



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

I Background Information:

A 510(k) Number

K243374

B Applicant

Instrumentation Laboratory (IL) Co.

C Proprietary and Established Names

HemosIL CL HIT-IgG_(PF4-H)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LCO	Class II	21 CFR 864.7695 - Platelet Factor 4 Radioimmunoassay	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:

Clearance of a new device

B Measurand:

Anti-PF4/Heparin IgG Antibodies

C Type of Test:

Qualitative assay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

HemosIL CL HIT-IgG_(PF4-H) is a qualitative, fully automated, chemiluminescent immunoassay (CIA) for the detection of IgG antibodies that react with Platelet Factor 4 (PF4) when complexed to heparin. The assay is for use in human 3.2% citrated plasma on the ACL TOP 970 CL in a laboratory setting.

The result provided by the assay should be interpreted as either positive or negative based on the assay cut-off (1.00 U/mL). The positive or negative result aids in determining the risk for heparin-induced thrombocytopenia (HIT) when used in conjunction with other laboratory and clinical findings.

Anti-PF4/Heparin antibodies are commonly found in patients with HIT. For use in adult population suspected of HIT. Not for use in isolation to exclude HIT.

For prescription use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

ACL TOP 970 CL (K221359)

IV Device/System Characteristics:**A Device Description:**

The HemosIL CL HIT-IgG_(PF4-H) kit consists of:

- CL HIT-IgG_(PF4-H) Cartridge: 1 cartridge containing 1 vial of magnetic particle suspension coated with PF4/PVS complex, 1 vial of assay buffer, 1 vial of tracer consisting of an mAb anti-human IgG antibody labeled with isoluminol, and 1 vial of sample diluent used for the regular predilution of the sample. The reagents are in a phosphate or HEPES buffer containing bovine serum albumin, bovine fetal serum, PF4/PVS complex, mouse monoclonal IgG, stabilizers, and preservative.
- CL HIT-IgG_(PF4-H) Calibrator 1: 1 x 1.2 mL barcoded vial of a solution with humanized mAb anti-PF4-Heparin in Tris buffer containing bovine serum albumin, stabilizers and preservative.
- CL HIT-IgG_(PF4-H) Calibrator 2: 1 x 1.2 mL barcoded vial of a solution with humanized mAb anti-PF4-Heparin in Tris buffer containing bovine serum albumin, stabilizers, and preservative.

The calibrators are lot specific, and they cannot be used with other lots of reagents.

B Principle of Operation:

HemosIL CL HIT-IgG_(PF4-H) assay is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with PF4 complexed to polyvinyl sulfonate (PVS) which capture PF4/H antibodies from the sample, if present.

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 PF4/H IgG on the

particles. After a second incubation, magnetic separation, and a 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. The RLUs are directly proportional to the PF4/H IgG concentration in the sample.

The HemosIL CL HIT-IgG_(PF4-H) assay utilizes a 4 Parameter Logistic Curve fit (4PLC) 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 reagent kit calibrator value sheet 2D barcode.

V Substantial Equivalence Information:

A Predicate Device Name(s):

HemosIL AcuStar HIT-IgG_(PF4-H), HemosIL AcuStar HIT Controls

B Predicate 510(k) Number(s):

K170854

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K243374</u>	<u>K170854</u>
Device Trade Name	HemosIL CL HIT-IgG _(PF4-H)	HemosIL AcuStar HIT-IgG _(PF4-H) HemosIL AcuStar HIT Controls
General Device Characteristic Similarities		
Intended Use/Indications For Use	HemosIL CL HIT-IgG _(PF4-H) is a qualitative, fully automated, chemiluminescent immunoassay (CIA) for the detection of IgG antibodies that react with Platelet Factor 4 (PF4) when complexed to heparin. The assay is for use in human 3.2% citrated plasma on the ACL TOP 970 CL in a laboratory setting. The result provided by the assay should be interpreted as either positive or negative	HemosIL AcuStar HIT-IgG _(PF4-H) is a qualitative, fully automated, chemiluminescent immunoassay (CIA) for the detection of IgG antibodies that react with Platelet Factor 4 (PF4) when complexed to heparin. The assay is for use in human 3.2% or 3.8% citrated plasma and serum on the ACL AcuStar instrument in a laboratory setting. The result provided by the assay should be interpreted

	based on the assay cut-off (1.00 U/mL). The positive or negative result aids in determining the risk for heparin induced thrombocytopenia (HIT) when used in conjunction with other laboratory and clinical findings. Anti-PF4/Heparin antibodies are commonly found in patients with HIT. For use with adult population suspected of HIT. Not for use in isolation to exclude HIT. For prescription use only.	as either positive or negative based on the assay cut-off (1.00 U/mL). The positive or negative result aids in determining the risk for heparin induced thrombocytopenia (HIT) when used in conjunction with other laboratory and clinical findings. Anti-PF4/Heparin antibodies are commonly found in patients with HIT. For use in adult population suspected of HIT. Not for use in isolation to exclude HIT. For prescription use.
Product code	LCO	LCO
Measurand	Anti-PF4/Heparin IgG Antibodies	Same
Methodology	Chemiluminescent immunoassay (CIA)	Same
Reagents	Cartridge containing magnetic particle suspension coated with PF4 complexed to polyvinyl sulfonate (PVS)	Same
Antibodies	Mouse monoclonal anti-human IgG antibody	Same
Conjugate	Isoluminol conjugated anti-human IgG	Same
Cut-off	Positive: ≥ 1.00 U/mL Negative: < 1.00 U/mL	Same
General Device Characteristic Differences		
Sample Type	Human 3.2% citrated plasma	Human citrated (3.2 and 3.8%) plasma and serum
Instrumentation	ACL TOP 970 CL	ACL AcuStar
Calibrators	Two calibrator levels (included in the kit): Calibrator 1 target value: 1.00 U/mL	Two calibrator levels (included in the kit): Calibrator 1 target value: 1.00 U/mL

	Calibrator 2 target value: 16.50 U/mL	Calibrator 2 target value: 16.00 U/mL
Controls	Two control levels (sold separately, liquid format): Low Control target value: 0.60 U/mL High Control target value: 3.50 U/mL	Two control levels (sold separately, liquid format): Low Control target value: 0.60 U/mL High Control target value: 3.00 U/mL

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition
- CLSI EP07-Ed.3: Interference Testing in Clinical Chemistry—Third Edition
- CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition.
- CLSI EP25-A2: Evaluation of Stability of In Vitro Medical Laboratory Test Reagents—Second Edition
- CLSI EP28-A3: Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition
- CLSI EP37-Ed1: Supplement Tables for Interference Testing in Clinical Chemistry—First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Repeatability:

An internal precision study was performed using two (2) controls and five (5) plasma sample pools on two ACL TOP 970 CL instruments (The controls were run on one (1) ACL TOP 970 CL instrument and the plasma sample pools on a second ACL TOP 970 CL). Each sample was tested with three (3) lots of HemosIL CL HIT-IgG_(PF4-H) reagent cartridges for 20 days, with two (2) runs a day and two (2) replicates per run, yielded N=80 replicates per sample for each reagent cartridge lot as summarized below.

Sample	Mean (U/mL)	N	Repeatability		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Sample A (pool)	0.272	240	0.018	6.7%	0.000	0.0%	0.012	4.2%	0.022	8.1%	0.031	11.3%
Plasma Sample B (pool)	0.777	240	0.029	3.7%	0.027	3.5%	0.023	2.9%	0.112	14.5%	0.122	15.6%
Plasma Sample C (pool)	1.210	240	0.042	3.4%	0.053	4.4%	0.020	1.7%	0.118	9.7%	0.137	11.3%
Plasma Sample D (unadulterated)	1.817	240	0.062	3.4%	0.067	3.7%	0.062	3.4%	0.082	4.5%	0.137	7.6%
Plasma Sample E (pool)	21.451	240	0.618	2.9%	0.386	1.8%	0.632	2.9%	2.031	9.5%	2.248	10.5%
High Multi-Ab Control	3.260	240	0.105	3.2%	0.073	2.2%	0.075	2.3%	0.064	2.0%	0.161	4.9%
Low Multi-Ab Control	0.440	239	0.019	4.4%	0.011	2.5%	0.012	2.7%	0.009	2.1%	0.027	6.1%

Qualitative Agreement Summary

Sample Description	Expected Status	Total Replicates	Positive	Negative
Plasma Sample A	Negative	320	0/320	320/320
Plasma Sample B	Negative	320	3/320	317/320
Plasma Sample C	Positive	320	320/320	0/320
Plasma Sample D	Positive	320	320/320	0/320
Plasma Sample E	Positive	320	320/320	0/320
Low Multi-Ab Control	Negative	319*	0/319	319/319
High Multi-Ab Control	Positive	320	320/320	0/320

*Failed result due to instrument error. Replicate not repeated.

2. Reproducibility:

Reproducibility studies were conducted at three (3) external clinical sites by four operators (minimum of 1 operator per site), on three (3) ACL TOP 970 CL instruments (one instrument per site), using three (3) lots of HemosIL CL HIT-IgG_(PF4-H) with six (6) plasma sample pools (samples 1–6) and two (2) controls (low (sample 7) and high (samples 8)). Each sample was tested in triplicate, twice a day for 5 days, for a total of 270 replicates per sample. The pooled data for each sample is presented below:

Sample ID	N	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Between-Site		Between-lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	270	0.405	0.052	12.9%	0.005	1.2%	0.010	2.4%	0.024	6.0%	0.000	0.0%	0.059	14.4%
2	270	0.050	All replicates < 1.00 U/mL											
3	270	0.845	0.051	6.0%	0.023	2.7%	0.026	3.0%	0.020	2.4%	0.000	0.0%	0.065	7.6%
4	270	1.336	0.081	6.1%	0.042	3.2%	0.000	0.0%	0.033	2.4%	0.025	1.9%	0.101	7.5%
5	270	2.622	0.102	3.9%	0.057	2.2%	0.051	1.9%	0.032	1.2%	0.096	3.7%	0.162	6.2%
6	270	19.082	0.665	3.5%	0.422	2.2%	0.315	1.7%	0.853	4.5%	2.882	15.1%	3.123	16.4%
7	270	0.515	0.021	4.1%	0.024	4.7%	0.000	0.0%	0.020	3.8%	0.011	2.1%	0.039	7.6%
8	270	3.487	0.105	3.0%	0.121	3.5%	0.053	1.5%	0.046	1.3%	0.080	2.3%	0.193	5.5%

Qualitative Agreement Summary

Sample ID	Expected Status of Sample	N	Positive	Negative
1	Negative	270	0/270	270/270
2	Negative	270	0/270	270/270
3	Negative	270	2/270	268/270
4	Positive	270	270/270	0/270
5	Positive	270	270/270	0/270
6	Positive	270	270/270	0/270
7	Negative	270	0/270	270/270
8	Positive	270	270/270	0/270

3. Linearity:

HemosIL CL HIT-IgG_(PF4-H) assay is a qualitative assay and linearity is not applicable.

4. Analytical Specificity/Interference:

Interference study was conducted using a paired-bias approach testing each potential interfering substance on either two or three lots of HemosIL CL HIT-IgG_(PF4-H) on a minimum of one ACL TOP 970 CL at three sample levels for each substance: baseline (no interfering substance), at the intended claim, and at 20% above the intended claim (or C₀, C_{max}, and C_{max}+20%, respectively). Interfering substances were tested in three citrated plasma pools (Negative, MDL, and High Positive) with a minimum of 14 replicates per sample.

A dose-response interference study was performed for unfractionated heparin (UFH), low molecular weight heparin (LMWH), rheumatoid factor (RF), fondaparinux, and protamine. Five levels of each interferent were tested in three citrated plasma pools (Negative, MDL, and High Positive), with five (5) replicates per each level.

A cross-reactivity study was conducted with 24 patients diagnosed with Antiphospholipid Syndrome (APS) and 20 patient samples negative for APS. Samples were tested with one lot of HemosIL CL HIT-IgG_(PF4-H) on the ACL TOP 970 CL.

The results of the above studies demonstrate that the HemosIL CL HIT-IgG_(PF4-H) on the ACL TOP 970 CL analyzer is not affected by the interferents up to the levels indicated in table below:

Limitations/ Interfering Substances	Concentrations up to:
Hemoglobin	1000 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Bilirubin (conjugated)	40 mg/dL
Triglycerides	1500 mg/dL
Unfractionated heparin	1.2 IU/mL
Low Molecular weight heparin	2.5 IU/mL
HAMA	1 µg/mL
Rheumatoid Factor	160 IU/mL
Acid Citric Dextrose	0.45 g/dL
Argatroban	1.2 µg/mL
Fondaparinux	0.102 mg/dL
Dabigatran	0.900 mg/dL
Rivaroxaban	0.270 mg/dL
Protamine	5 mg/dL
Antiphospholipid Syndrome	Twenty-four citrated plasma samples from patients diagnosed with Antiphospholipid Syndrome (APS) were tested with the HemosIL CL HIT-IgG _(PF4-H) assay and four commercially available antiphospholipid antibody assays (anti-cardiolipin IgG, anti-cardiolipin IgM, anti-β2 glycoprotein I IgG, anti-β2 glycoprotein I IgM). Three out of the twenty-four samples recovered above the cut-off of 1.00 U/mL (positive) for HemosIL CL HIT-IgG _(PF4-H) . These three samples also tested positive for anti-cardiolipin IgG but tested negative for the other three antiphospholipid assays.

5. Assay Reportable Range:

Not applicable

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

Traceability

There is currently no International Standard available for Anti-PF4/Heparin (PF4/H) antibodies; therefore, each manufacturer must establish their own internal standardization and reportable units. Two different types of calibrator materials are prepared for the HemosIL CL HIT-IgG_(PF4-H) assay standardization:

- Internal Primary Reference Materials: Used internally to prepare the kit calibrators

- Kit Calibrators: Used by the ACL TOP 970 CL instrument to prepare the Working Calibration Curve

HemosIL CL HIT-IgG_(PF4-H) is standardized to Internal Primary Reference Material (humanized monoclonal anti PF4-Heparin antibody diluted in Tris-buffer containing negative human serum, stabilizers, and preservative). The Primary Reference Materials are shared among HemosIL CL HIT-IgG_(PF4-H) and HemosIL AcuStar HIT-IgG_(PF4-H) (K170854). The values were initially set on HemosIL AcuStar HIT-IgG_(PF4-H) according to the following process: A population of HIT suspected samples was studied and a Receiver Operating Characteristics (ROC) analysis vs Serotonin Release Assay (SRA) showed a clear separation of negative and positive results with an optimal sensitivity and specificity using a cut-off of 1.00 U/mL. This same value was proposed for HemosIL CL HIT-IgG_(PF4-H) which was confirmed as optimal with a validation cut-off study on HemosIL CL HIT-IgG_(PF4-H) assay using a population of HIT suspected samples and assessing the overall agreement vs SRA.

Expected values

The Kit Calibrators (component of assay test kit) are prepared and assigned versus internal Primary Reference Material (RMI). The assigned concentration is obtained for each calibrator by analyzing the Kit Calibrators being manufactured referenced to their respective internal Primary Reference Material over multiple runs and instruments.

The assigned concentration must fall within the following acceptability ranges:

- Calibrator 1: 1.00 ± 0.24 U/mL
- Calibrator 2: 16.50 ± 3.30 U/mL

Reagent stability

Reagent real-time shelf-life stability and continuous on-board stability testing was conducted using three (3) lots of HemosIL CL HIT-IgG_(PF4-H) reagent cartridges with a panel of four citrated plasma samples (two sample pools and two unadulterated samples) and two control levels (low and high) at different time points, spanning the claimed stability range. Each material was analyzed in 13 replicates and compared to the results obtained from baseline day 0. The reagent stability study results support the following claim:

- On-Board Stability was established at 30 days for the HemosIL CL HIT-IgG_(PF4-H) at 2–8°C continuously on-board the ACL TOP 970 CL.
- Shelf-life was established at 12 months for the HemosIL CL HIT-IgG_(PF4-H) when stored at 2–8 °C.

Calibrator stability

The calibrator on-board was conducted with three (3) lots of HemosIL CL HIT-IgG_(PF4-H) calibrators (1 & 2) by performing one calibration at different time points (0, 2, 4 and 5 hours) after the placement of the assay calibrators on board the ACL TOP 970 CL. Each of the HemosIL CL Multi-Ab Controls (control low and control high) was analyzed with 11 replicates. The calibrator open-vial stability studies was conducted with three (3) lots of HemosIL CL HIT-IgG_(PF4-H) calibrators (1 & 2A) first placed on board the ACL TOP 970 CL for 1 hour and then re-capped and placed at +2-8°C. Calibrations were performed at time 0, 14 days, and 15 days (at +2-8°C) with 10 replicates each of Multi-Ab Control (Low and

High). The mean values obtained for each Multi-Ab Control at each time point were compared versus the corresponding mean values obtained with the calibration at time 0. The calibrator on-board and open-vial study results support that the calibrators are stable for 4 hours on-board the ACL TOP 970 CL in the open original vial or 14 days at 2–8 °C in the closed original vial.

Transport stability

A shipping (transport simulation) study was conducted using a total of 30 kits of HemosIL CL HIT-IgG_(PF4-H) which were packed in two boxes (15 kits per box) at two stressed transport conditions. The data collected from these two shipment conditions were compared to non-stressed kits (reference kits) which were continuously maintained at the recommended storage condition of 2–8°C. The results support warm shipment: Two days at room temperature (15–30°C), three days at 37°C in heater followed by 2–8°C storage until testing. HemosIL CL HIT-IgG_(PF4-H) does not tolerate freezing conditions. A temperature logging device is always included in the packaging to ensure that kits can be deemed valid on receipt.

Sample Stability

The sample stability study was performed to support the recommended storage and handling instructions found in the device labeling. Three (3) citrated plasma samples (negative sample, MDL sample and high positive sample) were tested at 2–8°C and 15–25°C. The study was performed using one reagent lot, with thirteen (13) replicate measurements at each time point for each sample on one ACL TOP 970 CL instrument. The study data demonstrate that citrated plasma samples can be stored at 2–8°C and 15–25°C for 8 hours before testing with the HemosIL CL HIT-IgG_(PF4-H) assay.

7. Detection Limit:

The Limit of the Blank (LoB) and Limit of Detection (LoD) studies were performed using three different lots of HemosIL CL HIT-IgG_(PF4-H) reagents on two ACL TOP 970 CL instruments, according to CLSI EP17-A2. The LoB and LoD were established at 0.09 U/mL and 0.14 U/mL, respectively.

8. Assay Cut-Off:

Refer to section D. Clinical Cut-Off.

B Comparison Studies:

1. Method Comparison with Predicate Device:

The method comparison study was conducted at three U.S. sites with 341 samples from intended use population with confirmed medical history of a suspicion of HIT and calculation of a 4T score. Each sample was run in singlicate using the candidate assay on the ACL TOP 970 CL instrument and the predicate assay on the ACL AcuStar instrument (K170854).

		HemosIL AcuStar HIT-IgG_(PF4-H) Results		
		Positive	Negative	Total
HemosIL CL HIT IgG_(PF4-H) Results	Positive	91	1	92
	Negative	3	246	249
	Total	94	247	341
		Estimate	Wilson 95% CI	
PPA (Positive Percent Agreement)		97% (91/94)	91%	99%
NPA (Negative Percent Agreement)		100% (246/247)	98%	100%
Total Percent Agreement		99% (337/341)	97%	100%

2. Matrix Comparison:

Fresh vs. Frozen Sample Study

Ninety-four 3.2% citrated plasma samples covering the range of 0.24–14020 U/mL were tested for fresh/frozen equivalence at two external US sites after storage at $\leq -20^{\circ}\text{C}$ for at least 24 hours. The study confirmed equivalence between matrices and up to two freeze/thaw cycles of the HemosIL CL HIT-IgG_(PF4-H) measurement procedure on the ACL TOP 970 CL.

C Clinical Studies:

1. Clinical Sensitivity:

NA

2. Clinical Specificity:

NA

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

NA

D Clinical Cut-Off:

The cut-off validation was conducted by qualitatively comparing the presence of anti-PF4/H antibodies as determined by HemosIL CL HIT IgG_(PF4-H) to the presence of functionally relevant antibodies as determined by PF4-enhanced platelet activation assay, Serotonin Release Assay (SRA). Testing was performed with two (2) lots of HemosIL CL HIT-IgG_(PF4-H) using 91 3.2% citrated plasma samples (45 SRA positive and 46 SRA negative) from patients suspected of HIT with 4T scores of 1 to 3 (low), 4 to 5 (intermediate) or 6 to 8 (high) who were sent for SRA testing at one external US site.

HemosIL CL HIT-IgG(PF4-H) vs. SRA	Estimate	95% CI
PPA	100.0% (45/45)	92.1%, 100.0%
NPA	97.8% (45/46)	88.7%, 99.6%
PPV	97.9% (45/46)	86.9%, 99.7%
NPV	100.0% (45/45)	92.1%, 100.0%
Overall Agreement	98.9% (90/91)	94.0%, 99.8%

E Expected Values/Reference Range:

Reference Interval – Healthy Donors Not Heparin Exposed: A total of 122 citrated plasma samples from ostensibly healthy donors with an age range of 20 to 71 years old were tested with two lots of HemosIL CL HIT-IgG(PF4-H) on one ACL TOP 970 CL instrument. The data were analyzed using the non-parametric quantile interval based on the order statistics. The reference interval was calculated, and the upper limit determined to be 0.45 U/mL (90% CI: 0.28 to 0.68 U/mL).

Reference Interval – Heparin Exposed, Not- HIT Suspected Patients (HIT Negative): A total number of 132 samples from patients exposed to heparin (UFH and LMWH) but not suspected of HIT with an age range of 21 to 88 years old were tested with two lots of HemosIL CL HIT-IgG(PF4-H) on one ACL TOP 970 CL instrument. The statistical method applied to analyze the data was the non-parametric quantile interval based on the order statistics. The reference interval was calculated, and the upper limit determined to be 1.42 U/mL (90% CI: 0.49 to 3.88 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.