510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k091556

B. Purpose for Submission:
New device

C. Measurand:
Anti-β2 Glycoprotein-I IgG and IgM autoantibodies

D. Type of Test:
Automated chemiluminescent immunoassay

E. Applicant:
Instrumentation Laboratory Co.

F. Proprietary and Established Names:
HemosIL AcuStar Anti-β2 Glycoprotein-I IgG
HemosIL AcuStar Anti-β2 Glycoprotein-I IgM
HemosIL AcuStar Anti-β2 Glycoprotein-I IgG Controls
HemosIL AcuStar Anti-β2 Glycoprotein-I IgM Controls

G. Regulatory Information:

1. Regulation section:
   21 CFR § 866.5660, Multiple autoantibodies immunological test system
   21 CFR § 862.1660, Single (specified) analyte controls (assayed and unassayed)
2. Classification:
   Class II (Assays)
   Class I (Controls)
3. Product code:
   MSV - Antibodies, β_2 Glycoprotein I
   JJX – Quality control material (assayed and unassayed)
4. Panel:
   Immunology (82)
   Chemistry (75)

H. Intended Use:

1. Intended use(s):
   HemosIL AcuStar Anti-β2 Glycoprotein-I IgG: Fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-β2 Glycoprotein-I (anti-β2GPI) IgG antibodies in human citrated plasma and serum on the ACL AcuStar™, as an aid in the diagnosis of thrombotic disorders related to primary and secondary Antiphospholipid Syndrome (APS) when used in conjunction with other laboratory and clinical findings.

   HemosILTM AcuStar Anti-β2 Glycoprotein-I IgG Controls: For the quality control of the Anti-β2 Glycoprotein-I IgG assay performed on the ACL AcuStar.

   HemosIL AcuStar Anti-β2 Glycoprotein-I IgM: Fully automated

chemiluminescent immunoassay for the semi-quantitative measurement of anti-β2 Glycoprotein-I (anti-β2GPI) IgM antibodies in human citrated plasma and serum on the ACL AcuStar™ as an aid in the diagnosis of thrombotic disorders related to primary and secondary Antiphospholipid Syndrome (APS) when used in conjunction with other laboratory and clinical findings.

HemosIL AcuStar Anti-β2 Glycoprotein-I IgM Controls: For the quality control of the Anti-β2 Glycoprotein-I IgM assay performed on the ACL AcuStar.

2. Indication(s) for use:
   See Intended Use above.
3. Special conditions for use statement(s):
   For prescription use only.
4. Special instrument requirements:
   For use on the ACL AcuStar automated chemiluminescent immunoassay analyzer (k083518).

I. Device Description:

The device consists of three components:

1) anti-β2GPI IgG or IgM Cartridge containing: a vial of magnetic particle suspension coated with purified human β2GPI, a vial of assay buffer, a vial of tracer consisting of a monoclonal mouse anti-human IgG or IgM antibody labeled with isoluminol, and a vial of sample diluent used for the regular pre-dilution of the sample and automatic dilution in rerun. The reagents are in a phosphate or borate buffer containing bovine serum albumin, stabilizers and preservative.
2) anti-β2GPI IgG or IgM Calibrator containing: one 1 mL barcoded tube of a solution with anti-β2GPI IgG or IgM in a phosphate buffer containing bovine serum albumin, stabilizers and preservative.
3) anti-β2GPI IgG or IgM Calibrator 2: one 1 mL barcoded tube of a solution with anti-β2GPI IgG or IgM in a phosphate buffer containing bovine serum albumin, stabilizers and preservative.

The IgG and IgM control materials are sold separately. Each device consists of: three barcoded ready-to-use 1-mL Low anti- β2GPI IgG or IgM Low Control and three 1-mL Low anti- β2GPI IgG or IgM High Control. In addition to anti-- β2GPI antibodies, each control contains phosphate buffer, bovine serum albumin, stabilizers and preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   REAADS IgG anti-β2 GPI Test Kit
   REAADS IgM anti-β2 GPI Test Kit
2. Predicate K number(s):
   k031208
   k031208
3. Comparison with predicate:

HemosIL™ AcuStar Anti- β2 GPI IgG

Similarities		
Item	Device	Predicate
Intended Use/Indications for Use	For the semi-quantitative measurement of anti-β_2 Glycoprotein-I (anti-β_2GPI) IgG antibodies in human citrated plasma and serum on the ACL AcuStar, as an aid in the diagnosis of thrombotic disorders related to primary and secondary Antiphospholipid Syndrome (APS) when used in conjunction with other laboratory and clinical findings.	For the detection and semi-quantitation of IgG anti-β_2 GPI antibodies in human serum or plasma as an aid for assessing the risk of thrombosis in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (anti-phosph
Sample type	Serum or Citrated Plasma	Same

Differences		
Item	Device	Predicate
Technology	Two-step chemiluminescent immunoassay	ELISA
Calibrator	Two calibrator levels (included in test kit)	Three calibrator levels (included in test kit)
Quality control	Low and high controls (sold separately)	Normal and positive controls (included in test kit)
Assay range	6.4 – 6100 U/mL	Up to 100 GPL
Cut-off	20.0 U/mL	20 GPL

HemosIL™ AcuStar Anti- β2 GPI IgM

Similarities		
Item	Device	Predicate
Intended Use/Indications for Use	For the semi-quantitative measurement of anti-β_2 Glycoprotein-I (anti-β_2GPI) IgM antibodies in human citrated plasma and serum on the ACL AcuStar, as an aid in the diagnosis of thrombotic disorders related to primary and secondary Antiphospholipid Syndrome (APS) when used in conjunction with other	For the detection and semi-quantitation of IgM anti-β_2 GPI antibodies in human serum or plasma as an aid for assessing the risk of thrombosis in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (anti-phosph

Similarities		
Item	Device	Predicate
	laboratory and clinical findings.	
Sample type	Serum or Citrated Plasma	Same

Differences		
Item	Device	Predicate
Technology	Two-step chemiluminescent immunoassay	ELISA
Calibrator	Two calibrator levels (included in test kit)	Three calibrator levels (included in test kit)
Quality control	Low and high controls (sold separately)	Normal and positive controls (included in test kit)
Assay range	1.1 – 841 U/mL	Up to 100 MPL
Cut-off	20.0 U/mL	20 MPL

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

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition
CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation Approved Guideline
CLSI EP07-A2: Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition

L. Test Principle:

The devices are chemiluminescent two-step immunoassays consisting of magnetic particles coated with human purified β2GPI which capture, if present, the anti-β2GPI antiphospholipid antibodies from the sample. After incubation, magnetic separation, and a wash step, a tracer consisting of an isoluminol-labeled anti-human IgG or IgM antibody is added and may bind with the captured anti-β2GPI IgG or IgM on the particles. After a second incubation, magnetic separation, and wash step, reagents that trigger the luminescent reaction are added, and the emitted light is measured as relative light units (RLUs) by the ACL AcuStar optical system. The RLUs are directly proportional to the anti-β2GPI IgG or IgM concentration in the sample. The ACL AcuStar anti-β2GPI IgG and IgM assays utilize a 4 Parameter Logistic Curve (4PLC) fit data reduction method to generate a Master Curve. The Master Curve is predefined and lot dependent and it is stored in the instrument through the cartridge barcode. With the measurement of calibrators, the predefined Master Curve is transformed to a new, instrument specific 4PLC Working Curve. The concentration values of the calibrators are included in the calibrator tube barcodes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*

HemosIL™ AcuStar Anti-β2-GPI IgG

Precision testing was performed in accordance with CLSI Approved

Guideline EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.

Sample	Mean (U/mL)	CV (%) (within run)	CV (%) (total)
Low anti-β2-GPI IgG Control	19.1	7.8	11.2
High anti- β2-GPI IgG Control	429	3.0	3.8
Anti-β2-GPI IgG plasma sample	58.1	3.2	5.0

An additional experiment was performed with two clinical plasma samples close to the cut-off and three clinical plasma samples in the upper end of the reportable range:

Sample	Mean (U/mL)	CV (%) (within run)	CV (%) (total)
A	14.7	6.9	10.9
B	20.9	4.7	7.3
C	508	2.5	3.3
D	1470	2.5	3.7
E	2694	3.7	3.7

HemosIL™ AcuStar Anti-β2-GPI IgM

Precision testing was performed in accordance with CLSI Approved Guideline EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.

Sample	Mean (U/mL)	CV (%) (within run)	CV (%) (total)
Low anti-β2-GPI IgM Control	4.3	3.4	6.4
High anti- β2-GPI IgM Control	63.0	2.4	4.3
Anti-β2-GPI IgM plasma sample	11.0	3.6	5.8

An additional experiment was performed with two clinical plasma samples close to the cut-off and three clinical plasma samples in the upper end of the reportable range:

Sample	Mean (U/mL)	CV (%) (within run)	CV (%) (total)
A	13.6	4.5	8.3
B	16.3	2.7	6.6
C	91.9	2.4	5.7
D	302	3.0	5.2
E	510	4.1	6.0

b. Linearity/assay reportable range:

Linearity of both the HemoSIL anti-β2-GPI IgG and IgM assays was assessed by preparing a series of dilutions of three different samples from patients suspected to have APS to cover the reportable range. The linearity tests were

performed with two different lots of reagents; results were comparable for both lots. Results from one lot are shown below:

Assay	Sample	Regression	r^2	Dilution Range (U/mL)
IgG	1	y = 0.92x + 3.8	0.986	7.2 – 47.0
	2	y = 1.04x – 16.5	0.996	21.4 – 469.7
	3	y = 0.995x – 36.9	0.996	417 – 5632.0
IgM	1	y = 0.98x + 0.5	0.996	1.5 – 25.8
	2	y = 0.98x + 1.6	0.992	5.3 – 105.8
	3	y = 1.05x – 33.5	0.990	23.4 – 727.3

The sponsor has established the linear range of the assays as 6.4 – 6100 U/mL for the anti- β2-GPI IgG assay and 1.1 – 841 U/mL for the anti- β2-GPI IgM assay.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):* An international reference material for anti-β2-GPI antibodies is not available. The assay is calibrated in relative arbitrary units (U/mL).

The two calibrator values are assigned using in-house standards and a four-parameter Master Curve. The assignment values of the two calibrators are used to create a lot-specific four-parameter logistic curve, using two stored parameters from the Master Curve and two lot-specific parameters based on the calibrator values.

Control values are assigned by analyzing the material with previous batches of reagents on two different ACL AcuStar instruments. The controls are analyzed in replicates of 10 on both instruments, then the batches of reagent are switched between instruments and the controls are re-analyzed.

Stability studies were performed that support a shelf-life of 15 months at 2-8°C storage for the HemosIL AcuStar Anti- **β2-GPI** IgG and the HemosIL AcuStar Anti- **β2-GPI** IgM reagent cartridges, as well as calibrators and controls for both assays.

The on-board stability of the reagent cartridge after opening on the AcuStar System was demonstrated to be at least 6 weeks. On-board stability of calibrators was shown to be 3.5h.

The sponsor recommends following the recommendations for storage conditions for samples in CLSI H18-A3 “Procedures for the Handling and Processing of Blood Specimens: Third Edition”.

d. Detection limit:

The Limit of Detection (LoD) was determined to be 6.4 U/mL for the anti-β2-GPI IgG assay and 1.1 U/mL for the anti-β2-GPI IgM assay. The LoB and LoD were determined using the procedure described in CLSI EP17-A. Briefly, LoB was determined by performing 60 replicates of saline buffer. The results were ordered according to their value and the mean of position 57 and 58 (representing the 95th percentile) was determined as the LoB. The LoD was determined by reading 60 replicates of two low concentration samples and determining the standard deviation. LoD was calculated as LoD = LoB + 1.65 SD where SD is the pooled standard deviation of the two low positive sample readings.

e. Analytical specificity:

Both the IgG and the IgM assays were tested with the following endogenous interferents at two levels and showed less than ± 10% interference at the concentration noted: Hemoglobin, 500 mg/dL: Bilirubin, 18 mg/dL: Triglycerides, 1250 mg/dL: Heparin, 2 IU/mL: Rheumatoid Factor (RF), 500 IU/mL.

Cross-reactivity: 10 samples positive for RF, 10 samples positive for antinuclear (ANA) antibodies, and 10 samples positive for rapid plasma reagin test (RPR, a form of syphilis antibody test) were tested with the HemosIL anti-β2 GPI IgG and the IgM assays. None of the RF or RPR samples tested positive by the HemosIL anti-β2 GPI assays. Two ANA samples (# 6 and #7) tested positive by the IgG assay and one sample (#7) tested positive by the IgM assay. Sample #7 tested positive by another anti-β2 GPI commercial assay.

f. Assay cut-off:

The assay cut-offs were determined to be 20 U/mL in a reference range study described in the clinical cut-off section below.

2. Comparison studies:

a. Method comparison with predicate device:

HemoSIL AcuStar anti-β2GPI IgG: 150 clinically defined patient samples with results in the reportable range of both the test and the predicate (REAADS) were compared. There is no equivocal range for either test.

Comparison of HemoSIL AcuStar anti-β_2GPI IgG and Predicate

		Predicate Assay		
		+	-	Total
HemoSIL AcuStar anti-β2 GPI IgG	+	51	19*	70
	-	0	80	80
	Total	51	99	150

* 12 SLE, 7 SAPS

Positive agreement (51/51) = 100.0% (95% CI: 93.0% to 100.0%)
Negative agreement (80/99) = 80.8% (95% CI: 71.7% to 88.0%)
Overall agreement (131/150) = 87.3% (95% CI: 80.9% to 92.2%)

HemoSIL AcuStar anti-β2GPI IgM: 205 clinically defined patient samples with results in the reportable range of both the test and the predicate (REAADS) were compared. There is no equivocal range for either test.

Comparison of HemoSIL AcuStar anti-β_2GPI IgM and Predicate

		Predicate Assay		
		+	-	Total
HemoSIL AcuStar anti-β2 GPI IgM	+	30	5**	35
	-	17*	153	170
	Total	47	158	205

* 3 SLE, 7 SAPS, 3 PAPS, 4 Control
** 1 SLE, 2 SAPS, 1 PAPS, 1 Control

Positive agreement (30/47) = 63.8% (95% CI: 48.5% to 77.3%)
Negative agreement (76/101) = 96.8% (95% CI: 92.8% to 99.0%)
Overall agreement (183/205) = 76.5% (95% CI: 84.2% to 93.2%)

b. Matrix comparison:
The suitability of citrated plasma and serum was determined by assaying plasma/serum paired samples covering the assay range. The results of the Passing-Bablok regression analysis were following:

AcuStar anti-β2GPI	n =	regression	r value
IgG assay	28	y = 1.00x + 1.25	1.00
IgM assay	48	y = 0.99x + 0.02	0.996

3. Clinical studies:
   a. Clinical Sensitivity and Specificity:
   321 frozen citrated plasmas were tested with the AcuStar anti-β2GPI IgG and IgM assays. These plasmas were from individuals diagnosed as primary APS (PAPS), secondary APS (SAPS), systemic lupus erythematosus (SLE) without APS and SLE-like by standard objective tests. Another group was patients with cardiovascular disorders but not classified in the previous four groups. A group of apparently healthy people was also included. The results summarized below are based on a cut-off of 20 U/mL:

AcuStar anti-β2GPI IgG:

AcuStar anti-β2GPI IgG	Clinical Diagnosis		
	POS	**NEG**	**Total**
POS	59	21	80
NEG	33	208	241
Total	92	229	321

Sensitivity (59/92) = 64.1% (95% CI: 53.5% to 73.9%)
Specificity (208/229) = 90.8% (95% CI: 86.3% to 94.2%)

The results for each clinical subgroup are shown in the table below:

Disease category	N	N Positive	% Positive
PAPS	23	14	60.9%
SAPS	69	45	65.2%
SLE	115	20	17.4%
SLE-like	5	0	0%
Others	6	1	16.7%
Normals	103	0	0%

AcuStar anti-β2GPI IgM:

AcuStar anti-β2GPI IgM	Clinical Diagnosis		
	POS	**NEG**	**Total**
POS	27	11	38
NEG	65	218	283
Total	92	229	321

Sensitivity (27/92) = 29.3% (95% CI: 20.3% to 39.8%)
Specificity (218/229) = 95.2% (95% CI: 91.6% to 97.6%)

Disease category	N	N Positive	% Positive
PAPS	23	7	30.4%
SAPS	69	20	29.0%
SLE	115	10	8.7%
SLE-like	5	0	0%
Others	6	1	16.7%
Normals	103	0	0%

b. Other clinical supportive data (when a. is not applicable):
Not applicable.

4. Clinical cut-off**:**
   The upper limits of normal (and cut-off) of the HemosIL anti-β2 GPI IgG and IgM assays were determined by assaying 262 citrated plasma samples from apparently healthy blood bank volunteers. Following the recommendations of the international consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS)[1] the threshold for positive anti- β2 GPI antibodies was set at the 99th percentile of the tested population. This was determined to be 20 U/mL for both assays.

5. Expected values/Reference range:
   See Clinical cut-off discussion above.

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.

[1] Miyakis S, et al. "International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS)." J Thromb Haemost. 4:295-306 2006.