

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k072032

**B. Purpose for Submission:**

New Device

**C. Measurand:**

IgG oxidized low-density lipoprotein (oxLDL) beta2-glycoprotein 1 complex (anti-oxidized LDL/ $\beta$ 2GPI) antibodies in human serum.

**D. Type of Test:**

Semiquantitative ELISA

**E. Applicant:**

Corgenix, Inc.

**F. Proprietary and Established Names:**

IgG Anti-AtherOx™ Test Kit

**G. Regulatory Information:**

1. Regulation section:

21 CFR 866.5660 Multiple autoantibodies immunological test system

2. Classification:

Class II

3. Product code:

MSV - system, test, antibodies,  $\beta$ 2-glycoprotein 1 ( $\beta$ 2-GP1)

4. Panel:

82 - Immunology

**H. Intended Use:**

1. Intended use(s):

An enzyme-linked immunoassay (ELISA) for the detection of IgG antibodies to complexes formed by oxidized low-density lipoprotein (oxLDL) with  $\beta$ 2-glycoprotein I ( $\beta$ 2GPI) in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (antiphospholipid syndrome).

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Microplate reader capable of measuring OD 450 nm.

**I. Device Description:**

Each device contains the following: stabilized oxLDL- $\beta$ 2GPI antigen (human) coated microwell strips, with frame, sample diluent, IgG calibrator sera (human) (1-high, 2-moderate, 3-low), IgG positive control serum (human), IgG normal control serum (human), IgG anti-human (goat) HRP-conjugated antibody solution, one-component substrate (TMB and H<sub>2</sub>O<sub>2</sub>), stopping solution, and wash concentrate.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

REAADS Anti-Cardiolipin IgG/IgM Semi-Quantitative kit

2. Predicate K number(s):  
k022992
3. Comparison with predicate:

<b>Similarities</b>		
Item	Device	Predicate
Indications for Use	For individuals with SLE and lupus-like disorders (anti-phospholipid syndrome)	Same
Specimen Type	Serum	Same
Technology	ELISA	Same
Assay format	Semi-quantitative	Same
Solid phase	Coated polystyrene microwell plates	
Enzyme-conjugate	Horseradish peroxidase conjugated to goat anti-human IgG	Same
Substrate	TMB chromogen	Same
Results determination	Multipoint calibration curve	Multipoint calibration curve (or single point calibrator)

<b>Differences</b>		
Item	Device	Predicate
Intended Use	An enzyme-linked immuno-assay (ELISA) for the detection of IgG antibodies to complexes formed by oxidized low-density lipoprotein (oxLDL) with $\beta_2$ -glycoprotein I ( $\beta_2$ GPI) in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (anti-phospholipid syndrome).	For the detection and semi-quantitation of anti-cardiolipin antibodies in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (anti-phospholipid syndrome).
Analyte	IgG anti-oxidized LDL/ $\beta_2$ GPI antibodies	IgG anti-cardiolipin antibodies
Capture antigen	OxLDL/ $\beta_2$ -GPI complexes	Purified cardiolipin
Positive & negative controls	Dilute 1:100	Ready to use
Assay time	150 min at RT	40 min at RT
Quantitation range	10-100 G Units	0-100 GPL Units
Sample type and dilution	Human serum at 1:100	1:50

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

EP5-A2 *Evaluation of Precision Performance of Quantitative Measurement Methods*

EP6-A *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*

EP17-A *Protocols for Determination of Limits of Quantitation; Approved Guideline*

**L. Test Principle:**

Diluted serum samples, calibrator(s), and controls are incubated in microwells coated with the oxLDL- $\beta_2$ GPI complex. Incubation allows the IgG anti-oxLDL- $\beta_2$ GPI antibody present in the samples to react with the immobilized antigen complex. After the removal of unbound serum proteins by washing, anti-human IgG antibodies, labeled with horseradish peroxidase (HRP), are added forming complexes with the bound IgG anti-oxLDL- $\beta_2$ GPI antibody. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of a solution containing tetramethylbenzidine (TMB) and hydrogen peroxide ( $H_2O_2$ ) as the chromogenic substrate. Color develops in the wells at an intensity proportional to the serum concentration of IgG anti-oxLDL- $\beta_2$ GPI antibody. Results are obtained by reading the OD (optical density or absorbance) of each well in a spectrophotometer. Calibrator sera are provided, with the IgG anti-oxLDL- $\beta_2$ GPI antibody concentration expressed in G Units. A log-log regression analysis is performed with calibrator values plotted against calibrator mean ODs. Controls and patient results are determined from the calibration curve.

**M. Performance Characteristics (if/when applicable):****1. Analytical performance:****a. Precision/Reproducibility:**

Precision: Serum samples with concentrations spanning the range of the assay were tested in the Corgenix laboratory by 2 operators in duplicate on each of 20 days over 30 calendar days. One reagent lot was tested, and assays were calibrated each day.

Sample	Mean Conc. (G Units)	Inter-assay Precision (%CV)	Within-laboratory Precision (%CV)
Low	9.6	3%	13%
Med-Low	20.2	4%	12%
Med	28.1	7%	13%
Med-High	38.4	5%	12%
High	76.8	4%	10%

Reproducibility: Three operators at one site ran three clinical samples (low, medium, and high) over four consecutive days. The medium and high samples were run in triplicate and the low was run in quadruplicate. Results were determined from 48 measurements for the low sample and 36 for the medium and high sample.

	Samples		
	Low	Medium	High
Mean (G Units)	19	48	94
Intra-assay %CV	4%	1%	2%

- b. *Linearity/assay reportable range:*  
Linearity: Independently duplicate dilutions of a strongly-positive sample, mixed at various fixed ratios with an IgG-depleted pooled serum, were prepared to produce 11 evenly-spaced concentrations that were predicted to extend past the linear range of the assay. Acceptance criterion was set at  $\leq 5$  G units between predicted and recovered values. Evaluation of the data indicated that the linear range of the assay was 10 – 100 G units.
- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*  
 No recognized reference material for IgG anti-oxLDL- $\beta_2$ GPI. The results are reported in arbitrary units.  
Controls: The standards are prepared in-house and values are assigned during the development process. Positive and negative controls are prepared in-house.  
Stability: Calibrators, controls, a sample diluent alone were used to assess the real-time stability of four kits every 4-6 months for a 24 month period. All kits were shown to be stable up to 12 months.
- d. *Detection limit:*  
Limit of Blank (LoB): Three serum samples were mixed and depleted of IgG using a protein G column. The IgG-depleted serum, four clinical samples, and a negative sample (sample diluent) were tested multiple times on two different plate readers. Ninety-two results were generated from the negative samples and 228 for the clinical samples. The frequency distribution were tabulated and the 95<sup>th</sup> percentile of the negative results at 6.1 G units  
Limit of Detection (LoD): was defined the lowest level where 5% or fewer of the observed measurements are below the LoB based on a sample set showing non-Gaussian distribution. Since none of the results for the positive data set were at 7.0 G units or below, so the LoD was set as 7 G units.
- e. *Analytical specificity:*  
 Five clinical samples with the addition of increasing amounts of hemoglobin (to 500 mg/dL), conjugated bilirubin (to 20 mg/dL), triglycerides (to 3 g/dL), and rheumatoid factor (to 500 units/ml) were tested and showed that these levels had no effect on IgG Anti-AtherOx test kit results. However, it is recommended in the package insert that results obtained from grossly hemolyzed or lipemic specimens be interpreted with caution and that grossly lipemic samples can be clarified by centrifugation.
- f. *Assay cut-off:*  
 A cut-off value of 20 G Units was established by testing 75 serum samples from healthy volunteers in duplicate. Values ranged from 3.5-17.8 G Units.

N	Range [G units]	Mean (Median) [G units]	SD	95 <sup>th</sup> percentile	Mean + 3 SD
75	3.5-17.7	8.8 (8.9)	3	13.8	17.7

## 2. Comparison studies:

- a. *Method comparison with predicate device:*  
 Serum samples from 143 Systemic lupus erythematosus (SLE), 99 rheumatoid

arthritis and 205 normal healthy controls were tested for elevated IgG antiphospholipid antibody levels using the Corgenix IgG Anti-AtherOx and REAADS IgG anti-cardiolipin kits. Each group is summarized below:

SLE: Serum samples from 143 SLE patients (94 SLE with secondary APS, 19 SLE only and 30 with unknown APS status) were tested on the IgG Anti-AtherOx™ Test Kit and the REAADS IgG Anti-Cardiolipin Test. The mean IgG anti-oxLDL-β<sub>2</sub>GPI antibody level of this group was determined to be 24.4 G Units. Of the SLE/APS subgroup, the mean IgG anti-oxLDL-β<sub>2</sub>GPI antibody level was 28.8 G Units. Twenty five SLE/APS samples were positive when tested with the new device and 17 of these were also positive for anti-cardiolipin antibodies by the predicate device. Since the new and predicate devices detect different analytes, it is likely that some patients have one and not the other autoantibodies. Comparison with the predicate device for the SLE/APS group is shown below:

<b>SLE/APS</b> (N = 94)		<b>REAADS IgG Anti-Cardiolipin</b>		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	17	8	25
	Neg	6	63	69
	Total	23	71	94

Positive Percent Agreement = 73.9% (17/23)

Negative Percent Agreement = 88.7% (63/71)

Overall % Agreement = 85.1% (80/94)

The APS patients were then further analyzed according to their clinical history of APS manifestations: arterial thrombosis, venous thrombosis, or pregnancy morbidity. Results, including the SLE only group are summarized in the following tables:

<b>Pregnancy Morbidity</b> (N = 15)		<b>REAADS IgG Anti-Cardiolipin</b>		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	0	1	1
	Neg	2	12	14
	Total	2	13	15

Positive Percent Agreement = 0% (0/2)

Negative Percent Agreement = 92.3% (12/13)

Overall % Agreement = 80.0% (12/15)

<b>Arterial Thrombosis</b> (N = 42)		REAADS IgG Anti-Cardiolipin		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	11	1	12
	Neg	3	27	30
	Total	14	28	42

Positive Percent Agreement = 78.6% (11/14)

Negative Percent Agreement = 96.4% (27/28)

Overall % Agreement = 90.5% (38/42)

<b>Venous Thrombosis</b> (N = 37)		REAADS IgG Anti-Cardiolipin		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	6	6	12
	Neg	1	24	25
	Total	7	30	37

Positive Percent Agreement = 85.7% (6/7)

Negative Percent Agreement = 80.0% (24/30)

Overall % Agreement = 81.1% (30/37)

For the 19 SLE patients without APS, the mean IgG anti-oxLDL-  $\beta_2$ GPI antibody level was 12.0 G Units. One sample was positive with the new device which was negative with the predicate.

<b>SLE only</b> (N = 19)		REAADS IgG Anti-Cardiolipin		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	0	1	1
	Neg	0	18	18
	Total	0	19	19

Summary results for the SLE group are shown below:

<b>SLE and SLE/APS</b> (N = 143)		REAADS IgG Anti-Cardiolipin		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	19	16	35
	Neg	6	102	108
	Total	25	118	143

Positive Percent Agreement = 76.0% (19/25)

Negative Percent Agreement = 86.4% (102/118)

Overall % Agreement = 84.6% (121/143)

Normal Healthy controls: Samples from 205 normal, healthy controls were tested and six samples were positive for IgG anti-AtherOx assay but negative for IgG anti-cardiolipin using the cut-off established in the initial study. The overall % agreement with the predicate device is 97.1% (199/205).

Healthy Controls (N = 205)		REAADS IgG Anti-Cardiolipin		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	0	6	6
	Neg	0	199	199
	Total	0	205	205

Rheumatoid arthritis: Serum samples from 99 rheumatoid arthritis patients were tested on the IgG Anti-AtherOx™ Test Kit and the REAADS IgG Anti-Cardiolipin Test. The mean IgG value of this population was determined to be 16.4 G Units. One sample was positive for both device but there were 17 samples positive only with the new device, where 58.8% (10/17) were near the cut-off for the new device. For this group of patients, the overall % agreement is 82.8% (82/99).

Rheumatoid Arthritis (N = 99)		REAADS IgG Anti-Cardiolipin		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	1	17	18
	Neg	0	81	81
	Total	1	98	99

A summary of all samples tested in the method comparison study is as follows:

All Subjects (N = 447)		REAADS IgG Anti-Cardiolipin		
		Pos	Neg	Total
Corgenix IgG Anti-AtherOx	Pos	20	39	59
	Neg	6	382	388
	Total	26	421	447

Positive Percent Agreement = 76.9% (20/26)

Negative Percent Agreement = 90.7% (382/421)

Overall % Agreement = 89.9% (404/447)

*b. Matrix comparison:*

Both assays use serum as the matrix.

3. Clinical studies:

*a. Clinical Sensitivity and Specificity:*

The tables below show the same samples from the above comparison data, but presented according to the diagnosis.

Disease state	N	Corgenix anti-AtherOx % positive
SLE + Secondary APS		
Preg. Morbidity	42	28.6% (12/42)
Atrial thrombosis	37	32.4% (12/37)
Venous thrombosis	15	6.7% (1/15)
Total	94	26.6% (25/94)

Disease state	N	Corgenix anti-AtherOx % positive
No APS		
Rheumatoid Arthritis (RA)	99	18.2% (18/99)
SLE (no secondary APS)	19	5.3% (1/19)
SLE (APS status unknown)	30	26.7% (8/30)
Total	148	18.2% (27/148)
Total number of patient samples		242

		APS		
		(+)	(-)	Total
Corgenix IgG Anti-AtherOx	(+)	25	27	52
	(-)	69	121	190
	Total	94	148	242

Sensitivity: 26.6% (25/94)  
Specificity: 81.8% (121/148)

4. Clinical cut-off:  
See Assay cut-off.
5. Expected values/Reference range:  
Expected value in the normal population is negative. Six (6) samples were positive by the Corgenix IgG Anti-AtherOx™ assay.

**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.