

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

A. 510(k) Number:

K153137

B. Purpose for Submission:

Clearance of a new device

C. Measurand:

Anti-PF4/Heparin Total Antibodies

D. Type of Test:

Automated, latex enhanced immuno-turbidimetric assay

E. Applicant:

Instrumentation Laboratory (IL) Co.

F. Proprietary and Established Names:

HemosIL HIT-Ab(PF4-H)

HemosIL HIT-Ab(PF4-H) 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 HIT-Ab_(PF4-H) is a qualitative, fully automated, latex enhanced immunoassay for the detection of anti-platelet factor 4/heparin (PF4/H) antibodies. The assay is for use in human 3.2% or 3.8% citrated plasma on the ACL TOP® Family of instruments in a laboratory setting.

The result provided by the assay should be interpreted as either positive or negative based on the assay cut-off (1.0 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.

HemosIL HIT-Ab_(PF4-H) Controls are for the Quality Control of the HemosIL HIT-Ab_(PF4-H) assay as performed on the ACL TOP® Family of instruments.

For prescription use.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use

4. Special instrument requirements:

ACL TOP® Family Instruments

I. Device Description:

The HemosIL HIT-Ab_(PF4-H) kit is a latex particle enhanced immuno-turbidimetric assay to detect total anti-PF4/Heparin antibodies found in HIT patients. A monoclonal

antibody that mimics human HIT antibodies is coated onto latex particles.

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

Latex Reagent: Suspension of polystyrene latex particles coated with purified mouse monoclonal anti-PF4-Heparin in Tris buffer, containing bovine serum albumin, stabilizers and preservative.

Stabilizer: PBS buffer containing bovine serum albumin, stabilizers and preservative.

Complex: Solution of PF4-PVS complex (PF4 from human platelets complexed to PVS), in PBS buffer containing bovine serum albumin, stabilizers and preservative. Contains 0.02% Bronidox™ as a preservative.

Calibrator: Lyophilized solution of a monoclonal anti- PF4-Heparin antibody in Tris buffer containing bovine serum albumin, stabilizers and preservative.

Controls: The Low and High HIT-Ab_(PF4-H) Controls are prepared by means of a dedicated process and contain different concentrations of humanized monoclonal anti-PF4-Heparin human IgG.

- Low HIT Control: Control intended for the assessment of precision and accuracy of the assay at PF4/H antibody levels at or below the cut-off.
- High HIT Control: Control intended for the assessment of precision and accuracy of the assay at abnormal PF4/H antibody levels.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Asserachrom HPIA Test kit from Diagnostica Stago

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

K003767

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Trade Names	HemosIL HIT-Ab _(PF4-H) HemosIL HIT-Ab _(PF4-H) Controls (K153137)	Asserachrom HPIA Test Kit (kit includes two control levels) (K003767)
Measurand	Anti-PF4/Heparin Total Antibodies	Anti-PF4/Heparin Total Antibodies
Detection Method	Absorbance (Turbimetric)	Absorbance (Colorimetric)
Intended Use	<p>HemosIL HIT-Ab_(PF4-H) is a qualitative, fully automated, latex enhanced immunoassay for the detection of anti-platelet factor 4/heparin (PF4/H) antibodies. The assay is for use in human 3.2% or 3.8% citrated plasma on the ACL TOP® Family of instruments in a laboratory setting.</p> <p>The result provided by the assay should be interpreted as either positive or negative based on the assay cut-off (1.0 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.</p> <p>HemosIL HIT-Ab_(PF4-H) Controls are for the Quality Control of the HemosIL HIT-Ab_(PF4-H) assay as performed on the ACL TOP Family of instruments. For prescription use.</p>	<p>The ASSERACHROM® HPIA Test Kit is intended for use as a qualitative procedure for the detection of anti-heparin-platelet factor 4 (anti-Heparin-PF4) antibodies in citrated plasma or serum by the sandwich technique of enzyme-linked immunosorbent assay (ELISA).</p> <p>The presence in plasma or serum of anti-Heparin-PF4 antibodies, together with a concurrent drop in platelet count, is generally associated with Type II heparin-induced thrombocytopenia (Type II HIT), a condition that occurs during heparin therapy, leading to arterial or venous thrombosis.</p>
Assay Type	Qualitative	Qualitative

Differences		
Item	Device	Predicate
Sample Types	Citrated human plasma only	Citrated human plasma or serum
Cut-off	Fixed clinical cut-off: ≥ 1.0 U/mL	Variable clinical cut-off Cut-off is lot and plate dependent. Every time a plate is processed, the cut-off for this plate is calculated as the percentage (X%) of the value

Differences		
Item	Device	Predicate
		obtained for the reagent supplied with the kit. This percentage is provided for each lot through the insert sheets.
Methodology	Latex-enhanced immuno-turbidimetric assay	Two-step enzyme immunoassay (EIA) sandwich method with a final colorimetric detection.
Antibodies	Purified mouse monoclonal anti-PF4-Heparin	Goat anti-human antibodies to IgG, IgA and IgM
Controls	Controls sold separately: - Low Level at or below the cut-off - High Level at abnormal anti-PF4/H antibody level.	Controls included in test kit: - Negative level - Positive level
Calibrator Traceability	The reported values for the kit calibrator are determined over multiple runs on the ACL TOP Family of instruments using specific lots of reagents and against an internal House Standard. Since an HIT International Standard is not currently available, arbitrary units (U/mL) have been established.	Not Applicable

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

EP05-A3; Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline; 2014

EP06-A; Evaluation of the Linearity of Quantitative Measurement Procedures; a Statistical Approach; Approved Guideline; 2003

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

EP09-A3; Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline; 2013

EP12-A2; User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline; 2008

EP14-A3; Evaluation of Commutability of Processed Samples; Approved Guideline; 2013

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

EP24-A2; Assessment of Diagnostic Accuracy of Laboratory Tests Using receiver Operating

Characteristic Curves; Approved Guideline; 2011

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

EP28-A3C; Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline; 2010

L. Test Principle:

The HemosIL HIT-Ab_(PF4-H) kit is a latex particle enhanced immuno-turbidimetric assay to detect total Anti-PF4/Heparin (PF4/H) antibodies found in HIT patients. A monoclonal antibody that mimics human HIT antibodies is coated onto latex particles. In the presence of PF4 from human platelets complexed to polyvinyl sulfonate (PVS), and the patient sample, a competitive agglutination reaction occurs. The degree of agglutination is inversely proportional to the concentration of antibodies in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.

The Low and High HIT-Ab_(PF4-H) Controls contain different concentrations of humanized monoclonal anti-PF4-Heparin-human IgG and are intended for the assessment of precision and accuracy of the assay at PF4/H antibody levels at or below the cut-off (Low control) and at abnormal PF4/H antibody levels (High control).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision

Three lots of HemosIL HIT-Ab_(PF4-H) reagents were tested on ACL TOP 700 analyzer. The study used a single lot of HemosIL HIT-Ab_(PF4-H) Controls (Low and High), as well as five patient pools prepared at different levels to span the assay range. Each material was tested with each reagent lot in duplicate, twice a day for 20 days, for a total of 80 replicates per level per lot as summarized below.

Reagent Lot 1			
Material	Mean (U/mL)	Within-Run (Repeatability) % CV	Total (Within-Device) % CV
Low HIT-Ab _(PF4-H) Control	0.8	9.0	9.9
High HIT-Ab _(PF4-H) Control	2.5	2.6	3.3
Plasma Sample 1	0.8	10.6	13.5
Plasma Sample 2	1.5	3.8	4.3
Plasma Sample 3	3.3	3.0	4.5
Plasma Sample 4	9.2	3.0	3.8
Plasma Sample 5	15.3	3.4	4.3

Reagent Lot 2			
Material	Mean (U/mL)	Within-Run (Repeatability) % CV	Total (Within-Device) % CV
Low HIT-Ab _(PF4-H) Control	0.7	7.9	9.3
High HIT-Ab _(PF4-H) Control	2.5	3.1	3.9
Plasma Sample 1	0.8	9.0	11.1
Plasma Sample 2	1.5	3.9	4.4
Plasma Sample 3	3.4	7.2	7.2
Plasma Sample 4	8.8	2.9	3.6
Plasma Sample 5	14.7	4.8	5.3
Reagent Lot 3			
Material	Mean (U/mL)	Within-Run (Repeatability) % CV	Total (Within-Device) % CV
Low HIT-Ab _(PF4-H) Control	0.7	8.4	9.9
High HIT-Ab _(PF4-H) Control	2.3	3.5	4.0
Plasma Sample 1	0.7	10.1	10.2
Plasma Sample 2	1.4	3.8	4.7
Plasma Sample 3	3.0	4.4	5.9
Plasma Sample 4	8.3	2.3	3.8
Plasma Sample 5	13.3	3.2	4.3

Aggregated data (Reagent Lots 1, 2 and 3)		
Material	Mean (U/mL)	Lot-to-Lot Variability %CV
Low HIT-Ab _(PF4-H) Control	0.7	4.1
High HIT-Ab _(PF4-H) Control	2.4	3.7
Plasma Sample 1	0.8	5.2
Plasma Sample 2	1.5	6.3
Plasma Sample 3	3.3	6.1
Plasma Sample 4	8.8	5.3
Plasma Sample 5	14.4	7.2

Reproducibility

Reproducibility studies were conducted at three external clinical sites using different operators (one operator per site), on three different ACL TOP 500 CTS instruments (one instrument per site), with three different lots of HemosIL HIT-Ab_(PF4-H) reagents and HemosIL HIT-Ab_(PF4-H) Controls (low and high). To span the assay range, three patient pools (2 positive and 1 negative) and a manufactured material containing a citrated plasma sample spiked with monoclonal anti-PF4-Heparin antibody were also tested (Plasma Sample 4). Each material was tested in triplicate, twice a day for 5 days, for a total of 30 replicates per level. The pooled data for each reagent lot is presented below.

Pooled 3-Site Data: Reagent Lot 1 of HemosIL HIT-Ab _(PF4-H)											
Level	Mean (U/mL)	Repeatability (within-run)		Between-Run		Between-Day		Between-Site		Reproducibility (Total)	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Low HIT-Ab _(PF4-H) Control	0.9	0.11	11.7	0.07	7.1	0.00	0.0	0.06	6.7	0.14	15.3
High HIT-Ab _(PF4-H) Control	2.6	0.13	5.1	0.06	2.1	0.07	2.7	0.00	0.0	0.16	6.1
Plasma Sample 2	2.1	0.11	5.2	0.08	3.9	0.00	0.0	0.05	2.2	0.14	6.8
Plasma Sample 3	4.0	0.21	5.2	0.05	1.2	0.12	2.9	0.00	0.0	0.25	6.1
Plasma Sample 4	13.4	0.88	6.5	0.00	0.0	0.33	2.5	0.73	5.5	1.19	8.9
Level	Mean (U/mL)	Result									
Plasma Sample 1	0.4	All Replicates < 1.0 U/mL									

Pooled 3-site Data: Reagent Lot 2 of HemosIL HIT-Ab _(PF4-H)											
Level	Mean (U/mL)	Repeatability (within-run)		Between-Run		Between-Day		Between-Site		Reproducibility (Total)	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Low HIT-Ab _(PF4-H) Control	0.9	0.08	9.2%	0.02	2.8%	0.00	0.0%	0.05	6.1%	0.06	7.4%
High HIT-Ab _(PF4-H) Control	2.6	0.09	3.4%	0.05	1.9%	0.02	0.8%	0.13	4.8%	0.13	5.2%
Plasma Sample 2	1.9	0.08	4.2%	0.03	1.4%	0.00	0.0%	0.04	1.8%	0.04	2.3%
Plasma Sample 3	3.9	0.17	4.3%	0.08	2.1%	0.00	0.0%	0.31	7.9%	0.32	8.1%
Plasma Sample 4	12.5	0.56	4.5%	0.33	2.6%	0.01	0.1%	0.70	5.6%	0.77	6.2%
Level	Mean (U/mL)	Result									
Plasma Sample 1	0.3	All Replicates < 1.0 U/mL									

Pooled 3-site Data: Reagent Lot 3 of HemosIL HIT-Ab _(PF4-H)											
Level	Mean (U/mL)	Repeatability (within-run)		Between-Run		Between-Day		Between-Site		Reproducibility (Total)	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Low HIT-Ab _(PF4-H) Control	0.8	0.07	8.8%	0.06	6.5%	0.00	0.0%	0.00	0.0%	0.09	11.0%
High HIT-Ab _(PF4-H) Control	2.7	0.11	4.0%	0.06	2.1%	0.00	0.0%	0.08	3.1%	0.15	5.5%
Plasma Sample 2	1.7	0.09	5.3%	0.02	1.1%	0.07	3.7%	0.04	2.2%	0.12	6.9%
Plasma Sample 3	3.5	0.17	4.8%	0.09	0.2%	0.03	0.8%	0.17	4.8%	0.24	6.8%
Plasma Sample 4	11.8	0.61	5.1%	0.11	0.9%	0.00	0.0%	0.58	4.9%	0.85	7.2%
Level	Mean (U/mL)	Result									
Plasma Sample 1	0.2	All Replicates < 1.0 U/mL									

b. Linearity/assay reportable range:

A linearity study was conducted as described in CLSI EP6-A “Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach”. The

AMR was established at standard range 0.6–5.7 U/mL and at auto re-run range up to 16 U/mL. Two lots of the assay reagent were tested on ACL TOP 700, ACL TOP 500 CTS and ACLTOP 300 CTS instruments. Human plasma samples were used to assess linearity across the claimed range.

Note: The HemosIL HIT-Ab_(PF4-H) assay is claimed as a qualitative assay with the cut-off value at 1.0 U/mL. However, the assay output is quantitative in terms that the numeric data is calculated based on the calibration curve. The medical technologist will determine whether the quantitative data is below or above the cut-off value. Only qualitative results (normal/abnormal) will be reported to physicians.

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

Traceability

Since an HIT International Standard is not currently available, an arbitrary unit (U/mL) has been established.

HemosIL HIT-Ab_(PF4-H) is standardized to a House Standard Calibrator diluted in Tris buffer containing bovine serum albumin (BSA), stabilizers and preservative. The value of the House Standard Calibrator was set after studying a population of HIT suspected samples. Receiver Operating Characteristics (ROC) analysis vs. Serotonin Release Assay (SRA) showed a clear separation of negative and positive results with optimal sensitivity and specificity using a cut-off of 1 U/mL. The Primary Standard was set at 5.7 U/mL (5.7 times the cut-off). The Kit Calibrator (component of assay test kit) is prepared and assigned versus the House Standard Calibrator. An assigned concentration is obtained by analyzing the Kit Calibrator being manufactured referenced to House Standard Calibrator over multiple runs, days and instruments. The assigned concentration must fall within an acceptability range (5.7 ± 0.5 U/mL).

Stability

Reagent open vial stability, continuous on board stability and cumulative stability testing was performed using three lots of HemosIL HIT-Ab_(PF4-H) kit reagents and analyzed on a representative member of the ACL TOP 700 instrument.

Open-vial stability: 2 months at 2–8°C in the original vial.

Continuous on-board stability: up to 24 hours.

Cumulative on-board stability: 60 days when reagents are used for two 1-hour test sessions on-board the ACL TOP Family and all vials are returned well-capped to 2–8°C between sessions.

The kit reagents do not survive a freeze/thaw cycle. Therefore the reagents are shipped refrigerated at 2–8°C with a delta-track temperature monitor in each pack.

Control stability was tested using three lots of the HemosIL HIT-Ab_(PF4-H) Controls (low and high).

Unopened controls are stable until the expiration date shown on the vial when stored at 2–8°C. Do not freeze.

Stability after opening: 2 months at 2–8°C, 24 hours in the original vial when placed continuously on-board the ACL TOP Family analyzers.

Cumulative on-board stability: When controls are used for 1 hour on-board per test session and the vials are returned well-capped to 2–8°C between sessions; the controls are stable for an on-board cumulative time of up to 8 hours. This cumulative time on-board study was distributed over a period of 12 days.

For optimal stability, controls should be removed from the system and stored at 2–8°C in the original vial.

HemosIL HIT-Ab_(PF4-H) Controls Low and High

Value assignment: The values for HemosIL HIT-Ab_(PF4-H) Controls are assigned with respect to the House Standard Controls according to the internal protocol. The control value is lot-specific.

Precision: Precision was assessed using three lots of HemosIL HIT-Ab_(PF4-H) Controls (low and high) run on ACL TOP 700 analyzer, with a single lot of HemosIL HIT-Ab_(PF4-H) reagent. Each level of control material from each lot was tested in duplicate, twice a day for 20 days, for a total of 80 replicates per level per lot as summarized below.

Material	Mean (U/mL)	Within-Run (Repeatability) % CV	Total (Within-Device) % CV
Low HIT-Ab _(PF4-H) Control Lot No. 1	0.8	9.0	9.9
High HIT-Ab _(PF4-H) Control Lot No. 1	2.5	2.6	3.3
Low HIT-Ab _(PF4-H) Control Lot No. 2	0.8	6.6	9.2
High HIT-Ab _(PF4-H) Control Lot No. 2	2.7	2.1	3.0
Low HIT-Ab _(PF4-H) Control Lot No. 3	0.9	6.9	8.9
High HIT-Ab _(PF4-H) Control Lot No. 3	2.7	2.1	2.6

Reproducibility: Reproducibility studies were conducted at three external clinical sites using different operators, on three different ACL TOP 500 CTS instruments, using three different lots of HemosIL HIT-Ab_(PF4-H) reagents and HemosIL HIT-Ab_(PF4-H) Controls (low and high). Each material was tested in triplicate, twice a day for 5 days, for a total of 30 replicates per level. The pooled 3-site data for each reagent lot is presented in the table below.

Pooled 3-Site Data: Reagent Lot 1 of HemosIL HIT-Ab_(PF4-H)											
Level	Mean (U/mL)	Repeatability (within-run)		Between-Run		Between-Day		Between-Site		Reproducibility (Total)	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Low HIT-Ab _(PF4-H) Control	0.9	0.11	11.7	0.07	7.1	0.00	0.0	0.06	6.7	0.14	15.3
High HIT-Ab _(PF4-H) Control	2.6	0.13	5.1	0.06	2.1	0.07	2.7	0.00	0.0	0.16	6.1
Pooled 3-site Data: Reagent Lot 2 of HemosIL HIT-Ab_(PF4-H)											
Low HIT-Ab(PF4-H) Control	0.9	0.08	9.2%	0.02	2.8%	0.00	0.0%	0.05	6.1%	0.06	7.4%
High HIT-Ab(PF4-H) Control	2.6	0.09	3.4%	0.05	1.9%	0.02	0.8%	0.13	4.8%	0.13	5.2%
Pooled 3-site Data: Reagent Lot 3 of HemosIL HIT-Ab_(PF4-H)											
Low HIT-Ab(PF4-H) Control	0.8	0.07	8.8%	0.06	6.5%	0.00	0.0%	0.00	0.0%	0.09	11.0%
High HIT-Ab(PF4-H) Control	2.7	0.11	4.0%	0.06	2.1%	0.00	0.0%	0.08	3.1%	0.15	5.5%

HemosIL HIT-Ab_(PF4-H) Calibrator

The HemosIL HIT-Ab_(PF4-H) Calibrator is included as a component of the reagent kit. The calibrator value assignment is performed with respect to the House Standard Calibrator, according to the internal protocol. The assigned value is lot specific. A precision study was conducted using three calibrator lots and reproducibility was evaluated using one calibrator lot.

d. Detection limit:

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were assessed using two lots of the assay reagent and contrived human plasma samples.

LoB	LoD	LoQ
0.2U/mL	0.3U/mL	0.6U/mL

e. Analytical specificity:

Analytical specificity for detecting anti-HIT antibodies was tested using 10 µL of PF4/ Heparin complex to inhibit reactivity of the assay in 18 citrated patient plasma samples. Inhibition of a positive reaction by $\geq 50\%$ was considered confirmatory for the assay specificity to heparin-dependent antibody characteristic of HIT.

Interferences: Dose-response interference study was performed for hemoglobin, free bilirubin, conjugated bilirubin, rheumatoid factor and human anti-mouse antibody (HAMA). HIT-Ab_(PF4-H) results on the ACL TOP Family analyzers are not affected by the tested interferents up to the levels indicated in table below:

Hemoglobin	Bilirubin	Triglycerides	Rheumatoid Factor	HAMA
500 mg/dL	19 mg/dL	375 mg/dL	1000 IU/mL	1 µg/mL

Heparin Sensitivity: 126 samples from non-HIT suspected, heparin treated patients (either UFH or LMWH) were tested with the HemosIL HIT-Ab_(PF4-H). For LMWH, 115 samples with a heparin concentration range between 0–2.47 IU/mL were tested. For UFH, 11 samples with a heparin concentration range between 0.04–1.08 IU/mL were tested. There was no dose-response correlation between the HIT results and heparin (UFH and LMWH) concentrations.

Antiphospholipid Syndrome (APS): Forty samples from patients diagnosed with APS were tested with the HemosIL HIT-Ab_(PF4-H). All 40 samples reported negative with HemosIL HIT-Ab_(PF4-H), demonstrating that the assay is not affected by APS antibodies.

f. Assay cut-off:

Cut-off Determination: A method comparison with the Serotonin Release Assay (SRA) performed on 63 plasma samples from HIT suspected patients (31 SRA positive and 32 SRA negative) indicated that 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 HIT-Ab(PF4-H) results equal to or higher than 1.0 U/mL may indicate the presence of HIT antibodies.

2. Comparison studies:

a. Method comparison with predicate device:

An in-house method comparison was performed on 118 frozen samples from HIT suspected patients using a commercially available ELISA assay. The results summarized below are based on a cut-off of 1.0 U/mL for the HemosIL HIT-Ab_(PF4-H) assay.

		HPIA ELISA results		
		+	-	Total
HemosIL HIT-Ab _(PF4-H) results	+	68	8	76
	-	13	29	42
	Total	81	37	118

HemosIL HIT-Ab_(PF4-H) vs Asserachrom HPIA	Proportion	Wilson 95% CI	
PPA (Positive Percent Agreement)	84% (68/81)	74%	91%
NPA (Negative Percent Agreement)	78% (29/37)	62%	90%
Total Percent Agreement	82% (97/118)	74%	89%

b. Matrix comparison:

- i. A comparison study between two citrate anticoagulant concentrations (3.2% vs. 3.8%) were conducted according to CLSI EP14-A2 Evaluation of matrix effect; Approved Guideline, 2nd edition. The study demonstrated matrix equivalence.
- ii. A comparison study between fresh vs. frozen plasma samples demonstrated matrix equivalence.

3. Clinical studies:

A multi-site clinical study was performed with a total of 639 patients suspected of HIT and treated with low molecular weight heparin (LMWH) and unfractionated heparin (UFH), as well as fondaparinux. Studies were performed in three clinical sites located in the United States. Testing included comparison of HemosIL HIT-Ab_(PF4-H) to the Asserachrom HPIA Test Kit (K003767). The study was expanded to also test samples using Serotonin Release Assay (SRA) as the reference method.

The performance of the HemosIL HIT-Ab(PF4-H) assay with regard to the SRA method was further evaluated in sub-groups of HIT suspected patients categorized by pre-test probability (low and intermediate-high). The pre-test probability of HIT was assessed according to the 4T score classification system, including:

- Thrombocytopenia
- Timing of platelet fall
- Thrombosis (or other sequelae of HIT)
- Absence of other causes of thrombocytopenia

Any patient with recent heparin exposure (within 100 days of antibody testing) from various units within the hospital was considered eligible for enrollment in the study based on HIT suspicion.

The results summarized below are based on a cut-off of 1.0 U/mL for the HemosIL HIT-Ab_(PF4-H) assay and the manufacturer's cut-off value for the ELISA.

HemosIL HIT-Ab_(PF4-H) vs. ELISA

		HPIA ELISA results		
		+	-	Total
HemosIL HIT-Ab _(PF4-H) results	+	67	39	106
	-	22	504	526
	Total	89	543	632

HemosIL HIT-Ab _(PF4-H) vs Asserachrom HPIA	Proportion	Wilson 95% CI	
PPA (Positive Percent Agreement)	75% (67/89)	65%	83%
NPA (Negative Percent Agreement)	93% (504/543)	90%	95%
Total Percent Agreement	90% (571/632)	88%	92%

HemosIL HIT-Ab_(PF4-H) vs. SRA and ELISA vs. SRA

A subset of the study population (n=537) was compared against SRA.

HemosIL HIT-Ab_(PF4-H) Assay vs. SRA:

		SRA Results		
		+	-	Total
HemosIL HIT-Ab _(PF4-H) results	+	33	56	89
	-	59	389	448
	Total	92	445	537

HemosIL HIT-Ab _(PF4-H) vs SRA	Proportion	Wilson 95% CI	
PPV (Positive Predictive Value)	37% (33/89)	28%	47%
NPV (Negative Predictive Value)	87% (389/448)	83%	90%

Asserachrom HPIA Test Kit (Predicate) vs. SRA:

		SRA Results		
		+	-	Total
HPIA ELISA results	+	31	37	68
	-	61	408	469
	Total	92	445	537

Asserachrom HPIA vs SRA	Proportion	Wilson 95% CI	
PPV (Positive Predictive Value)	46% (31/68)	34%	57%
NPV (Negative Predictive Value)	87% (408/469)	84%	90%

HemosIL HIT-Ab_(PF4-H) vs. 2013 American Society of Hematology (ASH) guidelines

The results of the subset of the study population (n=537) summarized below are based on the 2013 ASH guideline¹⁶ for determining clinical probability. The diagnostic algorithm classifies samples as HIT Likely or HIT Unlikely based on clinical probability score (i.e. 4T score), ELISA result (predicate device), and SRA result. In this retrospective study using the multicenter data, the results of the ASH diagnostic algorithm were compared with the data obtained with HemosIL HIT-Ab_(PF4-H).

The performance results are not based on a confirmed diagnosis of HIT.

		Clinical Probability According to 2013 ASH		
		HIT Likely	HIT Unlikely	Total
HemosIL HIT-Ab_(PF4-H) results	+	17	72	89
	-	2	446	448
	Total	19	518	537

Clinical Probability according to ASH 2013	Proportion	Wilson 95% CI	
PPA (Positive Percent Agreement)	89% (17/19)	69%	97%
NPA (Negative Percent Agreement)	86% (446/518)	83%	89%

Clinical Probability according to ASH 2013	Proportion	Wilson 95% CI	
PPV (Positive Predictive Value)	19% (17/89)	12%	28%
NPV (Negative Predictive Value)	100% (446/448)	98%	100%

4. Clinical cut-off:

See Clinical Study above

5. Expected values/Reference range:

The presence of anti-PF4/H antibodies in a normal population is not expected. Therefore,

results may be flagged as below linearity (< 0.6 U/mL). A set of studies was performed to assess the optimal cut-off of HemosIL HIT-Ab_(PF4-H) on the ACL TOP Family systems as described below.

Reference Interval – Heparin Exposed, HIT Suspected Patients (HIT Negative): A population of 122 samples from HIT-suspected, but negative by a commercially available ELISA was tested. The 95% reference interval was 0 – 0.9 U/mL.

Reference Interval – Healthy Donors: A population of 131 plasma samples from apparently healthy individuals was tested. The 95% reference interval was 0–0.7 U/mL.

N. Instrument Name: ACL TOP Family

To demonstrate instrument model equivalence, a study was performed using 51 citrated plasma samples from individual clinical patients suspected of having HIT. Each clinical sample was analyzed in singlet with a single lot of HemosIL HIT-Ab_(PF4-H) reagents on representative ACL TOP Family members: ACL TOP 700, ACL TOP 500 CTS and ACL TOP 300 CTS. Samples were distributed along the measuring range. The results obtained on the ACL TOP 700, the ACL TOP 500 CTS and the ACL TOP 300 CTS are summarized below:

Instrument	Slope (95% CI)	Intercept (95% CI)	R (95% CI)
ACL TOP 700 vs. ACL TOP 500 CTS	0.97 (0.92 to 1.02)	0.14 (0.05 to 0.23)	0.9947 (0.9909 to 0.9969)
ACL TOP 700 vs. ACL TOP 300 CTS	1.03 (1.00 to 1.06)	-0.13 (-0.20 to -0.10)	0.9981 (0.9966 to 0.9989)

The Bland-Altman plot of the 95% limits of agreement between the ACL TOP Instruments supported that the instrument models provide statistically equivalent results.

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 or No

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

Yes or No

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:

Bar code; manual entry

4. Specimen Sampling and Handling:

Automated

5. Calibration:

Automated, the calibrator is a component of the assay kit.

6. Quality Control:

Two levels of control are recommended for a complete quality control program. HemosIL HIT-Ab_(PF4-H) Controls Low and High are designed for this program. Each laboratory should establish its own mean and standard deviation, and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift, in accordance with good laboratory practice. Refer to the instrument's Operator's Manual for additional information.

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.