49	102%	0.9925	5.8824	0.9987
Pair 6 Human High, BCS Low; 50 ng	465	45	102%	0.9962	5.0256	0.9995
Pair 7 Human High, BCS Low; 50 ng	540	53	101%	0.9847	5.6655	0.9993
Pair 8 Human High, BCS Low; 25 ng	507	20	104%	0.9951	6.4466	0.999
Pair 9 Human High, BCS Low; 25 ng	465	21	103%	1.0017	3.7621	0.9996
Pair 10 Human High, BCS Low; 25 ng	540	25	101%	0.9907	3.8576	0.9993

Lot 3 Results:

Serum Samples Used	High Sample (ng/mL)	Low Sample (ng/mL)	Mean % Recovery	Linear Regression Parameters		
				Slope	Intercept	R2
Pair 1 Human High, Human Low	486	57	103%	1.0076	5.7815	0.999
Pair 2 Human High, Human Low	497	58	101%	1.0031	0.7276	0.9998
Pair 3 Human High, Human Low	472	58	103%	1.0018	6.4618	0.9991
Pair 4 Human High, BCS Low; 50 ng	486	54	103%	0.999	7.3023	0.9992
Pair 5 Human High, BCS Low; 50 ng	475	44	104%	0.9938	9.1189	0.9988
Pair 6 Human High, BCS Low; 50 ng	436	42	102%	1.0053	3.6708	0.9995
Pair 7 Human High, BCS Low; 50 ng	507	50	102%	0.9913	5.356	0.9995
Pair 8 Human High, BCS Low; 25 ng	475	20	104%	1.0044	5.8435	0.999
Pair 9 Human High, BCS Low; 25 ng	436	20	102%	1.0114	2.0716	0.9995
Pair 10 Human High, BCS Low; 25 ng	507	24	102%	0.9973	3.7246	0.9995

Additionally, the zero calibrator was spiked with varying levels of recombinant Lp-PLA₂ up to 3,000 ng/mL and tested to assess the prozone or high hook dose effect. The results of the hook effect study supports the sponsor's claimed measuring range of 25-500 ng/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The PLAC Test Calibrator Kit and Lp-PLA₂ Control Kit were previously cleared and have not been modified, see k072599 for traceability, stability, and expected values.

Shelf life stability testing was performed on 3 reagent lots stored capped at 37°C which was compared to the established PLAC Test Reagent Kit (k072599) also stored at 37°C. Each reagent was calibrated at each time point before testing samples. Each sample was tested at days 1,6,8,15,21, and 42 with two replicates per sample. Two buffer based controls and 5 serum samples ranging from 55-455 ng/mL were assayed. The reagent fill volumes

for each reagent kit were equivalent to a 100 test fill volume. The control reagent met the acceptance criteria. Real-time stability study is on going.

On-board stability testing of the reagent was performed with the reagent stored uncapped on-board the Hitachi® 917 analyzer. One lot of reagent was tested for on-board stability. Two controls and 5 human samples ranging from 58 to 448 ng/mL were run once a week for three weeks with a calibration performed at time 0. Five replicates were run for each sample. The results met the acceptance criteria with 90-110% average recovery from time 0 for at least 2 weeks. This supports the claimed on-board stability claim of 2 weeks.

d. Detection limit:

The sponsor conducted a Limit of Blank (LoB), Limit of Detection (LoD) and a Limit of Quantitation (LoQ) study. The detection limit was defined as the lowest quantity of Lp-PLA₂ that is significantly different from the absence of any Lp-PLA₂. In order to determine the LoD, the LoB was first defined by running 60 replicates of the 0 level calibrator using three reagent lots. Then, six low serum samples were prepared by diluting serum samples with fetal bovine serum (Lp-PLA₂ negative serum). Each sample was tested in duplicate over 5 days (10 results per sample, n=60).

The results of the LoB and LoD studies are summarized in the following table:

Lot	LoB (ng/mL)	LoD (ng/mL)
1	1.99	4.50
2	3.17	5.84
3	1.65	4.15

The LOQ was defined as the lowest concentration at which an analyte can be measured accurately without exceeding 20% CV. Three low serum samples were diluted with fetal bovine serum in a series of four dilutions per sample (1x, 0.5x, 0.25x, and 0.125x). Each sample was tested in duplicate in 10 runs by three reagent lots on one analyzer. The sponsor's predetermined total error goal was ≤ 5 ng/mL for $\text{LoQ} \leq 20$ ng/mL. The total error estimate was ≤ 5 ng/mL for all three lots, therefore, $\text{LoQ}=\text{LoD}$ (4.5, 5.8, 4.2 ng/mL).

The measuring range of the assay was established as 25 - 500 ng/mL based on the linearity studies and detection limit studies.

e. Analytical specificity:

Five endogenous substances (hemoglobin, triglycerides, total cholesterol, bilirubin, and human serum albumin) and exogenous substances (common and prescription drugs in the package insert) were evaluated for interference in the

assay. Potential interfering substances were spiked with interferent into four different serum samples with endogenous Lp-PLA₂ ranging from 140-500 ng/mL. Defined amounts of the interferents at and exceeding a relevant physiological range were added to the serum samples, assayed in duplicate with three lots of reagents. Interferent test concentrations were selected according to CLSI Protocol EP7-A2. Controls were prepared for each sample by spiking with solvent used for each substance stock solution. Controls were matched to test samples for volume and solvent concentration. Recoveries of test samples were calculated as result/matched control value x 100%. Testing results met the sponsor's acceptance criteria of less than 10% error in detected Lp-PLA₂ in the presence of the compound. There was no significant (less than or equal to 10%) interference detected for any of the substances listed below.

Endogenous Substances	
Potential Interferent	Test Concentration
Bilirubin	20 mg/dL
Cholesterol	500 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3,000 mg/dL
Albumin	5,000 mg/dL

Exogenous (OTC Drugs, etc.)	
Potential Interferent	Test Concentration
Acetaminophen	1654 µmol/L
Aspirin	3620 µmol/L
Atorvastatin	20 µmol/L
Clopidogrel bisulfate	100 µmol/L
Diphenhydramine	19.6 µmol/L
Fenofibrate	125 µmol/L
Lisinopril	0.74 µmol/L
Metformin	310 µmol/L
Niacin	4804 µmol/L
Tolbutamide	2370 µmol/L
Vitamin C	227 µmol/L
Warfarin	64.9 µmol/L

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The current PLAC Test turbidimetric immunoassay method testing 3 lots on the Hitachi 917 was compared to the PLAC Test ELISA microplate assay (k062234). The singlet point method comparison for the 742 unaltered stored human serum samples ranged from 25-500 ng/mL, obtained from banked study sets were within the sponsor's linear range. The results are shown in the table below.

Method Comparisons of PLAC Test Reagent Kit Lots vs. ELISA (Lots 1, 2 and 3)

PLAC Test Reagent Kit Lots	Slope	Intercept	R	# samples
Lot 1	1.1	0.1	0.92	742
Lot 2	1.1	-2.9	0.92	742
Lot 3	1.0	-2.2	0.92	742

b. *Matrix comparison:*

The sponsor conducted a matrix comparison study with the PLAC turbidimetric immunoassay. Twenty fresh samples were collected in each of the four tube types (Serum with separator (SST), Serum, EDTA Plasma, and EDTA Plasma with separator) and an aliquot of each sample was stored at 4°C and -70°C for 1 day post collection. An additional twenty banked and paired SST/K2EDTA samples obtained from a commercial sample bank were used to cover the upper assay range. These samples were stored at -70°C until use. Each sample was tested in triplicate using one lot of reagent on a single Hitachi 917 analyzer. Samples tested ranged from 59 to 418 ng/mL. The resulting recoveries ranged from 95% to 110%. The results of the regression analyses are shown in the table below.

Regression Parameters			
	Correl. Coeff (r)	Slope	Intercept
Serum vs. SST	0.9980	0.9998	0.0097
Plasma gel vs. SST	0.9837	0.9460	-1.747
Plasma vs. SST	0.9931	0.9048	2.968

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):

See clinical data provided in k030477. Also see method comparison in 2. a. above and the reference range study presented in 5. below.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

A screened normal healthy population who met the following inclusion criteria for the study: not on any lipid-modifying medications and have no reported evidence of cardiovascular disease. In addition, the following exclusion criteria were utilized: age <35, currently on lipid-modifying medications, and with a reported history of cardiovascular disease. The sponsor collected a set of 312 screened samples from a clinical research organization. Samples were of men and women between the ages of 35-75 years old (median age of 50 years).

The demographics are as followed:

Population	Number	Age Median (Range)
All	312	50 (35-75)
Males	168	52 (35-75)
Females	144	47 (35-69)

The results of this study and the results previously obtained with the predicate device in k062234 are presented in the following table:

Results of Reference Range Study				Predicate Device –k062234		
	All (n=312)	Males (n=168)	Females (n=144)	All (n=425)	Females (n=174)	Males (n=251)
Percentile	Lp-PLA2 (ng/mL)	Lp-PLA2 (ng/mL)	Lp-PLA2 (ng/mL)	Lp-PLA2 (ng/mL)	Lp-PLA2 (ng/mL)	Lp-PLA2 (ng/mL)
5	131	147	112	126	120	131
20	185	194	182	174	169	179
33	206	215	194	201	188	205
50	228	234	215	235	228	244
67	252	259	244	262	252	268

80	277	285	273	289	285	293
95	339	355	332	369	342	376

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.