

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

A. 510(k) Number:

k092181

B. Purpose for Submission:

New device

C. Analyte:

Anti-Cardiolipin IgG

Anti-Cardiolipin IgM

D. Type of Test:

Automated chemiluminescent immunoassay for semi-quantitative measurement of autoantibodies

E. Applicant:

Instrumentation Laboratory Co.

F. Proprietary and Established Names:

HemosIL™ AcuStar Anti-Cardiolipin IgG

HemosIL™ AcuStar Anti-Cardiolipin IgM

HemosIL™ AcuStar Anti-Cardiolipin IgG Controls

HemosIL™ AcuStar Anti-Cardiolipin IgM Controls

G. Regulatory Information:

1. Regulation section:

21CFR§866.5660 – Multiple autoantibodies immunological test system

21CFR§862.1660 – Single (specified) analyte controls (assayed and unassayed)

2. Classification:

Class II (Assays)

Class I (Controls)

3. Product code:

MID – System Test, Anti-Cardiolipin Immunological

JJX – Controls

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

HemosIL™ AcuStar Anti-Cardiolipin IgG: Fully automated chemiluminescent assay for the semi-quantitative measurement of anti-cardiolipin (aCL) 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).

HemosIL™ AcuStar Anti-Cardiolipin IgM: Fully automated chemiluminescent assay for the semi-quantitative measurement of anti-cardiolipin (aCL) 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).

HemosIL™ AcuStar Anti-Cardiolipin IgG Controls: For the quality control of the Anti-Cardiolipin IgG assay performed on the ACL AcuStar.

HemosIL™ AcuStar Anti-Cardiolipin IgM Controls: For the quality control of the Anti-Cardiolipin IgM assay performed on the ACL AcuStar.

2. Indication(s) for use:
Same as intended use
3. Special conditions for use statement(s):
Prescription use only
4. Special instrument requirements:
For use on the ACL AcuStar automated chemiluminescent immunoassay analyzer (k083518).

I. Device Description:

HemoSIL AcuStar Anti-Cardiolipin (aCL) IgG is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with cardiolipin and human purified β 2GPI which capture, if present, the aCL antiphospholipid antibodies from the sample. After incubation, magnetic separation and a wash step, a tracer consisting of an isoluminol-labeled anti-human IgG antibody is added and may bind with the captured aCL IgG 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 aCL IgG concentration in the sample.

The ACL AcuStar aCL IgG assay utilizes 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.

The **aCL IgG** kit consists of:

- **aCL IgG Cartridge for 50 determinations:** 1 cartridge containing 1 vial of magnetic particle suspension coated with bovine cardiolipin and human purified β 2GPI, 1 vial of assay buffer, 1 vial of tracer consisting of an anti-human IgG antibody labeled with isoluminol, and 1 vial of sample diluent used for regular predilution of the sample and automated dilution in the rerun. The reagents are in a phosphate or borate buffer containing bovine serum albumin, bovine cardiolipin, human β 2GPI, mouse monoclonal IgG, stabilizers, and preservative.
- **aCL IgG Calibrator 1:** 1 x 1 mL barcoded tube of a solution with aCL IgG in saline solution containing bovine fetal serum, stabilizers and preservative.
- **aCL IgG Calibrator 2:** 1 x 1 mL barcoded tube of a solution with aCL IgG in saline solution containing bovine fetal serum, stabilizers and preservative.

HemoSIL AcuStar Anti-Cardiolipin (aCL) IgM is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with cardiolipin and human purified β 2GPI which capture, if present, the aCL antiphospholipid antibodies from the sample. After incubation, magnetic separation and a wash step, a tracer consisting of an isoluminol-labeled anti-human IgM antibody is added and may bind with the

captured aCL 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 aCL IgM concentration in the sample.

The ACL AcuStar aCL IgM assay utilizes 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.

The **aCL IgM** kit consists of:

- **aCL IgM Cartridge for 50 determinations:** 1 cartridge containing 1 vial of magnetic particle suspension coated with bovine cardiolipin and human purified β 2GPI, 1 vial of assay buffer, 1 vial of tracer consisting of an anti-human IgM antibody labeled with isoluminol, and 1 vial of sample diluent used for regular predilution of the sample and automated dilution in the rerun. The reagents are in a phosphate or borate buffer containing bovine serum albumin, bovine cardiolipin, human β 2GPI, mouse monoclonal IgM, stabilizers, and preservative.
- **aCL IgM Calibrator 1:** 1 x 1 mL barcoded tube of a solution with aCL IgM in saline solution containing bovine fetal serum, stabilizers and preservative.
- **aCL IgM Calibrator 2:** 1 x 1 mL barcoded tube of a solution with aCL IgM in saline solution containing bovine fetal serum, stabilizers and preservative.

HemosIL AcuStar Anti-Cardiolipin IgG Controls: The Low and High Anti-Cardiolipin IgG Controls are prepared by means of a dedicated process and contain different concentrations of human aCL IgG antibodies.

- **Low Anti-Cardiolipin IgG Control:** Control intended for the assessment of precision and accuracy of the assay at the normal or around cut-off aCL IgG levels. 3 x 1 mL barcoded tubes of a solution with aCL IgG in saline solution containing bovine fetal serum, stabilizers and preservative.
- **High Anti-Cardiolipin IgG Control:** Control intended for the assessment of precision and accuracy of the assay at the abnormal aCL IgG levels. 3 x 1 mL barcoded tubes of a solution with aCL IgG in saline solution containing bovine fetal serum, stabilizers and preservative.

HemosIL AcuStar Anti-Cardiolipin IgM Controls: The Low and High Anti-Cardiolipin IgM Controls are prepared by means of a dedicated process and contain different concentrations of human aCL IgM antibodies.

- **Low Anti-Cardiolipin IgM Control:** Control intended for the assessment of precision and accuracy of the assay at the normal or around cut-off aCL IgM levels. 3 x 1 mL barcoded tubes of a solution with aCL IgM in saline solution containing bovine fetal serum, stabilizers and preservative.

- **High Anti-Cardiolipin IgM Control:** Control intended for the assessment of precision and accuracy of the assay at the abnormal aCL IgM levels. 3 x 1 mL barcoded tubes of a solution

J. Substantial Equivalence Information:

1. Predicate device names:
 REAADS Anti-Cardiolipin IgG Semi-Quantitative Test Kit
 REAADS Anti-Cardiolipin IgM Semi-Quantitative Test Kit
2. Predicate 510(k) number:
 k022992
3. Comparison with predicate:

HemosIL™ AcuStar Anti-Cardiolipin IgG

Similarities		
Item	Device	Predicate
Intended Use	Fully automated chemiluminescent assay for the semi-quantitative measurement of anti-cardiolipin (aCL) 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).	An enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative determination of anti-cardiolipin (aCL) IgG antibodies in human citrated plasma and serum in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (antiphospholipid syndrome)
Indications for Use	Same as Intended Use	Same as Intended Use
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)
Clinical cut-off	20 U/mL	23 GPL
Assay range	2.6 –40,480 U/mL	Up to 100 GPL

HemosIL™ AcuStar Anti-Cardiolipin IgM

Similarities		
Item	Device	Predicate
Intended Use	Fully automated chemiluminescent assay for the semi-quantitative measurement of anti-cardiolipin (aCL) 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).	An enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative determination of anti-cardiolipin (aCL) IgM antibodies in human citrated plasma and serum in individuals with systemic lupus erythematosus (SLE) and lupus-like disorders (anti-phospholipid syndrome)
Indications for Use	Same as Intended Use	Same as Intended Use
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)
Clinical cut-off	20 U/mL	11 MPL
Assay range	1.0-15,480 U/mL	Up to 60 MPL

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

- EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition
- EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition

L. Test Principle:

The test is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with cardiolipin and human purified β_2 GPI which capture, if present, the aCL antiphospholipid antibodies from the sample. After incubation, magnetic separation, and a wash step, a tracer consisting of an isoluminol-labeled anti-human IgG (or anti-human IgM, as appropriate) is added and may bind with the captured aCL 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 aCL IgG (or IgM) concentration in

the sample. The instrument utilizes a 4 Parameter Logistic Curve (4PLC) fit data reduction method to generate a master curve.

The Low Controls are intended for the assessment of precision and accuracy of the assay at normal or around cut-off levels of aCL antibody. The High Controls are intended for assessment of precision and accuracy of the assay at abnormally high antibody levels.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

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

HemosIL™ AcuStar Anti-Cardiolipin IgG

	Mean (U/mL)	CV (%) (within run)	CV (%) (total)
Low anti-cardiolipin IgG Control	16.4	6.8 %	8.2%
High anti-cardiolipin IgG Control	158	6.1%	6.9%
Anti-cardiolipin IgG plasma sample	47.2	4.8%	7.2%

An additional experiment was performed with two samples around the cut-off and two in the upper end of the reportable range.

Instrument 1			Instrument 2		
Mean (U/mL)	CV (%) (within run)	CV (%) (total)	Mean (U/mL)	CV (%) (within run)	CV (%) (total)
13.5	4.9%	5.9%	13.8	4.0%	4.4%
19.0	2.8%	4.8%	19.1	3.7%	4.2%
511	2.5%	3.2%	515	3.7%	5.4%
958	3.9%	5.5%	1028	3.5%	6.7%

HemosIL™ AcuStar Anti-Cardiolipin IgM

	Mean (U/mL)	CV (%) (within run)	CV (%) (total)
Low anti-cardiolipin IgM Control	6.79	3.3%	4.9%
High anti-cardiolipin IgM Control	86.1	3.5%	4.0%
Anti-cardiolipin IgM plasma sample	19.2	2.6%	4.7%

A second experiment: performed with two additional samples around the cut-off and two to cover the upper portion of the reportable range.

Instrument 1			Instrument 2		
Mean (U/mL)	CV (%) (within run)	CV (%) (total)	Mean (U/mL)	CV (%) (within run)	CV (%) (total)
14.6	2.0%	2.5%	14.7	3.0%	3.3%
19.5	1.7%	1.7%	19.5	2.6%	2.9%
202	2.4%	2.8%	207	3.6%	4.4%
480	3.4%	4.8%	556	6.8%	8.4%

b. Linearity/assay reportable range:

When the rerun capability of the instrument is activated, the instrument makes an automatic dilution and corrects the final result for the dilution factor (20x), thereby expanding the test range 20-fold.

HemosIL™ AcuStar Anti-Cardiolipin IgG

	Value
Linearity (Rerun Off)	2.6 – 2024 U/mL
Linearity (Rerun On)	2.6 – 40,480 U/mL

HemosIL™ AcuStar Anti-Cardiolipin IgM

	Value
Linearity (Rerun Off)	1.0 – 774 U/mL
Linearity (Rerun On)	1.0 – 15,480 U/mL

Linearity of both the HemosIL aCL IgG and IgM assays was assessed by preparing a series of dilutions of three different samples to cover the reportable range. The linearity tests were performed with two different lots of reagents. Overall, slope = 0.99-1.00 and $r^2 > 0.99$ for both assays over this range.

Linearity data summary for AcuStar aCL IgG:

	Lot 1	Lot 2
Range	3.6 – 2213 U/mL	4.4 – 2212 U/mL
Slope	0.995	0.990
R ²	0.999	0.997

Linearity data summary for AcuStar aCL IgM:

	Lot 1	Lot 2
Range	1.5 – 807 U/mL	1.3 – 780 U/mL
Slope	0.999	1.002
R ²	0.995	1.000

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

An international reference material for anti-cardiolipin 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 assignments are performed using two QC approved batches of reagents (with corresponding calibrators) and 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-Cardiolipin IgG and the HemosIL AcuStar Anti-Cardiolipin 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. A comparison of fresh and frozen samples showed no difference in the performance of these samples in the assay.

d. Detection limit:

The Limit of Detection (LOD) was determined to be 2.6 U/mL for the aCL IgG assay and 1.0 U/mL for the aCL IgM assay. The LOD was determined using the procedure described in CLSI EP17-A.

e. Analytical specificity:

Interference studies were performed using negative and positive samples, as well as a sample with results close to the assay cutoff. No significant interference was observed for either the HemoSIL AcuStar aCL IgG or IgM assay with hemoglobin (up to 500 mg/dL), bilirubin (complexed and free, 18 mg/dL), triglycerides (1250 mg/dL), rheumatoid factor (800 IU/mL), LMW heparin (2 IU/mL), and unfractionated heparin (2 IU/mL). Recoveries were all in the range 96-103%.

The analytical specificities of the two kits were also evaluated by testing 30 defined serum samples, 10 positive for Rheumatoid Factor (RF), 10 positive for Antinuclear Antibodies (ANA) and 10 positive for Syphilis by the rapid plasma reagin test (RPR). One ANA sample gave a positive result on both the HemoSIL AcuStar aCL IgG and IgM test. None of the RF or RPR samples were positive on the AcuStar aCL tests.

f. Assay cut-off:

252 citrated plasma samples obtained from apparently healthy blood donors were tested in the HemoSIL AcuStar Anti-Cardiolipin IgG and IgM assays. Based on the recommendations of the International Committee in Sydney, the threshold for positive aCL IgG and IgM antibodies was established in the 99th percentile. The cut-off for both assays was set at 20 U/mL.

2. Comparison studies:

a. *Method comparison with predicate device:*

HemoSIL AcuStar aCL IgG: 136 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.

Method Comparison—HemoSIL AcuStar aCL IgG to REAADS aCL IgG

		Predicate Assay (REAADS aCL IgG)		
		+	-	Total
HemoSIL AcuStar aCL IgG	+	28	25**	53
	-	7*	76	83
	Total	35	101	136

*1 PAPS, 2 SAPS, 3 SLE and 1 control

**3 PAPS, 15 SAPS, and 7 SLE

Positive agreement (28/35) = 80.0% (95% CI: 63.1% to 91.6%)

Negative agreement (76/101) = 75.2% (95% CI: 65.7% to 83.3%)

Overall agreement (104/136) = 76.5% (95% CI: 68.4% to 83.3%)

HemoSIL AcuStar aCL IgM: 267 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.

Method Comparison—HemoSIL AcuStar aCL IgM to REAADS aCL IgM

		Predicate Assay (REAADS aCL IgM)		
		+	-	Total
HemoSIL AcuStar aCL IgM	+	32	4**	36
	-	41*	190	231
	Total	73	194	267

*5 PAPS, 9 SAPS, 19 SLE, 2 others and 6 controls. It should be noted that the positive SLE and control samples with the predicate device are false positives

**1 PAPS, 1 SAPS, 1 SLE and 1 control

Positive agreement (32/73) = 43.8% (95% CI: 32.2% to 55.9%)

Negative agreement (190/194) = 97.9% (95% CI: 94.8% to 99.4%)

Overall agreement (222/267) = 83.1% (95% CI: 78.1% to 87.4%)

b. *Matrix comparison:*

HemoSIL AcuStar aCL IgG: 21 paired citrated plasma/serum samples with values spanning the linear range were assayed and the mean values were compared using a Passing-Bablok regression analysis. The slope was 1.04, intercept -0.22, and r = 1.000.

HemoSIL AcuStar aCL IgM: 61 paired citrated plasma/serum samples with values spanning the linear range were assayed and the mean values were compared using a Passing-Bablok regression analysis. The slope was 1.01, intercept 0.08, and $r = 0.999$.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity*:

321 clinically defined patient samples (23 with primary APS, 69 with secondary APS, 120 with SLE, 103 controls, and 6 with other diagnoses) were tested by the HemoSIL AcuStar aCL IgG and IgM assays:

HemoSIL AcuStar aCL IgG:

AcuStar aCL IgG	Clinical Diagnosis		
	POS	NEG	Total
POS	50	10	60
NEG	42	219	261
Total	92	229	321

Sensitivity $(50/92) = 54.3\%$ (95% CI: 43.6% to 64.8%)

Specificity $(219/229) = 95.6\%$ (95% CI: 92.1% to 97.9%)

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

Disease category	N	N Positive	% Positive
PAPS	23	13	56.5%
SAPS	69	37	53.6%
SLE	115	9	7.8%
SLE-like	5	0	0.0%
Others	6	1	16.7%
Normals	103	0	0.0%

HemoSIL AcuStar aCL IgM:

AcuStar aCL IgM	Clinical Diagnosis		
	POS	NEG	Total
POS	31	12	43
NEG	61	217	278
Total	92	229	321

Sensitivity $(31/92) = 33.7\%$ (95% CI: 24.2% to 44.3%)

Specificity $(217/229) = 94.8\%$ (95% CI: 91.0% to 97.3%)

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

Disease category	N	N Positive	% Positive
PAPS	23	8	34.8%
SAPS	69	23	33.3%
SLE	115	10	8.7%
SLE-like	5	0	0.0%
Others	6	1	16.7%
Normals	103	1	1.0%

- b. Other clinical supportive data (when a. is not applicable):
Not applicable.
4. Clinical cut-off:
See Assay cut-off.
5. Expected values/Reference range:
See Assay cut-off. The expected value in the normal population is negative.
- 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.