510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K221359

B Applicant

Instrumentation Laboratory Co.

C Proprietary and Established Names

ACL TOP 970 CL, HemosIL CL Anti-Cardiolipin IgM, HemosIL CL Anti-ß2 Glycoprotein-I IgM

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JPA	Class II	21 CFR 864.5425 - Multipurpose system for in vitro coagulation studies	HE - Hematology
MID	Class II	21 CFR 866.5660 - Multiple autoantibodies immunological test system	IM - Immunology
MSV	Class II	21 CFR 866.5660 - Multiple autoantibodies immunological test system	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:
New instrument module evaluated with previously cleared assays

B Measurand:
Anti-Cardiolipin IgM
Anti-ß2 Glycoprotein-I IgM

C Type of Test:
Automated semi-quantitative chemiluminescent immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
ACL TOP 970 CL

The ACL TOP 970 CL is a bench top, fully automated, random access analyzer designed specifically for in vitro diagnostic use by health care professionals in a clinical laboratory. The system provides results for both direct measurements and calculated parameters.

HemosIL CL Anti-Cardiolipin IgM

HemosIL CL Anti-Cardiolipin IgM is a fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-cardiolipin (aCL) IgM antibodies in human 3.2% or 3.8% citrated plasma on the ACL TOP 970 CL in the laboratory setting by a healthcare professional, as an aid in the diagnosis of Antiphospholipid Syndrome (APS) when used in conjunction with other laboratory and clinical findings.
For use with adult population. For prescription use only.

HemosIL CL Anti-ß2 Glycoprotein-I IgM

HemosIL CL Anti-ß2 Glycoprotein-I IgM is a fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-ß2 Glycoprotein-I (anti-ß2 GPI) IgM antibodies in human 3.2% or 3.8% citrated plasma on the ACL TOP 970 CL in the laboratory setting by a healthcare professional, as an aid in the diagnosis of Antiphospholipid Syndrome (APS) when used in conjunction with other laboratory and clinical findings.

For use with adult population. For prescription use only.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM: For use with adult population.

D Special Instrument Requirements:
ACL TOP 970 CL

IV Device/System Characteristics:

A Device Description:

<u>Instrument</u>

The ACL TOP 970 CL is a mid to large volume (80 sample capacity) bench-top, fully automatic, random-access analyzer that is intended for analysis using both open and closed (capped) sample

tubes and having cap piercing capability. The ACL TOP 970 CL is an instrument that integrates new chemiluminescent test capability similar to the ACL AcuStar, K083518.

Control Module (CM): The CM provides a user interface and operation control. It consists of a personal computer running Windows 10 software, keyboard, touch screen display monitor, mouse, and communications interfaces to the AM and external devices/systems. The CM provides the major functionality associated with the user interface (UI) including data management, data reduction, LIS (Laboratory Information System) communications, sample identification, test materials management, fluid management, reporting, test tracking and QC management, and monitoring.

Analytical Module (AM): The AM consists primarily of sample and reagent handling hardware. It processes reagents and auxiliary materials. It consists of the following subsystem:

- **CL System**: This subsystem of the instrument performs chemiluminescence testing. This includes the cartridge-based CL reagent loading area, magnetic wash stations and the Luminometer. It is located on the right side of the instrument. The CL System module of the ACL TOP 970 CL is based on the ACL AcuStar (K083518), with the same reagent cartridge formulation for the HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM assays and the exact same calibrator formulation. However, there are differences in software and hardware, as well as packaging/container for calibrators and CL instrument rinse solution.

Assays

The HemosIL CL Anti-Cardiolipin IgM kit consists of:

- Anti-Cardiolipin IgM Cartridge for 50 determinations: 1 cartridge containing 1 vial of magnetic particle suspension coated with bovine cardiolipin and human purified ß2 GPI, 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. The reagents are in a phosphate or borate buffer containing bovine serum albumin, bovine cardiolipin, human ß2GPI, mouse monoclonal IgM, stabilizers, and preservative.
- aCL IgM Calibrator 1: 1 x 1.2 mL barcoded vial of a solution with aCL IgM in saline solution containing bovine fetal serum, stabilizers, and preservative.
- aCL IgM Calibrator 2: 1 x 1.2 mL barcoded vial of a solution with aCL IgM in saline solution containing bovine fetal serum, stabilizers, and preservative. The calibrators are lot dependent and cannot be used with other lots of reagents.

The HemosIL CL Anti-ß2 Glycoprotein-I IgM kit consists of:

- Anti-ß2 Glycoprotein-I IgM Cartridge for 50 determinations: 1 cartridge containing 1 vial of a magnetic particle suspension coated with human purified ß2GPI, 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. The reagents are in a phosphate buffer containing bovine serum albumin, human ß2GPI, mouse monoclonal IgM, stabilizers, and preservative.
- Anti-ß2 GPI IgM Calibrator 1: 1 x 1.2 mL barcoded vial of a solution with aß2 GPI IgM in a phosphate buffer containing bovine serum albumin, stabilizers, and preservative.

- Anti-ß2 GPI IgM Calibrator 2: 1 x 1.2 mL barcoded vial of a solution with aß2 GPI IgM in a phosphate buffer containing bovine serum albumin, stabilizers, and preservative. The calibrators are lot dependent and cannot be used with other lots of reagents.

B Principle of Operation:

The ACL TOP 970 CL instrument performs chemiluminescence measurements.

Assays:

HemosIL CL Anti-Cardiolipin IgM
HemosIL CL Anti-Cardiolipin IgM is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with cardiolipin and human purified ß2 Glycoprotein-I, which capture, if present, the anti-cardiolipin 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 anti-cardiolipin 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 (RLU) by the ACL TOP 970 CL optical system. RLUs are directly proportional to the anti-cardiolipin IgM concentration in the sample.

The HemosIL CL Anti-Cardiolipin IgM assay utilizes a 4 Parameter Logistic Curve (4PLC) fit data reduction method to generate a Master Curve. The Master Curve is predefined, lot dependent, and 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 reagent kit calibrator value sheet 2D barcode.

HemosIL CL Anti-ß2 Glycoprotein-I IgM
HemosIL CL Anti-ß2 Glycoprotein-I IgM is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with human purified anti-ß2 glycoprotein-I, which capture, if present, the anti-ß2 glycoprotein-I 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 anti-ß2 glycoprotein-I 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 TOP 970 CL optical system. RLUs are directly proportional to the anti-ß2 glycoprotein-I IgM concentration in the sample.

The HemosIL CL Anti-ß2 Glycoprotein-I IgM assay utilizes a 4 Parameter Logistic Curve (4PLC) fit data reduction method to generate a Master Curve. The Master Curve is predefined, lot dependent and 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 reagent kit calibrator value sheet 2D barcode.

C Instrument Description Information:

1. Instrument Name:
   ACL TOP 970 CL

2. Specimen Identification:
   Samples, reagents, and diluents are identified by a barcode reader.

3. Specimen Sampling and Handling:
   Draw blood into tubes containing 3.2% or 3.8% sodium citrate. Using other anticoagulants may cause invalid results. Note: Unless specifically directed, using a discard tube is not necessary before collecting a citrated plasma sample. Fill to the proper level, indicated by a fill line on the tube. Gently invert six times to mix. Process immediately.

4. Calibration:
   The ACL TOP 970 CL instrument makes dilutions, adds reagents, and takes readings, then generates a calibration curve. The instrument can store the ten most recent calibrations. However, only one calibration can be validated at any time.

5. Quality Control:
   HemosIL CL Multi-Ab Low and High Controls are not supplied with the reagents or device and must be purchased separately. The reported values were determined over multiple runs on the ACL TOP 970 CL using specific lots of reagents and against an internal House Standard. Following published recommendations,[1] the units of the internal House Standard for the aCL IgM and aß2 GPI IgM antibodies have been correlated with the reference monoclonal antibody EY2C9[2]. Controls should be analyzed at least once every 8 hours when testing is taking place, in accordance with good laboratory practice. Control materials consist of two levels at or near cut-off and at abnormal levels.

V Substantial Equivalence Information:

A Predicate Device Name(s):
ACL Acustar,
HemosIL AcuStar Anti- Cardiolipin IgM,
HemosIL AcuStar Anti-β2 Glycoprotein-I IgM Assay

B Predicate 510(k) Number(s):
K083518
K092181
K091556

C Comparison with Predicate(s):

Instrument:

[1] Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006; 4: 295-306.
[2] Ichikawa K, Tsutsumi A, Atsumi T, et al. A chimeric antibody with the human γ1 constant region as a putative standard for assays to detect IgG ß2 Glycoprotein-I-dependent anticardiolipin and anti-ß2 Glycoprotein-I antibodies. Arthritis Rheum. 1999; 42: 2461-2470.

Device & Predicate Device(s):	K221359	K083518
Device Trade Name	ACL TOP 970 CL	ACL AcuStar
General Device Characteristic Similarities		
Quality Control	Automated QC	Same
Methodology	Chemiluminescence reading unit and Photo Multiplier Tube (PMT) to the ACL AcuStar	Same
Test Menu	Chemiluminescent assays: HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM	Same
General Device Characteristic Differences		
Intended Use/Indications For Use	The ACL TOP 970 CL is a bench top, fully automated, random access analyzer designed specifically for in vitro diagnostic use by health care professionals in a clinical laboratory. The system provides results for both direct measurements and calculated parameters.	ACL AcuStar is an automated immunoassay analyzer designed specifically for in vitro diagnostic use in a clinical laboratory. The assay analysis is based on chemiluminescent technology. The system provides results for both direct measurements and calculated parameters.
Software Interface	Windows 10	Windows 7

HemosIL CL Anti-Cardiolipin IgM

Device & Predicate Device(s):	K221359	K092181
Device Trade Name	HemosIL CL Anti-Cardiolipin IgM	HemosIL AcuStar Anti-Cardiolipin IgM
General Device Characteristic Similarities		
Intended Use/ Indications For Use	HemosIL CL Anti-Cardiolipin IgM is a fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-cardiolipin (aCL) IgM antibodies in human 3.2% or 3.8% citrated plasma on the ACL TOP® 970 CL in the	Fully automated chemiluminescent assay for the semi-quantitative measurement of anticardiolipin (aCL) IgM antibodies in human citrated plasma and serum on the ACL AcuStar, as an aid in the diagnosis of thrombotic disorders

Device & Predicate Device(s):	K221359	K092181
Device Trade Name	HemosIL CL Anti-Cardiolipin IgM	HemosIL AcuStar Anti-Cardiolipin IgM
	laboratory setting by a healthcare professional, as an aid in the diagnosis of Antiphospholipid Syndrome (APS) when used in conjunction with other laboratory and clinical findings.	related to primary and secondary Antiphospholipid Syndrome (APS).
Methodology	Two-step chemiluminescent immunoassay	Same
Calibrator	Two calibrator levels (included in test kit)	Same
Clinical Cut-off	20 U/mL	Same
General Device Characteristic Differences		
Sample Type	Citrated Plasma	Serum or Citrated Plasma
Assay Range	2.7–500.0 U/mL	1.0–15,480 U/mL
Quality Control	Different control material, with two levels at or near cut-off and at abnormal level	Low and high controls (sold separately)

HemosIL CL Anti-ß2 Glycoprotein-I IgM

Device & Predicate Device(s):	K221359	K091556
Device Trade Name	HemosIL CL Anti-ß2 Glycoprotein-I IgM	HemosIL AcuStar Anti-β2 Glycoprotein-I IgM
General Device Characteristic Similarities		
Intended Use/ Indications For Use	HemosIL CL Anti-ß2 Glycoprotein-I IgM is a fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-ß2 Glycoprotein-I (anti-ß2GPI) IgM antibodies in human 3.2% or 3.8% citrated plasma on the ACL TOP® 970 CL in the laboratory setting by a healthcare professional, as an aid in the diagnosis of Antiphospholipid Syndrome (APS) when used in conjunction with other laboratory and clinical findings.	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
Calibrator	Two calibrator levels (included in	Same

Device & Predicate Device(s):	K221359	K091556
Device Trade Name	HemosIL CL Anti-ß2 Glycoprotein-I IgM	HemosIL AcuStar Anti-β2 Glycoprotein-I IgM
	test kit)	
Clinical Cut-off	20.0 U/mL	Same
Methodology	Two-step chemiluminescent immunoassay	Same
General Device Characteristic Differences		
Sample Type	Citrated Plasma	Serum or Citrated Plasma
Assay Range	1.9–400.0 U/mL	1.1–841 U/mL
Quality Control	Different control material, with two levels at or near cut-off and at abnormal level	Low and high controls (sold separately)

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures (3rd Edition)
CLSI EP06: Evaluation of the Linearity of Quantitative Measurement Procedures (2nd Edition)
CLSI EP07: Interference Testing in Clinical Chemistry (3rd Edition)
CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance (2nd Edition)
CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures (2nd Edition)
CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents (1st Edition)
CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory (3rd Edition)
CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry (1st Edition)
IEC 61010-1, Safety requirements for electrical equipment for measurement, control and laboratory use – Part 1: General requirements
IEC 61010-2-101, Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101
IEC 61326-1, Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements
IEC 61326-2-6, Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

HemosIL CL Anti-Cardiolipin IgM

Within-laboratory precision study was performed using three lots of HemosIL CL Anti-Cardiolipin IgM. To span the assay range, the study tested five 3.2% citrated plasma samples (3 positive and 2 negative), and three lots of HemosIL CL Multi-Ab Controls (low and high).

Each material was run in duplicate, twice per day over 20 days on an ACL TOP 970 CL. The standard deviation (SD) and %CV of the within-run (repeatability), between-run, between-day, and total within-laboratory imprecision were calculated for each sample and the results are summarized in the following table.

Sample	N	Mean (U/mL)	Repeatability		Between-run		Between-Day		Between-lot		Within-lab	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low QC	239*	8.35	0.31	3.7	0.09	1.1	0.12	1.5	0.14	1.6	0.37	4.4
High QC	240	86.13	1.68	1.9	1.76	2.0	0.83	1.0	1.05	1.2	2.78	3.2
Plasma A	240	5.50	0.62	11.3	0.27	5.0	0.00	0.0	0.53	9.6	0.86	15.7
Plasma B	240	15.68	0.42	2.7	0.21	1.4	0.32	2.1	0.82	5.2	1.00	6.4
Plasma C	240	24.33	0.72	3.0	0.13	0.6	0.37	1.5	0.65	2.7	1.05	4.3
Plasma D	240	107.46	2.51	2.3	2.04	1.9	0.00	1.0	1.75	1.6	3.67	3.4
Plasma E	240	396.44	9.79	2.5	4.03	1.0	4.80	1.2	9.27	2.3	14.87	3.8

*1 Low Control aggregate result excluded due to being an obvious visual and statistical outlier (Grubbs' test).

A multi-site reproducibility study was conducted to evaluate the reproducibility of HemosIL CL Anti-Cardiolipin IgM at three external U.S. clinical sites using three different lots of HemosIL CL Anti-Cardiolipin IgM with two controls (low and high) and four plasma sample pools. Each material was tested in triplicate, twice a day for five days, for a total of 30 replicates per level. Instrument and operator variables were nested within the multiple site component – i.e., a different operator and instrument was used at each of the three sites.

Multi-Site Multi-Lot Results for all Reagent Lot Combined:

Sample	N	Mean (U/mL)	Repeatability		Between-run		Between-Day		Between-site		Between-lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low QC	270	8.79	0.33	3.7	0.17	2.0	0.0	0.0	0.47	5.3	0.23	2.7	0.64	7.3
High QC	270	103.04	3.06	3.0	3.11	3.0	0.0	0.0	4.30	4.2	3.57	3.5	7.09	6.9
Clinical 1*	270	8.13	0.29	3.6	0.32	3.9	0.06	0.7	0.54	6.6	2.55	31.4*	2.65	32.5
Clinical 2	270	30.60	0.92	3.0	0.39	1.2	0.32	1.1	0.98	3.2	0.55	1.8	1.54	5.0
Clinical 3	270	101.44	3.38	3.3	1.71	1.7	0.0	0.0	3.61	3.6	2.98	2.9	6.02	5.9
Clinical 4	270	407.04	11.23	2.8	15.41	3.8	4.83	1.2	14.13	3.5	5.77	1.4	24.90	6.1

*The %CV for the multi-site reproducibility for this sample is 8.0%, 8.2% and 9.5% for each of three lots tested.

HemosIL CL Anti-ß2 Glycoprotein-I IgM

Within-laboratory precision study was performed using three lots of HemosIL CL Anti-ß2 Glycoprotein-I IgM. To span the assay range, the study tested five 3.2% citrated plasma samples (3 positive and 2 negative), and three lots of HemosIL CL Multi-Ab Controls (low and high). Each material was run in duplicate, twice per day over 20 days on an ACL TOP 970 CL.

Sample	N	Mean (U/mL)	Repeatability		Between-run		Between-Day		Between-lot		Within-lab	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low QC	238*	4.07	0.28	6.8	0.14	3.4	0.03	0.8	0.52	12.8	0.61	14.9
High QC	240	51.52	1.05	2.0	1.09	2.1	0.50	1.0	5.91	11.5	6.12	11.9
Plasma A	240	8.35	0.44	5.3	0.22	2.7	0.00	0.0	0.47	5.6	0.68	8.2

Sample	N	Mean (U/mL)	Repeatability		Between-run		Between-Day		Between-lot		Within-lab	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma B	240	15.0	0.47	3.1	0.30	2.0	0.13	0.9	0.66	4.4	0.87	5.8
Plasma C	240	21.68	0.50	2.3	0.11	0.5	0.32	1.5	0.77	3.6	0.97	4.5
Plasma D	240	96.85	2.24	2.3	2.57	2.7	0.00	0.0	7.02	7.2	7.80	8.1
Plasma E	240	297.29	6.81	2.3	7.11	2.4	0.00	0.0	21.24	7.1	23.41	7.9

*2 aggregate Low Control results excluded due to being obvious visual and statistical outliers (Grubbs' test).

A multi-site reproducibility study was conducted to evaluate the reproducibility of HemosIL CL Anti-ß2 Glycoprotein-I IgM at three external US clinical sites using three different lots of HemosIL CL Anti-Cardiolipin IgM with two controls (low and high) and four plasma sample pools. Each material was tested in triplicate, twice a day for 5 days, for a total of 30 replicates per level. Instrument and operator variables were nested within the multiple site component – i.e., a different operator and instrument was used at each of the three sites.

Multi-Site Multi-Lot Results for all Reagent Lot Combined:

Sample	N	Mean	Repeatability		Between-run		Between-Day		Between-site		Between-lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low QC	270	9.99	0.36	3.6	0.32	3.2	0.00	0.0	0.34	3.4	0.51	5.1	0.78	7.8
High QC	270	127.88	4.74	3.7	3.35	2.6	0.00	0.0	4.49	3.5	6.10	4.8	9.54	7.5
Clinical 1	270	8.30	0.27	3.2	0.22	2.7	0.00	0.0	0.37	4.4	0.34	4.1	0.61	7.3
Clinical 2	270	26.88	0.66	2.5	0.44	1.7	0.28	1.0	0.91	3.4	1.02	3.8	1.61	6.0
Clinical 3	270	90.30	2.40	2.7	1.99	2.2	1.76	1.9	3.60	4.0	3.0	3.3	5.90	6.5
Clinical 4	270	284.29	6.67	2.3	4.59	1.6	3.15	1.1	7.83	2.8	0.00	0.0	11.70	4.1

2. Linearity:
   Linearity was assessed per CLSI EP06 (2nd Edition) using three different lots of HemosIL CL Anti-Cardiolipin IgM and three different lots of HemosIL CL Anti-ß2 Glycoprotein-I IgM reagent cartridges. For each assay, a set of 11 linearity samples were prepared by diluting a high antibody plasma sample with a negative antibody plasma sample to create the required sample concentrations. Each level was measured in six replicates with three lots for each assay. The linearity range was determined such that all acceptance criteria were met.
   The results support the linearity of the claimed analytical measuring range: 2.7–500 U/mL for HemosIL CL Anti-Cardiolipin IgM; 1.9–400.0 U/mL for HemosIL CL Anti-ß2 Glycoprotein-I IgM.

3. Analytical Specificity/Interference:
   An interference study was performed with three lots of HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM on an ACL TOP 970 CL. Three different plasma samples at different antibody concentrations were tested. Normal pooled plasma (Negative:< 20.0 U/mL): unadulterated APS positive donor sample diluted in a normal pooled plasma (MDL: ~20.0 U/mL), and unadulterated APS positive donor sample (High Positive: > 20.0 U/mL). A minimum of 14 replicates was ran for each sample and compared to the control sample. Based on the results, the following tables display the highest concentration in which ≤ ±10% interference was observed for the two assays.

Endogenous Substances

Interferent	HemosIL CL Anti-Cardiolipin IgM	HemosIL CL Anti-ß2 Glycoprotein-I IgM
Bilirubin (Free & Conjugated)	40 mg/dL	40 mg/dL
Hemoglobin	1000 mg/dL	1000 mg/dL
Rheumatoid Factor	500 IU/mL	500 IU/mL
Triglycerides	1500 mg/dL	1500 mg/dL

Exogenous Substances

Interferent	HemosIL CL Anti-Cardiolipin IgM	HemosIL CL Anti-ß2 Glycoprotein-I IgM
Acetylsalicylic acid	3 mg/dL	3 mg/dL
Acid Citric Dextrose	4.5 g/dL	4.5 g/dL
Atorvastatin	0.075 mg/dL	0.075 mg/dL
Heparin (LMW and UF)	3.3 IU/mL	3.3 IU/mL
Hydroxychloroquine	777.6 ng/mL	777.6 ng/mL
Prednisone	9.90 E-03 mg/dL	9.90 E-03 mg/dL
Rituximab	318 mcg/mL	318 mcg/mL
Warfarin	7.5 mg/dl	7.5 mg/dL

4. Assay Reportable Range:

   The assay reportable range is as follows:
   - HemosIL CL Anti-Cardiolipin IgM: 2.7–500 U/mL;
   - HemosIL CL Anti-ß2 Glycoprotein-I IgM: 1.9–400.0 U/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Traceability:
   International reference materials for anti-cardiolipin antibodies and anti-β2 glycoprotein-I IgM antibodies are not available. The assays are 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.

   Sample Stability:
   Refer to Clinical and Laboratory Standards Institute (CLSI) Document H21, current edition, for further instructions on specimen collection, handling, and storage.

   Fresh versus Frozen Study
   A fresh versus frozen 3.2% citrated plasma sample study was performed at four external US sites to verify equivalence of the HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM measurement procedures when APS suspected specimens are tested fresh and then across two freeze-thaw cycles on the ACL TOP 970 CL. The first aliquot of fresh patient samples was tested in triplicate with the HemosIL CL Anti-Cardiolipin IgM and the HemosIL CL Anti-ß2 Glycoprotein-I IgM assays. For the first freeze-thaw cycle, the aliquots were kept frozen at ≤ -20 °C for a minimum of 24 hours prior to testing. Each frozen aliquot was rapidly thawed at 37°C for ≤ 10 minutes for retesting in triplicate with the assay. The freezing, thawing, and testing steps were repeated for a second freeze/thaw cycle. The

results suggest that there is no statistically significant difference between the results of fresh plasma samples and plasma samples frozen and thawed once or twice prior to being tested on the ACL TOP 970 CL with HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM assays.

Reagent Stability:

Shelf-Life Stability

A shelf-life stability study was performed to establish the real-time stability claim of 2–8°C for HemosIL CL Anti-Cardiolipin IgM reagent cartridge. Six lots of reagent cartridges were stored at 2–8°C and tested in triplicate using a panel of four citrated plasma sample pools and two control levels (low and high) at the following time intervals baseline, 3, 4, 6, 9, 10, 11, 12, and 13 months. Individual test results were compared to the baseline mean. All results were within the established acceptance criteria. The HemosIL CL Anti-Cardiolipin IgM reagent cartridge has a shelf-life claim of 12 months when stored at 2–8°C.

A shelf-life stability study was performed to establish the real-time stability claim of 2–8°C for HemosIL CL Anti-ß2 Glycoprotein-I IgM reagent cartridge. Three lots of reagent cartridges were stored at 2–8°C and tested in triplicate using a panel of four citrated plasma sample pools and two control levels (low and high) at the following time intervals baseline, 2, 3, 4, 6, 9, 10, and 11 months. Individual test results were compared to the baseline mean. All results were within the established acceptance criteria. The HemosIL CL Anti-ß2 Glycoprotein-I IgM reagent cartridge has a shelf-life of 10 months when stored at 2–8°C.

On-Board Stability

An on-board stability study was performed to establish the continuous on-board instrument stability claim for HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM reagent cartridges on the ACL TOP 970 CL at 2–8°C. Three lots of each reagent cartridge were placed on-board the ACL TOP 970 CL and tested in triplicate using a panel of four citrated plasma sample pools and two control levels at the following time intervals baseline, 1, 2, 3, 4, 5, 6, and 7 weeks. The baseline means were established with 12 replicates of each plasma and control samples. Results were compared to the baseline means and all results were within the acceptance criteria and support an on-board stability claim of six weeks at 2–8°C.

Shipping Stability

A transport simulation study was performed to assess the shipping stability of the HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM reagent cartridges during transport. The testing was performed with three lots of each reagent cartridge. The transport simulation test was assessed by comparing the performance of reagent cartridges boxed as final kits and packaged into two packs for regular shipment and held at two different temperatures:

- Stressed Pack 1: 4 days at room temperature (15–30°C) followed by one day at -20°C followed by 2–8°C storage until testing
- Stressed Pack 2: 2 days at room temperature (15–30°C), three days at 37°C followed by 2–8°C storage until testing

Testing was performed within seven days after respective stress termination of each pack compared to the baseline (cartridges stored continuously at 2–8°C). Reagent cartridges were

analyzed with a panel of four citrated plasma sample pools and two control levels (low and high), with each material run in triplicate. Results obtained in the transport simulation study were within acceptance criteria and supports that the product packaging mitigates the potential impact of temperature stressing during transport.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) studies were performed according to CLSI EP17-A2 by using three different lots of each HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM tested on an ACL TOP 970 CL.

The LoB was determined by the measuring five analyte-free plasma samples tested in five replicates over three days in a single run per day for each of three lots (75 observations per lot). The LoB was calculated as the 95th percentile using the non-parametric method for each lot as the dataset showed non-normal distribution. The claimed LoB was the highest 95th percentile value across the three lots.

The LoD was determined by measuring five low level plasma samples tested in replicates of five over three days in a single run per day for each of three lots (75 observations per lot). LoD was calculated as the LoB + 1.652 x SD of the replicates for the samples and the highest LoD across the three lots was the LoD claim.

The LoQ was determined by measuring five low level plasma samples tested in replicates of six over three days in two runs per day for each of three lots (60 replicates per sample per lot). The LoQ was defined as the lowest concentration of measurand that meets within-laboratory imprecision goal of <20% for each lot.

	LoB U/mL	LoD U/mL	LoQ U/mL
HemosIL CL Anti-Cardiolipin IgM	1.7	2.0	2.0
HemosIL CL Anti-ß2 Glycoprotein-I IgM	0.8	1.0	1.9

7. Assay Cut-Off:
The assay cut-offs were determined to be 20 U/mL in a clinical cut-off study below.

8. Accuracy (Instrument):
See Section F.

9. Carry-Over:
Sample carryover testing for HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-β_2 Glycoprotein-I IgM was performed on two ACL TOP 970 CL instruments. Carryover was determined by a two-step process. The first step was the determination of the expected baseline results for the low/recipient sample. The second step consisted of multiple runs which included the low recipient and high donor sample. The sample carryover was an average overall carryover of -0.35 U/mL for HemosIL CL Anti-Cardiolipin IgM and 0.93 U/mL for HemosIL CL Anti-β_2 Glycoprotein I IgM.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   a) An external method comparison study compared the performance of the HemosIL CL Anti-Cardiolipin IgM assay on the ACL TOP 970 CL instrument to the predicate HemosIL AcuStar Anti-Cardiolipin IgM assay (K092181) on the ACL AcuStar instrument (K083518). Only samples that were found within the measuring intervals of the HemosIL CL Anti-Cardiolipin IgM and/or the HemosIL AcuStar Anti-Cardiolipin IgM assays were used for the method comparison analysis. A total of 131 samples (128 native and 3 contrived) were analyzed in singlicate on both the predicate and candidate devices at one external site using one assay lot. The results were analyzed by a Passing-Bablok, regression and the positive percent agreement (PPA), negative percent agreement (NPA), and total agreement were calculated. The results are summarized in the tables below:

N	Min (U/mL)	Max (U/mL)	r	Slope (95%CI)	Intercept (95% CI)	%Bias at MDL (20.0 U/mL)
131	2.8	478.8	1.00	1.00 (0.98, 1.01)	-0.13 (-0.52, 0.26)	-1% (-2, 0%)

HemosIL CL Anti-Cardiolipin IgM	AcuStar Anti-Cardiolipin IgM				
	Positive	Negative	Total		Percent Agreement (95% CI)
Positive	102	0	102	PPA	100.0% (96.4%, 100.0%)
Negative	0	29	29	NPA	100.0% (88.3%, 100.0%)
Total	102	29	131		

   b) An external method comparison study compared the performance of the HemosIL CL Anti- β2 Glycoprotein-I IgM assay on the ACL TOP 970 CL instrument relative to the predicate HemosIL AcuStar Anti-β2 Glycoprotein-I IgM assay (K091556) on the ACL AcuStar instrument (K083518). Only samples that were found within the measuring intervals of the HemosIL CL Anti- β2 Glycoprotein-I IgM and/or the HemosIL AcuStar Anti- β2 Glycoprotein-I IgM assays were used for the method comparison analysis. A total of 123 native samples were analyzed in singlicate on both the predicate and candidate devices using one assay lot. The results were analyzed by a Passing-Bablok regression, and the positive percent agreement (PPA), negative percent agreement (NPA), and total agreement were calculated. The results are summarized in the tables below:

N	Min (U/mL)	Max (U/mL)	r	Slope (95%CI)	Intercept (95% CI)	%Bias at MDL (20.0 U/mL)
123	2.1	399.0	0.990	0.94 (0.92, 0.96)	0.13 (-0.22, 0.72)	-5% (-7, -3%)

HemosIL CL Anti-Cardiolipin IgM	AcuStar Anti-Cardiolipin IgM				
	Positive	Negative	Total		Percent Agreement (95% CI)
Positive	92	1	93	PPA	98.9% (94.2%, 99.8%)
Negative	1	29	30	NPA	96.70% (83.3%, 99.4%)
Total	93	30	123		

2. Matrix Comparison:
The study to assess the effect of different concentrations of sodium citrate (3.2% or 3.8%) found within the commercially available sample collection tubes on the performance of the HemosIL CL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM on the ACL TOP 970 CL.

Paired plasma samples were collected in 3.2% and 3.8% sodium citrate anticoagulant collection devices from adults ≥ 22 years of age who were diagnosed with APS or some other non-APS condition and tested on the ACL TOP 970 CL.

The study results suggest there is no difference in performance between 3.2% or 3.8% citrated plasma samples with HemosIL Anti-Cardiolipin IgM and HemosIL CL Anti-ß2 Glycoprotein-I IgM on the ACL TOP 970 CL.

3.2% Sodium Citrate Plasma vs 3.8% Sodium Citrate Plasma				
Assay:	**N**	**Range (U/mL)**	**Slope (95%CI)**	**Intercept (95%CI)**
HemosIL CL Anti-Cardiolipin IgM	61	2.8–485.3	1.01 (1.00, 1.03)	-0.04 (-0.32, 0.23)
HemosIL CL Anti-ß2 Glycoprotein-I IgM	46	2.0–376.9	1.01 (0.99, 1.03)	0.02 (-0.09, 0.14)

C Clinical Studies:

1. Clinical Sensitivity and Specificity:
A cohort of clinical samples evaluated the clinical sensitivity and specificity of the HemosIL CL Anti-ß2 Glycoprotein-I IgM assay. The study included APS samples from the patients diagnosed with APS and non-APS samples from patients diagnosed with a variety of clinical conditions. The samples were enrolled and tested at three clinical sites or were acquired and tested at an internal laboratory. Investigators at each clinical site enrolled subjects according to pre-specified inclusion/exclusion criteria. The diagnosis of APS was based on the 2006 classification criteria[1] for APS, while non-APS samples were enrolled based on a suspicion of APS or having related diseases, including autoimmune diseases, thrombotic disorders, infectious diseases, pre-eclampsia. Data from each site was compiled and the clinical sensitivity and specificity for HemosIL CL Anti-Cardiolipin IgM assay and the HemosIL CL Anti-ß2 Glycoprotein-I IgM assay in diagnosis of APS and non-APS were analyzed and results are shown in the following tables:

HemosIL CL Anti-Cardiolipin IgM:

		Diagnosis			**Clinical Analysis**
		APS	Non-APS	Total	
HemosIL CL Anti-Cardiolipin IgM	Positive	77	25	102	Sensitivity (95% CI): 40.5% (33.8%, 47.6%)
	Negative	113	285	398	
	Total	190	310	500	Specificity (95% CI): 91.9% (88.4%, 94.5%)

HemosIL CL Anti-B2 Glycoprotein-I IgM:

		Diagnosis			Clinical Analysis
		APS	Non-APS	Total	
HemosIL CL Anti-B2 Glycoprotein-I IgM	Positive	63	17	80	Sensitivity (95% CI): 33.0% (26.7%, 39.9%)
	Negative	128	295	423	
	Total	191	312	503	Specificity (95% CI): 94.6% (91.4%, 96.6%)

D Clinical Cut-Off:

The 20.0 U/mL clinical cut-off for the assays was previously determined, in K092181 for the HemosIL AcuStar Anti-Cardiolipin IgM assay and in K091556 for the HemosIL AcuStar Anti-ß2 Glycoprotein-I IgM assay.

E Expected Values/Reference Range:

A negative test result is expected in both assays for apparently healthy individuals. To determine the expected values, 100 normal donors (60 females and 40 males) were tested in singlicate on an ACL TOP 970 CL instrument following CLSI EP28-A3c. All results from the normal donors were below the cut-off of 20.0 U/mL. For the HemosIL CL Anti-Cardiolipin IgM assay, the range of samples was 0.0–7.7 U/mL and for the HemosIL AcuStar Anti-ß2 Glycoprotein-I IgM assay the range of sample concentrations was 0.0–2.6 U/mL.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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