DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

July 08, 2016

Instrumentation Laboratory (IL) Co.
Ms. Carol Marble
Regulatory Affairs Director
180 Hartwell Road
Bedford, MA 01730

Re: K153137
Trade/Device Name: HemosIL HIT-$Ab_{(PF4-H)}$; HemosIL HIT-$Ab_{(PF4-H)}$ Controls
Regulation Number: 21 CFR 864.7695
Regulation Name: Platelet factor 4 radioimmunoassay
Regulatory Class: Class II
Product Code: LCO, GGN
Dated: June 6, 2016
Received: June 8, 2016

Dear Ms. Marble:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21

CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Leonthena R. Carrington -S

Leonthena R. Carrington, MS, MBA, MT(ASCP)
Director
Division of Immunology and Hematology Devices
Office of *In Vitro* Diagnostics and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K153137

Device Name
HemosIL HIT-Ab(PF4-H)
HemosIL HIT- Ab(PF4-H) Controls

Indications for Use *(Describe)*

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.

HemoslL 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Instrumentation Laboratory
A Werfen Company

510(k) Summary

This 510(k) Summary of Safety and Effectiveness is being submitted in accordance with the requirements of the Safe Medical Device Act of 1990 and 21 CFR 807.92.

Submitter's Information	Instrumentation Laboratory (IL) Co. 180 Hartwell Road Bedford, MA 01730, USA	
Contact Person	Carol Marble, Regulatory Affairs Director Phone: 781-861-4467 Fax: 781-861-4207 Email: cmarble@ilww.com	
Preparation Date	June 28, 2016	
Device Trade Names	Reagent Kit (with Calibrator)	HemosIL HIT-Ab$_{(PF4-H)}$
	Controls	HemosIL HIT-Ab$_{(PF4-H)}$ Controls
Regulatory Information – Reagent Kit	Regulation Number	21 CFR 864.7695
	Regulation Description	Platelet Factor 4 Radioimmunoassay
	Classification	Class II
	Product Code	LCO
	Classification Panel	Hematology (81)
Regulatory Information – Controls	Regulation Number	21 CFR 864.5425
	Regulation Description	Plasma, Coagulation Control
	Classification	Class II
	Product Code	GGN
	Classification Panel	Hematology (81)
Predicate Device	K003767 (Asserachrom HPIA Test kit from Diagnostica Stago)	

Indications for Use / Intended Use	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. HemoslL 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.
Device Description **Reagent Kit with Calibrator**	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 HemosIL HIT- Ab$_{(PF4-H)}$ kit consists of: R 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. S Stabilizer: PBS buffer containing bovine serum albumin, stabilizers and preservative. Com 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. C 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-Ab$_{(PF4-H)}$ 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-Ab$_{(PF4-H)}$ Control:** Control intended for the assessment of precision and accuracy of the assay at abnormal PF4/H antibody levels. Use of both controls is recommended for a complete quality control program.

Comparison to Predicate		
Item	**Predicate**	**New Device**
Trade Names	Asserachrom HPIA Test Kit (Kit Includes 2 Control Levels)	HemosIL HIT-$Ab_{(PF4-H)}$ HemosIL HIT-$Ab_{(PF4-H)}$ Controls
Manufacturer	Diagnostica Stago	Instrumentation Laboratory Co.
Similarities		
Measurand	Anti-PF4/Heparin Total Antibodies	Same
Assay Type	Qualitative	Same
Detection Method	Absorbance (Colorimetric)	Absorbance (Turbidimetric)
Product Code	LCO	Same
Regulation Section	864.7695	Same
Classification	Class II	Same
Intended Use	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). 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.	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.

Differences		
Methodology	Two-step enzyme immunoassay (ELISA) sandwich method with a final colorimetric detection.	Latex-enhanced immuno-turbidimetric assay.
Sample Types	Citrated Human Plasma or Serum	Citrated Human Plasma Only
Antibodies	Goat anti-human antibodies to IgG, IgA and IgM	Purified mouse monoclonal anti-PF4-Heparin
Cut-off	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 obtained for the Reagent 6 supplied with the kit. This percentage is provided for each lot through the insert sheets.	Fixed clinical cut-off: ≥ 1.0 U/mL
Controls	Controls included in test kit: • Negative level • Positive level	Controls sold separately: • Low Level at or below the cut-off • High Level at abnormal anti-PF4/H antibody level.
Calibrator Traceability	Not Applicable	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 a HIT International Standard is not currently available, Arbitrary Units (U/ml) have been established.

Performance Summary

Multi-Reagent Lot Precision

An internal precision study was performed using three (3) different lots of HemosIL HIT-Ab$_{(PF4-H)}$ reagents run on a representative ACL TOP Family member (ACL TOP 700). The study used a single lot of HemosIL HIT-Ab$_{(PF4-H)}$ Controls (low and high), as well as five (5) 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 No. 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 No. 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 No. 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

Multi-Reagent Lot Site-to-Site Reproducibility

Reproducibility studies were conducted at three (3) external clinical sites by three (3) different operators (one operator per site), on three (3) different ACL TOP 500 CTS instruments (one instrument per site), using three (3) 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 (3) 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 are summarized below and on the next page:

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									

Multi-Control Lot Precision

An internal precision study was performed using three (3) different lots of HemosIL HIT-Ab$_{(PF4-H)}$ Controls (low and high) run on a representative ACL TOP Family member (ACL TOP 700), with a single lot of HemosIL HIT-Ab$_{(PF4-H)}$ reagents.

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

Multi-Control Lot 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 a representative reagent lot is presented below.

Pooled 3-Site Data											
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

Multi-Calibrator Lot Precision

An internal precision study was performed using three (3) different lots of HemosIL HIT-$Ab_{(PF4-H)}$ Calibrator (kit component from 3 different reagent lots) run on a representative ACL TOP Family member (ACL TOP 700), with a single lot of HemosIL HIT-$Ab_{(PF4-H)}$ reagents (with a different kit Calibrator lot).

Each calibrator lot was tested in duplicate, twice a day for 20 days, for a total of 80 replicates per lot as summarized below:

Material	**Mean (U/mL)**	**Within Run (Repeatability) % CV**	**Total (Within Device) % CV**
Kit Calibrator Lot No. 1	5.6	0.7	1.4
Kit Calibrator Lot No. 2	5.6	1.2	1.5
Kit Calibrator Lot No. 3	5.6	0.6	1.9

Unadulterated Patient Plasma Precision

An internal unadulterated (unmodified) patient plasma study was performed on a representative ACL TOP Family member (ACL TOP 700). The two (2) patient samples used in the study were collected at a hospital in the United States, with aliquots prepared and maintained at -70°C until use.

Each sample aliquot was tested in duplicate, twice a day for 10 days, for a total of 40 replicates per patient plasma sample as summarized below:

Material	**Mean (U/mL)**	**Within Run (Repeatability) % CV**	**Total (Within Device) % CV**
Patient Plasma Sample 1	2.1	3.0	5.4
Patient Plasma Sample 2	8.6	5.7	6.2

Instrument Model Equivalency

An instrument model equivalency study was performed using fifty-one (51) citrated plasma samples from individual clinical patients who were suspected of having HIT. Each clinical sample was analyzed in singlicate 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.

NOTE: Samples were distributed along the measuring range.

The reported values of the clinical sample results on ACL TOP 700 were plotted on a X-Y graph comparing the results obtained on the ACL TOP 700 to the ACL TOP 500 CTS and the ACL TOP 300 CTS results as 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.

Linearity and Test Ranges

A linearity study was performed using two (2) lots of HemosIL HIT-Ab$_{(PF4-H)}$ reagents, with each lot tested on three (3) representative ACL TOP Family members: ACL TOP 700, ACL TOP 500 CTS and ACL TOP 300 CTS.

Two (2) positive patient samples were spiked into donor plasma from a blood bank to prepare 10 concentrations ranging across the standard measurement range (0.6-5.7 U/mL). A third extremely high positive patient sample was used to prepare samples with concentrations ranging across the auto re-run linear range (2.1 to 16.0 U/mL).

The results support the following linearity claims:

Linearity Range:

	Auto Rerun off	Auto Rerun on
ACL TOP Family	0.6 – 5.7 U/mL	2.1 – 16.0 U/mL

Test Range:

	Auto Rerun off	Auto Rerun on
ACL TOP Family	0 – 5.7 U/mL	0 – 16.0 U/mL

Result Interpretation

HIT-Ab$_{(PF4-H)}$ results are reported in U/mL (HemosIL HIT-Ab$_{(PF4-H)}$ Arbitrary Units) on the ACL TOP Family instruments as follows:

System	Results	Interpretation
ACL TOP Family	$\geq$ 1.0 U/mL	HIT Antibody positive

ACL TOP Family < 1.0 U/mL HIT Antibody negative

IMPORTANT: The laboratory should report only the interpreted result (HIT Antibody positive or negative) to the clinician as the final reported result.

The positive or negative result should be used with other information, including the clinical context, in forming a diagnosis such as the 4T score and the 2013 American Society of Hematology guidelines.

Although a positive result obtained using this assay may indicate the presence of heparin-associated antibodies, a positive result **DOES NOT CONFIRM** the diagnosis of HIT. Some patients may have naturally occurring antibodies to PF4.

Interferences / Limitations

- **Interferences**

  Testing confirmed no interference for the HemosIL HIT-$Ab_{(PF4-H)}$ assay on the ACL TOP Family up to the following concentrations:

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

- **Heparin Sensitivity**

  One hundred twenty-six (126) citrated plasma samples from non-HIT suspected, heparin treated patients (either UFH or LMWH) were tested with the HemosIL HIT-$Ab_{(PF4-H)}$ assay. For LMWH, 115 samples with a heparin concentration range between 0-2.47 IU/mL. For UFH, 11 samples with a heparin concentration range between 0.04-1.08 IU/mL.

  There was no dose-response correlation between the HIT results and heparin (UFH and LMWH) concentrations.

- **Antiphospholipid Syndrome (APS)**

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

Reference Interval – Healthy Donors

A population of one hundred thirty-one (131) citrated plasma samples from apparently healthy individuals was tested with the HemosIL HIT-$Ab_{(PF4-H)}$ assay.

The upper limit of the reference interval was established as 0.7 U/mL (90% CI: 0.60-0.71 U/mL).

Reference Interval – Heparin Exposed, HIT Suspected Patients (HIT Negative)

A population of one hundred twenty-two (122) citrated plasma samples from HIT-suspected, but negative by a commercially available ELISA was tested with the HemosIL HIT-$Ab_{(PF4-H)}$ assay.

The upper limit of the reference interval was established as 0.9 U/mL (90% CI: 0.80-1.03 U/mL).

Cut-Off Determination

Sixty three (63) citrated plasma samples were obtained from hospitalized patients who had been exposed to heparin, and who displayed clinical signs consistent with Heparin Induced Thrombocytopenia (HIT). These patients were tested by the hospital with the Serotonin Release Assay (SRA) and the sample results (31 SRA positive and 32 SRA negative) were used to perform a Receiver Operating Characteristics (ROC) analysis to determine the clinical cut-off for HemosIL HIT-$Ab_{(PF4-H)}$.

The optimal cut-off as determined by ROC analysis was 1.0 U/mL (95.2% Agreement; CI% = 86.7% - 99.0%).

Method Comparison Definitions and Formulas

See below 2x2 contingency table with definitions and formulas.

		Reference	
		+	-
Test	+	TP	FP
	-	FN	TN

TP	*True Positives*
TN	*True Negatives*
FP	*False Positives*
FN	*False Negatives*

PPA Positive Percent Agreement

NPA Negative Percent Agreement

$$PPA = \frac{TP}{TP + FN} \qquad NPA = \frac{TN}{TN + FP}$$

NPV Negative Predictive Value

PPV Positive Predictive Value

$$PPV = \frac{TP}{TP + FP} \qquad NPV = \frac{TN}{TN + FN}$$

TPA Total Percent Agreement

$$TPA = \frac{TP + TN}{TP + TN + FP + FN}$$

Method Comparison (Internal)

An internal method comparison study was performed to compare the performance of the new HemosIL HIT-$Ab_{(PF4-H)}$ assay versus its predicate, the Asserachrom HPIA Test Kit (K003767), on a representative member of the ACL TOP Family (ACL TOP 700 model).

A total of 118 frozen citrated patient plasma samples from patients referred for HIT testing were obtained from a hospital in the US and a hospital in France. Samples were analyzed in singlicate, with no spiked samples used in this study.

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 lot- and plate-dependent cut-off for the Asserachrom HPIA Test Kit.

		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%

Multicenter Method Comparison

A multicenter method comparison study was conducted at three (3) hospitals on 632 patient samples, comparing the performance of the new HemosIL HIT-Ab$_{(PF4-H)}$ assay with the predicate device, the Asserachrom HPIA Test Kit (K003767) and with the reference device, Serotonin Release Assay (SRA). The samples were from patients exposed to heparin who showed HIT related symptoms. Samples were analyzed in singlicate, with no spiked samples used in this study.

HemosIL HIT-Ab$_{(PF4-H)}$ Assay vs. Predicate Device (Asserachrom HPIA Test Kit)

The pooled results below are based on a cut-off of 1.0 U/mL for the HemosIL HIT-Ab$_{(PF4-H)}$ assay and the lot- and plate-dependent cut-off for the Asserachrom HPIA Test Kit.

		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$_{(PF4-H)}$ Assay vs. SRA and Predicate vs. SRA

Patient samples in the multicenter study without a valid SRA result or with an indeterminate result (total N=95) were removed from calculations, bringing the final total to N = 537.

The pooled results summarized below compare both HemosIL HIT-Ab$_{(PF4-H)}$ assay results (cut-off of 1.0 U/mL) to the SRA test results and the Asserachrom HPIA Test Kit (lot- and plate-dependent cut-off) to the SRA test results.

- **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%

Conclusion: The diagnostic performance of the HemosIL HIT-Ab$_{(PF4-H)}$ assay was equivalent to the Asserachrom HPIA Test Kit (predicate) when compared to SRA test results.

HemosIL HIT-Ab$_{(PF4-H)}$ Assay vs. 2013 American Society of Hematology (ASH) Guidelines

The results summarized below are based on the 2013 ASH guideline for determining clinical probability. The ASH diagnostic algorithm classifies samples as HIT Likely or HIT Unlikely based on a clinical probability score (i.e. patient 4T score), the Asserachrom HPIA Test Kit (as the ELISA result) and the SRA results (as the functional assay). 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$_{(PF4-H)}$.

NOTE: 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%

Conclusion:

The analytical and clinical study results demonstrate that the HemosIL HIT-Ab$_{(PF4-H)}$ assay and HemosIL HIT-Ab$_{(PF4-H)}$ Controls are substantially equivalent to the predicate device, Asserachrom HPIA Test Kit (FDA cleared under K003767), and that the assay is safe and effective for its labeled intended use when compared to the SRA reference method.