510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K170854

B. Purpose for Submission:

Clearance of a new device

C. Measurand:

Anti-PF4/Heparin IgG Antibody

D. Type of Test:

Automated, chemiluminescent immunoassay (CIA)

E. Applicant:

Instrumentation Laboratory (IL) Co.

F. Proprietary and Established Names:

HemosIL AcuStar HIT-$IgG_{(PF4\text{-}H)}$ Assay

HemosIL AcuStar HIT Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.7695 Platelet factor 4 radioimmunoassay

   21 CFR 864.5425 Multipurpose system for in vitro coagulation studies

2. Classification:

   Class II

3. Product code:

   LCO, Platelet Factor 4 Radioimmunoassay

   GGN, Plasma, Coagulation Control

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   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 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.

   HemoslL AcuStar HIT Controls are for the quality control of the HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ assay as performed on the ACL AcuStar.

   For prescription use.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   ACL AcuStar

I. Device Description:

The HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ assay is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with PF4 complexed to polyvinyl sulfonate (PVS) which captures PF4/Heparin antibodies from the sample, if present.

The HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ kit consists of:

- HIT-IgG$_{(PF4-H)}$ Cartridge for 25 determinations: 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 Tris buffer containing bovine serum albumin, bovine fetal serum, PF4/PVS complex, mouse monoclonal IgG, stabilizers, and preservative.
- HIT-IgG$_{(PF4-H)}$ Calibrator: Barcoded tube of a solution with humanized mAb anti-PF4-Heparin in Tris buffer containing bovine serum albumin, stabilizers and preservative.
- HIT-IgG$_{(PF4-H)}$ Calibrator: Barcoded tube 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 cannot be used with other lots of reagents.

The HemosIL AcuStar HIT Controls consists of different concentrations of humanized monoclonal anti-PF4-Heparin. The Low HIT Control is intended for the assessment of precision and accuracy of the HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ assay below the cut-off. The High HIT Control is intended for the assessment of precision and accuracy of the HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ assay above the cut-off. Use of both controls is recommended for a complete quality control program.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Zymutest HIA IgG

2. Predicate 510(k) number(s):

   K071255

3. Comparison with predicate:

Similarities		
Item	Device HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ HemosIL AcuStar HIT Controls	Predicate Zymutest HIA IgG
Intended Use	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 ZYMUTEST HIA IgG ELISA is a qualitative assay itended for the detection of heparin-dependent antibodies of the IgG isotype, in human plasma, by clinical laboratories. It is intended for in vitro diagnostic use.

Similarities		
Item	Device HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ HemosIL AcuStar HIT Controls	Predicate Zymutest HIA IgG
	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. HemoslL AcuStar HIT Controls are for the quality control of the HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ assay as performed on the ACL AcuStar. For prescription use.	
Assay type	Qualitative	Same
Measurand	Anti-PF4/Heparin IgG Antibodies	Same

Differences		
Item	Device HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ HemosIL AcuStar HIT Controls	Predicate Zymutest HIA IgG
Sample type	Citrated (3.2% and 3.8%) human plasma and serum	Citrated (3.2% and 3.8%) human plasma
Reagents	Cartridge containing magnetic particle suspension coated with PF4 complexed to polyvinyl sulfonate (PVS)	Microtiter plate coated with unfractionated heparin
Antibodies/conjugate	Mouse monoclonal anti-human IgG antibody/ Isoluminol conjugated anti-human IgG	Goat antibody specific for human IgG/ Peroxidase conjugated anti-human IgG
Detection method	Chemiluminescence	Colorimetry (Absorbance)
Calibration	Lot specific Master Curve and two Calibrators	Not applicable
Cut-off	Fixed clinical cut-off: ≥ 1.00 U/mL	When the assay is run at 20±1°C, the results are as follows: Positive: $A_{450} > 0.50$ Weakly Positive: $0.30 \leq A_{450} \leq 0.50$ Negative: $A_{450} \leq 0.30$ (A_{450}=Absorbance at 450nm)

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

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurements Procedures; Approved Guideline; 2014

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline; 2005

CLSI EP12-A2, User Protocol For Evaluation ofQualitative Test Performance; Approved Guideline; 2008

CLSI EP14-A2, Evaluation of Matrix Effects; Approved Guideline; 2013

CLSI EP17-A2; Evaluation of Detection Capability For Clinical Laboratory Measurement Procedures; Approved Guideline; 2012

CLSI EP24-A2, Assessment of Diagnostic Accuracy ofLaboratory Tests Using Reveiver Operating Characteristic Curves; Approved Guideline; 2011

CLSI EP25-A3, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline; 2009

CLSI EP28-A3, Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline; 2010

L. Test Principle:

The HemosIL AcuStar HIT-$IgG_{(PF4\text{-}H)}$ assay is a chemiluminescent two-step immunoassay consisting of paramagnetic beads coated with PF4 complexed to polyvinyl sulfonate (PVS) which capture PF4/Heparin antibodies if present in the sample. Diluted patient plasma or serum, the magnetic beads suspension, and the assay buffer are combined into a second cuvette, and mixed. Following incubation, magnetic separation and a wash step, a tracer consisting of an isoluminol conjugated anti-human IgG antibodies is added to the cuvette and may bind the captured PF4/Heparin 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 (RLU) by the ACL AcuStar optical system. The RLUs are directly proportional to the PF4/Heparin IgG antibody concentration in the sample.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   <u>Precision</u>

Three lots of HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ reagent were tested on the ACL AcuStar analyzer. The study used five patient samples to span the assay range. Each sample was tested with each reagent lot in duplicate, twice a day for 22 days, for a total of 88 replicates per level per lot as summarized below.

HemosIL AcuStar HIT-IgG(PF4-H) Reagent Lot 1

Sample	Mean (U/mL)	Within-run %CV	Total (Within-device) %CV
Sample 1 (Pool)	0.62	8.3%	8.3%
Sample 2 (Pool)	2.23	6.5%	6.8%
Sample 3 (Unadulterated)	21.04	4.8%	5.6%
Sample 4 (Unadulterated)	46.15	4.7%	4.8%
Sample 5 (Pool)	90.35	4.4%	4.4%

HemosIL AcuStar HIT-IgG(PF4-H) Reagent Lot 2

Sample	Mean (U/mL)	Within-run %CV	Total (Within-device) %CV
Sample 1 (Pool)	0.52	8.4%	9.2%
Sample 2 (Pool)	1.98	7.5%	9.3%
Sample 3 (Unadulterated)	26.13	6.1%	6.1%
Sample 4 (Unadulterated)	54.88	6.9%	7.5%
Sample 5 (Pool)	111.74	4.1%	7.3%

HemosIL AcuStar HIT-IgG(PF4-H) Reagent Lot 3

Sample	Mean (U/mL)	Within-run %CV	Total (Within-device) %CV
Sample 1 (Pool)	0.49	13.0%	13.8%
Sample 2 (Pool)	1.69	7.1%	7.4%
Sample 3 (Unadulterated)	18.40	7.4%	8.1%
Sample 4 (Unadulterated)	36.85	4.8%	5.6%
Sample 5 (Pool)	86.79	6.8%	7.4%

Combined HemosIL AcuStar HIT-IgG(PF4-H) Reagent Lots

Sample	Mean (U/mL)	Lot-to-lot Variability %CV
Sample 1 (Pool)	0.54	11.8%
Sample 2 (Pool)	1.97	13.8%
Sample 3 (Unadulterated)	21.85	18.0%
Sample 4 (Unadulterated)	45.96	19.6%
Sample 5 (Pool)	96.30	14.0%

An additional internal precision study was performed with three different lots of HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ reagent cartridges, using three plasma samples at levels near the claimed assay cut-off (sample 1: negative sample pool, sample 2: positive sample pool, sample 3: unadulterated positive sample). Each sample was analyzed with each of the three reagent lots on two different ACL AcuStar instruments by two different operators over five days, two runs per day, three replicates per run, totaling an N=360 measurements per sample level. Obtained

precision data was within pre-established acceptance criteria. The data is summarized in the table below:

Sample	N	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Between-Lot		Between-Instrument		Between-Operator		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	360	0.79	0.04	4.7%	0.02	2.1%	0.02	2.0%	0.07	8.3%	0.00	0.0%	0.00	0.0%	0.08	10.0%
2	360	1.43	0.06	4.3%	0.04	3.0%	0.00	0.0%	0.07	4.8%	0.00	0.0%	0.00	0.0%	0.10	7.1%
3	360	3.61	0.13	3.6%	0.14	3.7%	0.05	1.5%	0.12	3.4%	0.00	0.0%	0.00	0.0%	0.23	6.4%

Reproducibility

Reproducibility studies were conducted at three external clinical sites by three operators (one operator per site), on three ACL AcuStar instruments (one instrument per site), using three lots of HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ reagent cartridges and three lots of HemosIL AcuStar HIT Controls (low and high). The same three patient citrated plasma sample pools were tested across all sites. Each sample was tested in triplicate, twice a day for five days, for a total of 30 replicates per level. The statistical analyses were summarized using a two-way nested ANOVA for individual site results and a three-way nested ANOVA for 3-site pooled results. The pooled data for each reagent lot and control lot is presented below:

HemosIL AcuStar HIT-IgG(PF4-H) Reagent Lot 1

Sample	N	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low HIT Control	90	0.64	0.02	2.5	0.03	4.7	0.01	1.7	0.06	9.2	0.07	10.7
High HIT Control	90	3.05	0.10	3.3	0.10	3.2	0.05	3.2	0.27	8.8	0.32	10.5
Plasma Sample 1	90	0.06	All Replicates < 1.00 U/mL									
Plasma Sample 2	90	3.52	0.17	4.8	0.09	2.7	0.07	1.8	0.16	4.6	0.26	7.4
Plasma Sample 3	90	21.33	1.53	7.2	0.00	0.0	0.37	1.7	0.77	3.6	1.75	8.2

HemosIL AcuStar HIT-IgG(PF4-H) Reagent Lot 2

Sample	N	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low HIT Control	90	0.56	0.02	3.6	0.02	2.9	0.02	3.9	0.03	5.7	0.05	8.2
High HIT Control	90	2.66	0.11	4.3	0.09	3.2	0.06	2.2	0.14	5.3	0.21	7.8
Plasma Sample 1	90	0.04	All Replicates < 1.00 U/mL									
Plasma Sample 2	90	3.23	0.15	4.6	0.14	4.4	0.10	3.1	0.24	7.3	0.33	10.2
Plasma Sample 3	90	20.79	1.41	6.8	0.42	2.0	0.50	2.4	0.88	4.2	1.79	8.6

HemosIL AcuStar HIT-IgG(PF4-H) Reagent Lot 3

Sample	N	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low HIT Control	90	0.47	0.02	3.6	0.02	4.6	0.02	3.2	0.01	2.7	0.03	7.2
High HIT Control	90	2.62	0.10	3.9	0.11	4.4	0.12	4.5	0.06	2.3	0.20	7.7
Plasma Sample 1	90	0.06	All Replicates < 1.00 U/mL									

Sample	N	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Sample 2	90	4.47	0.19	4.3	0.28	6.3	0.21	4.7	0.08	1.8	0.41	9.2
Plasma Sample 3	90	29.64	1.84	6.2	1.54	5.2	1.80	6.1	0.91	3.1	3.14	10.6

b. *Linearity/assay reportable range:*

HemosIL AcuStar HIT-IgG(PF4-H) assay is a qualitative assay therefore, linearity is not applicable.

c. *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 AcuStar HIT-$IgG_{(PF4-H)}$ assay standardization:

- House Standard Calibrators: Used internally to prepare the kit calibrators
- Kit Calibrators: Used by the ACL AcuStar instrument to prepare the Working Calibration Curve

HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ is standardized to House Standards (humanized monoclonal anti PF4-Heparin antibody) diluted in Tris buffer containing bovine serum albumin (BSA), stabilizers and preservative. The values of the House Standards were set after studying a population of HIT suspected samples. Receiver Operating Characteristics (ROC) analysis vs Serotonin Release Assay (SRA) demonstrated a clear separation of negative and positive results with an optimal sensitivity and specificity using a cut-off of 1.00 U/mL. The Primary Standard was set at 1.00 U/mL.

Stability

<u>HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ reagent</u>

Reagent real-time shelf-life stability and continuous on-board stability testing was performed using three lots of HemosIL AcuStar HIT-$Ab_{(PF4-H)}$ kit reagents and analyzed on the ACL AcuStar instrument. Shelf-life testing included four plasma samples and one lot of control material (low and high) analyzed with three reagent lots. The lots were manufactured at different time points, spanning the claimed stability range. Each material was analyzed in triplicate and compared to the results obtained with the newly manufactured lot (Reference Lot) as time zero.

The continuous on-board stability was established at 12 weeks, after opening and placed on board the ACL AcuStar.

The shelf-life stability was established at 12 months when stored at 2–8°C.

The HemosIL AcuStar HIT-IgG(PF4-H) kit is sensitive to temperature change. Therefore, the shipping procedure requires refrigeration at 2–8°C. Transport will include a delta-track temperature monitor in each shipment to record temperature values during transport.

HemosIL AcuStar HIT Controls

Three lots of HemosIL AcuStar HIT Controls were tested. The continuous on-board stability was established at 12 hours on-board the ACL AcuStar.

The cumulative on-board stability was established at 6 days when placed twice daily on-board (in an open barcoded tube) for 45 minutes and then recapped and placed at 2–8°C on the ACL AcuStar.

The shelf-life stability was established at 12 months when stored at 2-8°C.

The HemosIL AcuStar HIT Controls are stable at high temperatures and are unaffected by one freeze/thaw cycle. Controls can be frozen at -20°C and thawed once. Do not refreeze.

HIT-IgG(PF4-H) Calibrators

The continuous on-board stability was established at 4 hours, after opening and placed on-board the ACL AcuStar.

The shelf-life stability was established at 12 months when stored at 2–8°C.

Expected values (controls, calibrators)

HemosIL AcuStar HIT Controls

The values for HemosIL AcuStar HIT Controls are assigned with respect to the House Standard Controls according to internal protocol and are lot-specific.

An internal precision study was performed using three lots of HemosIL AcuStar HIT Controls (low and high) run on ACL AcuStar instrument with a single lot of HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ reagent. The control material was tested for 20 days x 2 runs x 2 replicates per run, for a total N=80 measurements per level, for each lot. The calculated precision data was within pre-established acceptance criteria. The data is summarized in the table below:

HemosIL AcuStar HIT Controls		Mean (U/mL)	Within-run %CV	Within-device %CV
Lot 1	Low HIT Control	0.58	4.6%	7.0%
	High HIT Control	3.16	3.5%	6.1%
Lot 2	Low HIT Control	0.49	3.2%	7.0%
	High HIT Control	2.77	3.4%	5.0%
Lot 3	Low HIT Control	0.60	4.1%	6.3%
	High HIT Control	2.96	2.8%	5.5%

HIT-IgG$_{(PF4-H)}$ Calibrators

The HemosIL AcuStar HIT Calibrators are included as a component of the reagent kit. The calibrator value assignment is performed with respect to the House Standard Calibrator, according to an internal protocol and are lot-specific.

A precision study was conducted using Calibrators 1 and 2 from three lots of HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ kits. The samples were tested with a single lot of the reagent following a 20 day x 2 runs per day x 2 replicates per run study design. The calculated precision data were within the pre-established acceptance criteria. The data is summarized in the table below:

HIT-IgG$_{(PF4-H)}$ Calibrators		Mean (U/mL)	Within-run %CV	Within-device %CV
Reagent Lot 1	Calibrator 1	0.92	3.9%	6.3%
	Calibrator 2	15.37	3.2%	3.9%
Reagent Lot 2	Calibrator 1	0.94	2.7%	5.4%
	Calibrator 2	14.85	2.2%	3.1%
Reagent Lot 3	Calibrator 1	0.90	2.4%	4.0%
	Calibrator 2	14.90	3.3%	5.0%

d. Detection limit:

The Limit of the Blank (LoB) and Limit of Detection (LoD) study was performed using two different lots of HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ reagents tested on an ACL AcuStar, according to CLSI EP17-A2. The LoB and LoD were established at 0.06 U/mL and 0.39 U/mL, respectively.

e. Analytical specificity:

Analytical specificity for detecting anti-HIT antibodies was tested in an inhibition assay with heparin, using 59 citrated plasma samples. Inhibition of a positive reaction by $\geq$ 50% was considered confirmatory for the assay specificity to heparin-dependent antibody characteristics of HIT. Four samples out of 59 were found not inhibited by the HIT antibody.

A dose-response interference study was performed for hemoglobin, unconjugated bilirubin, conjugated bilirubin, triglycerides, low-molecular weight (LMW) heparin, unfractionated (UF) heparin, rheumatoid factor, and human anti-mouse antibody (HAMA). Three samples were prepared from healthy donors and HIT-positive

patients to achieve a negative (0.6 U/mL), weakly positive (1.0 U/mL) and positive (7.0 U/mL) levels of the PF4-H antibody. The results of the study determined that the HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ on the AcuStar analyzer is not affected by the interferents up to the levels indicated in table below:

Interferent	Highest Concentration with no Significant Interference
Hemoglobin	500 mg/dL
Bilirubin (conjugated and unconjugated	18 mg/dL
Triglycerides	1250 mg/dL
LMW and UF Heparin	1 IU/mL
HAMA	1 µg/mL

Rheumatoid Factor was found to interfere with the assay. A limitation stating that Rheumathoid Factor may produce an over estimation of the test result with the HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ assay is included in the labeling.

Twenty-six samples from patients diagnosed with Antiphospholipid Syndrome (APS) were tested with a single lot of HemosIL AcuStar HIT-IgG(PF4-H) reagent. All samples reported negative, demonstrating that the assay is not affected by APS antibodies.

f. Assay cut-off:

A method comparison with the Serotonin Release Assay (SRA) was performed using 87 citrated plasma samples from HIT suspected patients (45 SRA positive and 42 SRA negative). The optimal cut-off determined by ROC analysis was 1.0 U/mL (95.2% Agreement, CI = 86.7% – 99.0%). Based on these studies, it was determined that for heparin treated patient samples, HemosIL AcuStar HIT-$IgG_{(PF4-H)}$ results ≥ 1.0 U/mL may indicate the presence of HIT antibodies.

2. Comparison studies:

a. Method comparison with predicate device:

A multi-site clinical study included a total of 802 patients exposed to heparin and suspected of HIT. The subjects enrolled in the study ranged from 18–97 years old. All subjects were categorized according to the 4Ts pretest probability scoring system for HIT. Studies were performed in three clinical test sites located in the United States. Testing included comparison of AcuStar HIT-$IgG_{(PF4-H)}$ to the Zymutest HIA IgG assay (K071255).

The combined sites data, summarized below, are based on a cut-off of 1.0 U/mL for the HemosIL HIT-Ab(PF4-H) assay and 0.5 OD for the Zymutest HIA IgG.

		Zymutest HIA IgG		
		Positive	Negative	Total
HemosIL AcuStar HIT-IgG$_{(PF4-H)}$	Positive	26	9	35
	Negative	48	719	767
	Total	74	728	802

HemosIL AcuStar HIT-IgG$_{(PF4-H)}$ vs. Zymutest HIA IgG	**Proportion**	**Wilson 95% CI**	
PPA (Positive Percent Agreement)	35% (26/74)	25%	47%
NPA (Negative Percent Agreement)	99% (719/728)	98%	99%
Total Percent Agreement	93% (745/802)	91%	94%

b. Matrix comparison:

3.2% vs. 3.8% Sodium Citrated Plasma

Twelve fresh, unadulterated, paired citrated 3.2% and 3.8% plasma samples were tested for equivalence. In addition, 29 frozen paired 3.2% and 3.8% citrated plasma samples were spiked with HIT-positive samples to achieve a concentration range covering 0.15–66.82 U/mL. The study confirmed equivalence between 3.2% citrated and 3.8% citrated plasma for fresh and frozen samples.

Sodium Citrated Plasma vs. Serum

Thirteen fresh unadulterated paired 3.2% citrated plasma and serum samples were tested for equivalence. Additionally, 29 paired samples were collected and shipped frozen to another location for testing. After thawing, the samples were spiked, with HIT positive samples to achieve concentration range 0.13–75.92 U/mL. The study confirmed matrix equivalence between 3.2% citrated plasma and serum for fresh and frozen samples.

Fresh vs. Frozen Sample Study:

Fifty-seven unadulterated samples covering the range of 0.0–32.16 U/mL were tested for fresh/frozen equivalence after storage at ≤ -20^{o}C for at least 24 hours. The study confirmed equivalence between matrices up to two freeze/thaw cycles.

3. Clinical studies:

a. Clinical Sensitivity:

The clinical study was expanded to test samples using the Serotonin Release Assay

(SRA) as the reference method. Frozen aliquots of samples tested in the multi-site predicate comparison study were shipped to and tested at one site. A total of 790 patients were tested. The results summarized below are based on a cut-off of 1.0 U/mL for the HemosIL AcuStar HIT- $IgG_{(PF4-H)}$ assay and the 20% serotonin-release cut-off value for the SRA method.

		Serotonin Release Assay		
		Postive	Negative	Total
HemosIL AcuStar HIT- $IgG_{(PF4-H)}$	Positive	26	8	34
	Negative	15	741	756
	Total	41	749	790

HemosIL AcuStar HIT- $IgG_{(PF4-H)}$ vs. Serotonin Release Assay	**Proportion**	**Wilson 95% CI**	
PPA (Positive Percent Agreement)	63% (26/41)	48%	76%
NPA (Negative Percent Agreement)	99% (741/749)	98%	99%

HemosIL AcuStar HIT- $IgG_{(PF4-H)}$ vs. Serotonin Release Assay	**Proportion**	**Wilson 95% CI**	
PPV (Positive Predictive Value)	76% (26/34)	60%	88%
NPV (Negative Predictive Value)	98% (741/756)	97%	99%
Total Percent Agreement	97% (767/790)	96%	98%

Zymutest HIA IgG vs. Serotonin Release Assay

The tables below show combined data from 3-sites from comparison between Zymutest HIA IgG (the predicate) and the reference method (SRA).

		Serotonin Release Assay		
		Positive	Negative	Total
Zymutest HIA IgG	Positive	26	47	73
	Negative	15	702	717
	Total	41	749	790

Zymutest HIA IgG vs. Serotonin Release Assay	**Proportion**	**Wilson 95% CI**	
PPA (Positive Percent Agreement)	63% (26/41)	48%	76%
NPA (Negative Percent Agreement)	94% (702/749)	92%	95%

Zymutest HIA IgG vs. Serotonin Release Assay	Proportion	Wilson 95% CI	
PPV (Positive Predictive Value)	36% (26/73)	26%	47%
NPV (Negative Predictive Value)	98% (702/717)	97%	99%
Total Percent Agreement	92% (728/790)	90%	94%

b. Clinical specificity:

Refer to Section M.3.a above.

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

Refer to Section M.3.a above.

5. Expected values/Reference range:

Reference Interval – Healthy Donors Not Heparin Exposed

A total of N=154 citrated plasma samples from apparently healthy adult donors who were not exposed to heparin were tested. The gender and age distribution was 88 females and 66 males, between 18 and 80 years of age. 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 0.37 U/mL (90% CI: 0.17 to 1.25 U/mL).

Reference Interval – Heparin Exposed, Not- HIT Suspected Patients (HIT Negative):

A total number of 91 samples from patients exposed to heparin but not suspected of HIT were tested. The gender and age distribution included 39 females and 52 males, between18 and 100 years of age. 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 0.75 U/mL (90% CI: 0.46 to 2.86 U/mL).

N. Instrument Name:

ACL AcuStar

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes ____X____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No ___X___

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   Barcode, manual entry

4. Specimen Sampling and Handling:

   Automated. The ACL AcuStar instrument's fluidic system performs the sampling, adding of diluents, reagent mixing, aspirating waste from cuvettes, cleaning of the magnetic particles and dispensing and aspirating of trigger agents. The sample carousel can hold 6 sample racks (numbered 1 to 6), each capable of holding 5 samples for a total capacity of 30 samples. The sample racks are moved by the carousel to dedicated locations where an aliquot of the sample can be aspirated and transferred for testing.

5. Calibration:

   The HemosIL AcuStar HIT-$IgG_{(PF4\text{-}H)}$ 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. The master curve is uploaded onto the ACL AcuStar instrument through the Reagent Cartridge barcode. The master curve is generated by analyzing, on different ACL AcuStar instruments (at least 2), on different days, a set of internal materials at concentrations that cover the range of 0.00 U/mL up to 128.00 U/mL. A minimum of 72 determinations per level are obtained. Every new Reagent Cartridge lot must be calibrated before first use with the HIT-IgG(PF4-H) Calibrators. The calibrators are ready to use in a barcoded tube and when loaded, the ACL AcuStar instrument automatically performs three replicates of each calibrator. Based on the results obtained with the both Calibrators, an instrument specific 4PLC Working Curve is created by the ACL AcuStar software, which is used to calculate the reported values (U/mL) from the instrument signal (RLU) obtained for each sample.

6. Quality Control:

   HemosIL AcuStar HIT Controls Low and High are recommended for a complete quality control program.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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