

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

A. 510(k) Number:

k102164

B. Purpose for Submission:

Clearance of a new device

C. Measurand:

Free Protein S Activity

D. Type of Test:

Quantitative

E. Applicant:

Instrumentation Laboratory Co.

F. Proprietary and Established Names:

HemosIL® Protein S Activity

G. Regulatory Information:

1. Regulation section:
21 CFR 864.7290, Factor Deficiency Test
2. Classification:
Class II
3. Product code:
GGP, Test Qualitative and Quantitative Factor Deficiency
4. Panel:
81 (Hematology)

H. Intended Use:

1. Intended use(s):
Automated coagulation functional assay for the quantitative determination of free Protein S in human citrated plasma as an aid in the diagnosis of hereditary and acquired Protein S deficiency, on the ACL TOP® Family of analyzers.
2. Indication(s) for use:
Same as intended use(s)
3. Special conditions for use statement(s):
For prescription use only
4. Special instrument requirements:
ACL TOP® Family of analyzers - ACL TOP, ACL TOP 700, ACL TOP 500 CTS, ACL TOP 700 CTS, and ACL TOP 700 LAS.

I. Device Description:

The HemosIL Protein S Activity Kit consists of the following:

- Protein S reagent: 3 x 2 mL vials of a lyophilized preparation containing recombinant human tissue factor, synthetic phospholipids, activated protein C, polybrene, buffer, stabilizers and preservatives.
- Calcium reagent: 3 x 6 mL vials of a liquid preparation containing calcium.
- Protein S deficient plasma: 3 x 2 mL vials of a lyophilized human plasma which has been artificially depleted of Protein S.

J. Substantial Equivalence Information:

1. Predicate device name(s):

HemosIL® ProS

2. Predicate K number(s):
k053499
3. Comparison with predicate:

Similarities		
Item	HemosIL® Protein S Activity	HemosIL® ProS (predicate)
Intended Use	Automated coagulation functional assay for the quantitative determination of free Protein S in human citrated plasma as an aid in the diagnosis of hereditary and acquired Protein S deficiency, on the ACL TOP® Family of analyzers.	Same
Reagent Shelf Life	2 years (when stored at 2 - 8°C)	Same

Differences		
Item	HemosIL® Protein S Activity	HemosIL® ProS (predicate)
Reagent Kit Composition	<p><u>Protein S reagent:</u> Lyophilized preparation containing recombinant human tissue factor, synthetic phospholipids, activated protein C, Polybrene, buffer, stabilizers, and preservatives.</p> <p><u>Calcium reagent:</u> included in a separate vial</p> <p><u>Protein S deficient plasma:</u> lyophilized human plasma which has been artificially depleted of protein S, vial size 2 mL.</p> <p><u>Protein S Control Plasma:</u> not sold with this product</p>	<p><u>Protein S reagent:</u> Lyophilized preparation containing recombinant rabbit tissue factor, calcium ions, synthetic phospholipids, activated protein C, Polybrene, buffer, stabilizers, preservatives,</p> <p><u>Calcium reagent:</u> included within Protein S reagent (separate calcium reagent vial absent).</p> <p><u>Protein S deficient plasma:</u> lyophilized human plasma which has been artificially depleted of protein S, vial size 1 mL.</p> <p><u>Protein S Control Plasma:</u> Lyophilized human plasma containing a low level of protein S.</p>
Open vial stability- after reconstitution (if applicable)	<p><u>Protein S reagent:</u> 24 hours at 2-8°C, 8 hours at 15°C on-board.</p> <p><u>Calcium reagent:</u> 24 hours at 2-8°C, 8 hours on-board.</p> <p><u>Protein S deficient plasma:</u> 6 hours at 15°C on-board.</p>	<p><u>Protein S reagent:</u> 2 days at 2-8°C, 2 days at 15°C on- board.</p> <p><u>Calcium reagent:</u> included within Protein S reagent</p> <p><u>Protein S deficient plasma:</u> 4 hours at 15-25°C.</p>

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

- CLSI, Evaluation of Precision of Quantitative Measurement Methods; Approved Guidelines-Second Edition (EP5-A2).
- CLSI, Evaluation of the Linearity of Quantitative Measurement Procedures: A statistical Approach (EP6-A).
- CLSI, Interference Testing in Clinical Chemistry (EP7-A2).

CLSI, Method Comparison and Bias Estimation Using Patient Samples (EP9-A2).
CLSI, Protocols for the Limit of Detection and Limits of Quantitation (EP17-A).
CLSI, Evaluation of Stability of In Vitro Diagnostic Reagents (EP25-A).
CLSI, Reference Range Data: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory (C28-A3).

L. Test Principle:

The test is based on the ability of endogenous protein S as a cofactor of activated protein C to prolong the clotting time. Protein S levels in patient plasma are measured automatically on the ACL TOP® Family of analyzers. The test determines the functional activity of free Protein S by measuring the degree of prolongation of a prothrombin time in the presence of the recombinant tissue factor, phospholipids, calcium ions, and activated protein C. The Protein S activity is correlated with the prolongation of the clotting time of a Protein S deficient plasma to which diluted sample has been added.

M. Performance Characteristics:

1. Analytical performance:

a. *Precision/Reproducibility:*

Within-run, between-run and total reproducibility: A precision study was performed with three lots of HemosIL Protein S Activity on an ACL TOP, ACL TOP 700 and ACL TOP 500 CTS analyzers, for 20 days, with 2 runs a day and 2 replicates each run for each sample level (N=80 per level for each instrument). Test samples consisted of High Abnormal Control (HAC), Normal Control (NC), and High Normal Donor (HND). The acceptance criteria were as follows for the within-run study: HAC, standard deviation (SD) ≤ 2.5 ; NC, coefficient of variation (CV) $\leq 6\%$; HND, CV $\leq 6\%$. The acceptance criteria were as follows for between-run study: HAC, SD ≤ 3.0 ; NC, CV $\leq 8\%$; HND, CV $\leq 8\%$. Acceptance criteria for total reproducibility were as follows: HAC, SD ≤ 3.0 ; NC, CV $\leq 8\%$; HND, CV $\leq 8\%$. The acceptance criteria for all samples within each study were achieved.

b. *Linearity/assay reportable range:*

Linearity testing was performed using two lots of HemosIL Protein S Activity reagent kits on two ACL TOP instruments. Eleven (11) samples with protein S % activity ranging from 7.5 to 150% were prepared. Each sample was run in 4 replicates using the two reagent lots, and the average activities for the 11 samples were plotted against their assigned values. Acceptance criteria were as follows: slope, 0.9 – 1.1; $R^2 \geq 0.95$. The results of the study fell within acceptable limits. The results support the claimed assay reportable range of 10 to 150% activity.

c. *Traceability, Stability, Expected values:*

Reagent stability

On-board instrument stability at 15°C: This study was performed using 2 different lots of Protein S and calcium reagents with 2 ACL TOP instruments (ACL TOP 700, ACL TOP 500 CTS). Vials of calcium reagent were opened, and vials of Protein S and Protein S Deficient Plasma reagent were reconstituted and maintained at 15°C on-board the instruments. At set intervals, controls were tested in 4 replicates and the results were compared to

the control values generated at time zero (i.e. baseline mean). Acceptance criteria were as follows: Normal Control, baseline mean \pm 15%; Special Test Control Level 2, baseline mean \pm 10%; High Abnormal Control, baseline mean \pm 10%. The resulting data supported an on-board 15°C stability claim for Protein S and calcium reagents of 8 hours. The Protein S Deficient Plasma was found to have an on-board 15°C stability of 6 hours.

Reconstituted reagent stability at 2-8°C: Vials of Calcium reagent were opened and vials of Protein S reagent were reconstituted and tested at time zero. The remaining vials were stored at 2-8°C for the duration of testing. At each time interval the three controls were tested in 4 replicates on an ACL TOP using the stored opened Calcium reagent and reconstituted Protein S Reagent. The results are compared to time zero. Acceptance criteria were as follows: Normal control, baseline mean \pm 15; Special test control level 1, baseline mean \pm 10; High abnormal control, baseline mean \pm 10. The data presented supported the following stability claims: Protein S reagent and Calcium- 24 hours at 2-8°C.

Accelerated closed vial stability: Protein S Reagent vials, stored at different temperatures were tested for Protein S Activity assay on ACL TOP instruments. The stability results at 37°C and 50°C were used to predict the estimated shelf-life. Testing was performed using Protein S Reagent vials stored at 50°C for 12, 24, 32, and 48 hours, as well as vials stored at 37°C for 3, 5, 7, 8, 9, 10, 11, 12 and 13 days and compared to vials stored at 4°C as the control for reagents. Reagents were placed at the desired temperatures at different times and then tested along with the reagents stored under normal storage (4°C) conditions. Control samples for each time/temperature point were analyzed in 4 replicates. The acceptance criteria were as follows: Normal Control, baseline mean \pm 15%; Special Test Control Level 2, baseline mean \pm 10%; High Abnormal Control, baseline mean \pm 10%. The results were evaluated and showed a predicted closed vial stability of 16.45 years.

Real-time closed vial stability: Real-time stability is on-going with 2 different lots of HemosIL® Protein S Activity kits stored at 2-8°C. At each time interval each control was run in 4 replicates and the mean value was compared to its established baseline value/range. The acceptance criteria were as follows: Normal Control, baseline mean \pm 15%; Special Test Control Level 2, baseline mean \pm 10%; High Abnormal Control, baseline mean \pm 10%. The real time stability has been tested up to the months of 18 and 6 for lots 1 and 2, respectively. All the real time stability tests performed are within the established stability acceptance range.

d. Detection limit:

Limit of Detection (LOD) and Limit of Blank (LOB) were performed using two lots of HemosIL Protein S Activity reagent kits on two ACL TOP instruments. Protein S Deficient plasma was used for determination of LOB and a 5% Activity Protein S plasma was used for LOD. The Protein S Deficient plasma and the 5% Activity Protein S plasma were analyzed each day (n=12 per day) for 5 days (n=60 total) using both lots of the HemosIL Protein S Activity reagents. The data supports the detection limit claim on the

ACL TOP family of 5%.

e. *Analytical specificity:*

An interference study was done with one lot of HemosIL Protein S Activity on an ACL TOP analyzer. Two samples, fresh frozen pool plasma and protein S depleted plasma were used to prepare three plasma pools with different protein S% activity. The three sample levels were spiked with multiple levels of the interferent, tested in quadruplicate, and the mean results compared to the unspiked control result. The acceptance criteria included the following: recovery of $\pm 15\%$ of the unspiked control result. The data support the claims in the package insert for no significant interference at the concentrations noted by unfractionated heparin (UFH), 1.6 IU/mL; low molecular weight heparin (LMWH), 2.1 IU/mL; hemoglobin, 250 mg/dL; bilirubin, 15 mg/dL; and triglyceride, 2360 mg/dL.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

An in-house method comparison study was conducted using the predicate and the HemosIL® Protein S Activity assay on an ACL TOP and ACL TOP 500 CTS. Results were calculated by comparing the first replicate values. The study included both normal (N=26) and abnormal (N=72) patient samples. A subset of these samples (N=47) was tested on an ACL TOP 500 CTS. The acceptance criteria were as follows: slope 0.9 – 1.1 and correlation coefficient (r) ≥ 0.95 . Deming regression analysis for the ACL TOP shows that the slope and correlation coefficient (r) are 0.966 and 0.968, respectively. ACL TOP 500 CTS demonstrates the slope and correlation coefficient (r) are 1.046 and 0.975, respectively.

Two field site exercises were completed to further evaluate the HemosIL® Protein S Activity assay against its predicate. A method comparison was conducted at a cancer center using 72 normal and 71 abnormal samples. The second comparison was produced at a medical center using 78 normal and 62 abnormal samples. The acceptance criteria for both studies were as follows: slope 0.9 – 1.1 and $r \geq 0.95$. The Deming regression analysis for the cancer center data performed on the ACL TOP shows that the slope and correlation coefficient (r) are 1.049 and 0.956, respectively. The medical center study performed on the ACL TOP produced a slope and correlation coefficient (r) of 0.984 and 0.965, respectively.

b. *Matrix comparison:*

Fresh v. Frozen Sample Study - Plasma samples were collected in evacuated glass tubes with 3.2% sodium citrate as an anticoagulant. Testing was performed on ACL TOP, as a representative family member, with the HemosIL® Protein S Activity assay. Fifty three normal donor samples, collected in-house, were used for the method comparison. Five different levels (10-70% of protein S activity) were prepared for each donor. As soon as the testing was completed for the fresh samples, they were stored at $\leq -70^\circ\text{C}$. The frozen plasma samples (stored for a minimum of 24 hours at $\leq -70^\circ\text{C}$) were

then thawed in a 37°C water bath for 3 to 4 minutes. The acceptance criteria are as follows: slope 0.9 – 1.1 and r (correlation coefficient) \geq 0.95. The study produced a slope = 0.992 and r = 0.99.

Comparison of 3.2% and 3.8% sodium citrate - From each of 28 donors, two blood samples were drawn, one in a 3.2% and the other in a 3.8% sodium citrate sample tube. Two pools, with different sodium citrate concentrations, were prepared using the same volume of citrated plasma from each donor. Protein S was depleted from each pool using a protein S monoclonal antibody resin. The donor samples then were diluted with the corresponding citrated protein S depleted plasma to produce a range of concentrations. The acceptance criteria are as follows: slope 0.9 – 1.1 and r \geq 0.95. The study yielded a slope of 1.028 and r = 0.993.

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity*

Not applicable

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

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

A reference interval study was performed using one lot of HemosIL Protein S Activity reagent kit on an ACL TOP analyzer. A total of 165 normal samples (76 male, 89 female) were obtained and a normal range of 63.5 – 149.0% Protein S Activity was established statistically.

N. Proposed Labeling:

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

O. Conclusion:

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