

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

A. 510(k) Number:

k072599

B. Purpose for Submission:

Clearance of new methodology (turbidimetric immunoassay) for previously cleared device (enzyme immunoassay)

C. Measurand:

Lipoprotein-Associated Phospholipase A₂ (Lp-PLA₂)

D. Type of Test:

Quantitative turbidimetric immunoassay

E. Applicant:

diaDexus, Inc.

F. Proprietary and Established Names:

PLAC[®] Test Reagent Kit
PLAC[®] Test Calibrator Kit
Lp-PLA₂ Control Kit

G. Regulatory Information:

1. Regulation section:

Product Code	Classification	Regulation Section	Panel
NOE-test, system, immunoassay, lipoprotein-associated phospholipase a2	Class II	21 CFR 866.5600	82 Immunology
JIT- calibrator, secondary	Class II	21 CFR 866.1150	75 Chemistry
JJX- quality control material	Class I	21 CFR 862.1660	75 Chemistry

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 plasma or serum 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.

The **PLAC[®] Test Calibrator Kit** is intended to establish points of reference that are used in the determination of values in the measurement of Lp-PLA₂ by the PLAC[®] Test Reagent Kit.

The **Lp-PLA₂ Control Kit** is intended for use as a quality control tool to monitor the performance within the clinical range of the PLAC[®] Test Reagent Kit, a turbidimetric immunoassay for the quantitative determination of Lp-PLA₂.

3. Special conditions for use statement(s):

For professional use only

4. Special instrument requirements:

The PLAC Test was validated on the Roche 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 Reagent Kit consists of two reagents. R1 is a tris-based buffer solution and R2 is a suspension of latex microparticles coated with mouse monoclonal antibodies specific to Lp-PLA₂ (2C10 and 4B4).
- The PLAC[®] Test Calibrator Kit is a five level set of Lp-PLA₂ calibrators made with recombinant Lp-PLA₂ in a protein stabilizing buffer and used to calibrate the PLAC assay.
- The Lp-PLA₂ Control Kit is a two level set of Lp-PLA₂ controls made with recombinant Lp-PLA₂ in a protein stabilizing buffer.

J. Substantial Equivalence Information:

1. Predicate device name(s):

diaDEXUS PLAC Test

2. Predicate K number(s):

k062234, k050523 and k030477

3. Comparison with predicate:

Similarities		
	Predicate PLAC[®] Test k062234	Current Device
Intended Use	<p>REAGENT KIT The diaDexus PLAC[®] Test is an enzyme immunoassay for the quantitative determination of Lp-PLA₂ (lipoprotein-associated phospholipase A₂) 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.</p> <p>(Calibrators and controls were included in kit.)</p>	<p>REAGENT KIT The PLAC[®] Test Reagent Kit is a turbidimetric immunoassay for the quantitative determination of Lp-PLA₂ (lipoprotein-associated phospholipase A₂) in human plasma or serum 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.</p> <p>CALIBRATOR KIT The PLAC[®] Test Calibrator Kit is intended to establish points of reference that are used in the determination of values in the measurement of Lp-PLA₂ by the PLAC[®] Test Reagent Kit.</p> <p>CONTROL KIT The Lp-PLA₂ Control Kit is intended for use as a quality control tool to monitor the performance within the clinical range of the PLAC[®] Test Reagent Kit, a turbidimetric immunoassay for the quantitative determination of Lp-PLA₂.</p>
Analyte	Lp-PLA ₂	Same
Laboratory Environment	Professional laboratory	Same

Differences		
	Predicate PLAC[®] Test k062234	Current Device
Sample	Serum	Serum, EDTA-plasma, heparin-plasma
Reagent Components	Dual monoclonal antibody sandwich ELISA: <ul style="list-style-type: none"> • anti-Lp-PLA₂ mAb (2C10) coated strip wells • Wash Buffer • Enzyme Conjugate : anti-Lp-PLA₂ mAb (4B4)-HRP • TMB Substrate Solution • Stop Solution 	Two-reagent system: <ul style="list-style-type: none"> • R1: Tris-based buffer solution • R2: Suspension of anti-Lp-PLA₂ (mAbs 2C10 and 4B4) coated latex beads
Calibration	Six calibrators made with recombinant Lp-PLA ₂ in a buffered protein matrix (included in ELISA kit)	Five calibrators made with recombinant Lp-PLA ₂ in a buffered protein matrix (sold separately)
Calibration Conc.	0, 50, 100, 250, 500, 1000 ng/mL	0, 50, 100, 250, 500 ng/mL
Methodology	Microplate Enzyme immunoassay (ELISA)	Latex particle-enhanced turbidimetric immunoassay (particle agglutination)
Detection Method	Microplate spectrophotometer read at 450 nm	Automated clinical chemistry analyzers read at 570 nm

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

STANDARDS
Title and Reference Number
CLSI: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (EP6-A).
CLSI: Interference Testing in Clinical Chemistry; Approved Guideline (EP 7-A)
CLSI: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2)
CLSI: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5-A)

L. Test Principle:

The diaDexus PLAC Test is based on turbidimetric immunoassay technology utilizing two Lp-PLA₂ specific monoclonal antibodies (2C10 and 4B4) coated to latex microparticles. 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 concentration in the test sample can be determined. Two levels of controls are assayed to monitor performance within the clinical range of the assay. The concentration of Lp-PLA₂ in each sample and control is interpolated from the standard curve using a spline curve fit with calibration curve fitting software.

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 controls (143.9 and 449.5 ng/mL) and one serum (68.5 ng/mL) sample were run in duplicates in 2 separate runs on the Hitachi 917 analyzer for 20 days. The results shown below met the sponsors acceptance criteria (intra-assay precision CV <8% and total precision CV <10%).

Reagent Lot	Sample	Mean Lp-PLA ₂ Conc. (ng/mL)	Intra-assay %CV n=80	Inter-assay %CV n=20	Total %CV n=80
1	1	69	2.9	0.6	3.0
	2	143	2.4	1.3	2.7
	3	449	1.4	1.0	1.7
2	1	69	2.6	1.4	2.9
	2	143	1.8	1.3	2.2
	3	449	1.7	0.6	1.8
3	1	69	2.4	2.1	3.2
	2	144	2.0	1.6	2.5
	3	450	1.6	0.8	1.8

b. *Linearity/assay reportable range:*

The recovery of reportable range was assessed according to CLSI EP6-A. Two high Lp-PA2 serum samples were mixed with low samples to product 11 concentrations/data points. The samples were assayed in duplicates with 3 lots on a single analyzer. The five level dilution series was conducted on a low serum sample. To further assess the lower end of the claimed assay range, the sponsor conducted an additional linearity study. Native human Lp-PLA2 was diluted with fetal bovine serum (used as a negative serum source) to the lowest level of detection. Percent recovery was determined and linear regression analysis was conducted. Additionally, the zero calibrator was titrated with an extremely high level of recombinant Lp-PLA2 up to 1500 ng/mL and dilutions were tested to asses the prozone or high dose hook effect. Three lots of reagents were evaluated over three separate runs on one analyzer. The results below met the sponsor’s acceptance criterion (percent recovery between 90 and 110% of the established mean and no hook effect below 1500 ng/mL). The linear range is defined as 7-500 ng/mL.

Reagent Lot	High Sample ng/mL	Low Sample ng/mL	Average % Recovery	Linear Regression Parameters		
				Slope	Intercept	R2
Human Serum (lot 1)	483.4	109.9	94	1.00	-13.6	0.995
	487.1	104.2	98	1.01	-5.9	0.998
Human Serum (lot 2)	432.6	94.7	96	1.01	-8.8	0.998
	461.8	89.7	99	1.01	-5.1	0.999
Human Serum (lot 3)	460.2	98.3	95	1.03	-15.8	0.994
	472.3	95.8	98	1.00	-5.6	0.999
Human Serum	89.0	12.3	94	1.02	-3.3	0.992
Human High, bovine low	438.8	0	100	1.04	-6.9	0.997
Human High, bovine low	312.7	0	99	1.04	-6.9	0.997
Human High, bovine low	213.6	0	95	1.04	-6.9	0.997

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

The purified recombinant Lp-PLA2 antigen is utilized as the analyte in the

PLAC test calibrators and controls. A master calibrator was prepared from purified primary stock solution of recombinant Lp-PLA₂ that was quantitated by amino acid analysis. A master calibrator was prepared and the value was assigned using a panel of stored human serum samples that were characterized by multiple runs with the predicate device. The calibrators are manufactured to match the absorbance levels of the master calibrator at each calibration level (0, 50, 100, 250 and 500 ng/mL). Controls are manufactured to a target value, but assigned the actual value range generated by multiple PLAC test runs. Quality control acceptance criteria are that the calibrators must quantitate replicates of the quality control panel serum samples within +/- 10% of the established mean. The formulation used for both the calibrators and controls has been validated with the predicate device. The sponsor states a real-time stability of 16 months at 2 to 8° C.

d. Detection limit:

The sponsor conducted a Limit of Detection (LOD) and a Limit of Quantitation (LOQ) study. The detection limit was defined as the lowest concentration that is significantly different from zero. Calibrator 1 (0 ng/mL) and Calibrator 2 (20 ng/mL) were assayed 20 times with three reagent lots in the same run. The mean of the zero calibrator plus 2 standard deviations was calculated as 4.5, 5.1 and 4.0 ng/mL. The limit of quantitation was defined as the lowest analyte concentration where the % CV of precision is less than 20%. The 50 ng/mL calibrator was titrated down to zero with calibrator diluent in a series of 10 incremental levels. Each sample was assayed in replicates of 3 in 10 runs by three reagent lots on one analyzer. The study was also performed with a serum sample diluted with bovine serum to 10 levels ranging from 56 ng/mL down to 8 ng/mL. The limit of quantitation was estimated to be 7 ng/mL in all three reagent lots using the calibrator series of samples and 11 ng/mL with the serum samples. The results from the above studies met the sponsor's acceptance criteria for LOD and LOQ of less than 20 ng/mL. The sponsor's limit of quantitation is estimated to be 7.0 ng/mL and limit of detection is 4.0 ng/mL.

e. Analytical specificity:

Five endogenous substances (hemoglobin, triglycerides, total cholesterol, bilirubin and human serum albumin) found in blood and exogenous substances (common and prescription drugs in the package insert) were evaluated for interference in the assay. Potential interfering substances were spiked with the interferent into four different serum samples with endogenous Lp-PLA₂ in the range of 160 to 470 ng/mL range. 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, and any resultant biases in analyte quantitation were reported. The highest levels were chosen according to CLSI Protocol EP7-A recommended high testing levels. Controls were prepared for each sample by spiking with the solvent used for

each substance stock solution. Controls were matched to test samples for both volume and solvent concentration. Recoveries of test samples were calculated as result/matched control value x 100%. The sponsor's acceptance criterion was less than 10% error in detected Lp-PLA2 in the presence of the compound. There was no interference detected for any of the substances listed below.

<u>Potential Interferent</u>	<u>Endogenous</u>	<u>Exogenous (OTC Drugs, etc.)</u>	
	<u>Test Concentration</u>	<u>Potential Interferent</u>	<u>Test Concentration</u>
Bilirubin	20 mg/dL	Acetaminophen	1.66 µmol/L
Cholesterol	500 mg/dL	Aspirin	3330 µmol/L
Hemoglobin	10,000 mg/dL	Atorvastatin	20 µmol/L
Triglycerides	3000 mg/dL	Clopidogrel bisulfate	100 µmol/L
Total Albumin*	~6500 mg/dL	Diphenhydramine	19.6 µmol/L
		Fenofibrate	125 µmol/L
		Lisinopril	0.74 µmol/L
		Metformin	310 µmol/L
		Niacin	4800 µmol/L
		Pravastatin	100 µmol/L
		Tolbutamide	2400 µmol/L
		Vitamin C	227 µmol/L
		Warfarin	64.9 µmol/L

* 2.5 g/dL albumin added to plasma pool of presumptively 4 g/dL albumin

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

The current PLAC Test turbidimetric immunoassay method was compared to the PLAC Test ELISA microplate assay (k062234). The singlet point method comparison for the 794 unaltered stored human serum samples ranged from <7 to 499 ng/mL, obtained from banked study sets were within the sponsor's linear range. The results are shown in the table below.

Regression Parameters				
	Correl Coeff (r)	Slope	Intercept	N
Results	0.92	1.02	-24.5	794

b. *Matrix comparison:*

The sponsor conducted a recovery matrix and anticoagulant comparison study with the PLAC turbidimetric immunoassay. Ten plasma samples ranging from 79.4 to 201.3 ng/mL. The recovery ranges from 89 – 111% for EDTA

plasma, 83-102% for Na heparin, 87- 106% for Li heparin. Specific recovery percentages linear equations for the 10 samples are shown in the table below.

Sample	Serum	Plasma_K2 EDTA	%Rec Plasma to Serum	Na Heparin	%Rec Na Heparin to Serum	Lithium Heparin	%Rec Li Heparin to Serum
S01	180.0	182.8	102%	187.0	104%	188.0	104%
S02	112.9	101.7	90%	92.5	82%	103.8	92%
S03	165.6	170.3	103%	182.3	110%	180.7	109%
S04	155.3	173.5	112%	152.0	98%	163.1	105%
S05	91.9	95.5	104%	79.4	86%	82.7	90%
S06	197.4	201.3	102%	204.9	104%	211.5	107%
S07	70.3	79.4	113%	80.7	115%	81.7	116%
S08	157.1	156.6	100%	162.6	103%	166.4	106%
S09	161.2	142.2	88%	149.5	93%	140.8	87%
S10	170.3	166.5	98%	172.2	101%	176.9	104%
			101%		100%		102%
			$y=.977x +4.10$		$y=1.09x-13.641$		$y=1.08x-8.76$
			y=plasma x=serum		y=Sodium Heparin x=serum		y=Lithium Heparin x=serum

Additionally, the sponsor conducted a matrix comparison study using 95 matched serum and EDTA samples that ranged from 132.1 to 402 ng/mL. The obtained recoveries ranged from 86.1 to 118.9% and the obtained linear regression equation was $Y(\text{serum}) = 0.986(\text{EDTA}) + 10.52$.

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.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The sponsor claims the same reference range as the initial device (k030477). The stored reference range samples were re-assayed by the PLAC test turbidimetric immunoassay to assess whether the results were statistically different from historical results. 155 plasma samples (61 females and 94 males) were assayed by the PLAC Test turbidimetric immunoassay in duplicate with one lot of reagents.

Additionally, the sponsor tested the samples against the ELISA PLAC test k050523. The mean results for both tests were compared with the historical reference results using the Two Sample t-Test for assessment of statistical differences. The sponsor’s acceptance criterion is a Two-Sample t-Test analysis result of $p \geq 0.05$, which states no statistical difference. The results are presented in the table below.

Method	Lp-PLA ₂ ng/mL		
	k072599 Turbidimetric immunoassay	k050523 ELISA	k030477 Historical Values
Lp-PLA ₂ Percentile			
5	172	195	157
25	212	229.7	201.7
50 (Median)	245.4	261	234.9
75	295	294	274
95	359	358	349
Mean	253	265	241
95% CI of Mean	244–263	256–273	232–250
N	155	155	155
P value turbidimetric vs. historical	0.0842		
P value turbidimetric vs. ELISA	0.0539		

The sponsor has chosen to use the previously obtained expected values from k030477 that are stated below.

“ Samples from apparently healthy males (n=251) and apparently healthy females (n=174), in the clinically relevant age range of 40 to 70 years, were evaluated with the diaDexus PLAC Test. The reference population was represented by the following ethnic backgrounds: African-American n=26, Caucasian n=390, Hispanic n=8 and not specified n=1. The distributions of Lp-PLA₂ values across the entire population and divided by gender appear in the following table:

Percentile	Lp-PLA ₂ ng/mL		
	All (n=425)	Females (n=174)	Males (n=251)
5	126	120	131
20	174	169	179
33	201	188	205
50	235	228	244
67	262	252	268
80	289	285	293
95	369	342	376

The reference interval calculated from the samples (central 90%) was found to be 120–342 ng/mL for females and 131–376 ng/mL for males.”

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.