

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

**A. 510(k) Number:**

k110237

**B. Purpose for Submission:**

New device

**C. Measurand:**

Factor VIII

**D. Type of Test:**

Quantitative

**E. Applicant:**

Instrumentation Laboratory Company

**F. Proprietary and Established Names:**

HemosIL® Factor VIII deficient plasma

**G. Regulatory Information:**

1. Regulation section:  
21 CFR 864.7290, Factor deficiency test
2. Classification:  
Class II
3. Product code:  
GJT, Plasma, Coagulation Factor Deficient
4. Panel:  
81 Hematology

**H. Intended Use:**

1. Intended use(s):  
HemosIL® Factor VIII deficient plasma is human plasma depleted of Factor VIII and intended for the in vitro diagnostic quantitative determination of Factor VIII activity in citrated plasma, based on the activated partial thromboplastin time (APTT) assay, on the ACL TOP® Family analyzers. HemosIL® Factor VIII deficient plasma is indicated for use on patients who are suspected of congenital or acquired deficiency based on the activated partial thromboplastin time (APTT) assay results.
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 TOP® Family analyzers: ACL TOP, ACL TOP 700, ACL TOP 500 CTS, ACL TOP 700 CTS, and ACL TOP 700 LAS.

**I. Device Description:**

The HemosIL® Factor VIII deficient plasma kit contains 10 x 1 mL vials of lyophilized human plasma that has been artificially depleted of Factor VIII containing buffer and stabilizers. The residual Factor VIII activity is less than or equal to 1% whereas von Willebrand Factor and the remaining intrinsic pathway factor levels are normal.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
HemosIL® Factor VIII deficient plasma
2. Predicate K number(s):  
k034007
3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device (k110237)</b>	<b>Predicate (k034007)</b>
Device Name	HemosIL® Factor VIII deficient plasma	Same
Manufacturer	Instrumentation Laboratory Co.	Same
Indications for Use	HemosIL® Factor VIII deficient plasma is human plasma depleted of Factor VIII and intended for the <i>in vitro</i> diagnostic quantitative determination of Factor VIII activity in citrated plasma, based on the activated partial thromboplastin time (APTT) assay, on the ACL TOP® Family analyzers.	Same

<b>Differences</b>		
<b>Item</b>	<b>Device (k110237)</b>	<b>Predicate (k034007)</b>
Kit Composition	Lyophilized human plasma deficient in Factor VIII and contains normal levels of von Willebrand factor.	Lyophilized human plasma deficient in Factor VIII and deficient in von Willebrand factor.

**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, Method Comparison and Bias Estimation Using Patient Samples (EP9-A2).

CLSI, Evaluation of Stability of In Vitro Diagnostic Reagents (EP25-A).

**L. Test Principle:**

The assay determines the functional activity of Factor VIII by measuring the degree of prolongation of activated partial thromboplastin time in the presence of a contact activator, thromboplastin, phospholipids and calcium ions. Factor VIII activity is correlated with the prolongation of the clotting time of the Factor VIII deficient plasma to which diluted patient sample has been added.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

Within-run and between-run: Precision studies were performed with three lots of HemosIL Factor VIII deficient plasma on the ACL TOP, ACL TOP 500 CTS, and ACL TOP 700 for 20 operational days, with 2 runs a day and 2 replicates per run for each sample level (N=80 per level for each reagent/instrument combination). The acceptance criteria are listed in the following table.

Sample	Range of sample means (FVIII % Activity)	Within-run % CV	Between-run % CV
HemosIL Normal Control	75.5 – 87.4	≤10%	≤10%
HemosIL ST Level 2 Control	23.8 – 29.7	≤15%	≤15%
Low Control I	7.4 – 12.1	≤20%	≤20%
Low Control II	5.1 – 6.8	≤20%	≤20%

The acceptance criteria were met for all samples within each study.

b. *Linearity/assay reportable range:*

Linearity testing was performed using two lots of HemosIL® Factor VIII deficient plasma on the ACL TOP and ACL TOP 500 CTS using the APTT reagents SynthASil and APTT SP. The Factor VIII activities of a high sample and an intermediate sample were determined from the mean of 8 replicates using the predicate HemosIL® Factor VIII deficient plasma. These two samples were diluted in order to prepare 12 samples with Factor VIII activities ranging from 0% to 170%. All Factor VIII samples were run in 4 replicates on the ACL TOP Family members for both reagent lots and the average activities for all the samples were plotted against their assigned values. Acceptance criteria were as follows: slope, 0.85 – 1.15;  $R^2 \geq 0.95$ . The results of the study fell within acceptable limits. The results support the claimed assay reportable range using SynthASil of 0.1 – 150% FVIII activity and a reportable range using APTT SP of 0.5 – 150% FVIII activity.

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

Reagent Stability

Real Time Stability: The general storage condition for HemosIL® Factor VIII Deficient Plasma is at 2-8°C. Three lots were stored and at periodic times sample vials were removed for stability testing. The study was performed on an ACL TOP for the recovery of Factor VIII activity for two plasma controls: Normal and ST Level 2, tested in quadruplicate. The acceptance criteria are found in the table below. To date of the submission, real time stability was tested for 12 months for the 1st and 2nd lots, and 6 months for the 3rd lot. All tests were within the established acceptance range. Based on the results of the accelerated stability study, which projects a 3-year shelf life, the 12 month shelf life claim is supported for the product when stored at 2-8°C. Ongoing real-time stability testing will be used to update the shelf life.

On board Stability (open vial): Open vials of two lots of reconstituted HemosIL® Factor VIII deficient plasma were placed in the reagent block of

an ACL TOP for 27 hours. Using on-board Factor VIII deficient plasma and SynthASil reagent, the Factor VIII activity assay was performed for Normal Control Plasma and ST Level 2 at 0, 2, 4, 8, 24 and 27 hour time points. Each control plasma sample was tested in quadruplicate. Using the mean of the Factor VIII activities of each control plasma sample at zero hour as a baseline, the change (%) in Factor VIII activity for each time point was calculated. The acceptance criteria are found in the table below. The data indicate that the change (%) at every time point tested met the specified limitation. Factor VIII deficient plasma is stable for 24 hours on board the ACL TOP at 15°C.

Reconstituted stability at 2-8° C: Two lots of HemosIL® Factor VIII deficient plasma were reconstituted and stored at 2-8°C for 27 hours. Using this plasma and SynthASil on the ACL TOP Base, the Factor VIII activity assay was performed for Normal Control Plasma and ST Level 2 at 0, 2, 4, 8, 24, and 27 hour time points. Each control plasma sample was tested in quadruplicate. Using the mean of the Factor VIII activities of each control plasma sample at zero hour as a baseline, the change (%) in Factor VIII activity for each time point was calculated. The acceptance criteria are found in the table below. The data indicate that the change (%) at every time point tested met the specified limitation. Factor VIII deficient plasma is stable for 24 hours at 2-8°C after reconstitution.

Frozen stability study at -20°C: Two lots of Factor VIII deficient plasma were reconstituted and stored at -20°C for 31 days. Using this plasma and APTT SP on the ACL TOP 500, the Factor VIII activity assay was performed for Normal Control Plasma and ST Level 2 at 0, 1, 4, 7, 14, 21, 28 and 31 day time points. Each control plasma sample was tested in quadruplicate. Using the mean of the Factor VIII activities of each control plasma sample at zero hour as a base line the change (%) in Factor VIII activity for each time point was calculated. The acceptance criteria are found in the table below. Results for both lots indicate that the change (%) met the specified limitation at every time point tested. Factor VIII deficient plasma is stable for 3 weeks at -20°C after reconstitution.

Stability Testing Acceptance Criteria:

Sample	Specification
HemosIL Normal Control	baseline mean ±10%
HemosIL SP Control	baseline mean ±15%

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

Interference studies were conducted using one lot of HemosIL® Factor VIII deficient plasma on the ACL TOP 700 analyzing normal plasma spiked with various concentrations of hemoglobin, triglyceride, bilirubin, lupus anticoagulant antibodies, and FVIII inhibitor. Both SynthASil and APTT SP reagents were employed. The percent differences in results of the spiked plasma samples and unspiked plasma sample were determined. Acceptance criterion was percent difference between the spiked and baseline (unspiked)

sample to be  $\leq 15\%$ . Interference tolerance limits were determined. Factor VIII results on the ACL TOP Family are not affected by hemoglobin up to 530 mg/dL, triglycerides up to 2000 mg/dL, bilirubin up to 150 mg/dL, and Factor VIII inhibitors up to 0.1 BU. Results are not affected by the presence of Lupus anticoagulant antibodies.

f. *Assay cut-off:*  
Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

An in-house method comparison study was conducted to compare the performance of the predicate Factor VIII Deficient Plasma to the new HemosIL® Factor VIII Deficient Plasma on an ACL TOP base model and an ACL TOP 500 CTS using each of the APTT reagents (SynthASil, APTT SP). A total of at least 88 samples were tested per instrument with each APTT reagent and the results were calculated by comparing the first replicate values. The study included apparently normal healthy donors and patients suspected of factor VIII deficiency. The clinical breakdown of samples is detailed in the table below:

Sample Type	Number on ACL TOP		Number on ACL TOP 500 CTS	
	SynthASil	APTT-SP	SynthASil	APTT-SP
Total (n)	98	88	90	90
Normal donors	40	33	37	38
Abnormal:	58	55	53	52
-FVIII	22	20	20	20
-Hemophilia	6	6	5	5
-OAC	6	6	6	6
-DIC	11	11	11	9
-Liver Disease	13	12	11	12

Acceptance criteria are as follows: slope must fall between 0.85 – 1.15 and  $r \geq 0.95$ . Results and analysis of the in-house study are listed in the table below.

ACL TOP (Base Model)	Reagent	
	SynthASil	APTT-SP
Slope (95% CI)	1.064 (1.031-1.097)	0.893 (0.864-0.923)
Intercept (95% CI)	-3.34 (-6.02 to -0.65)	+0.68 (-1.55 to +2.90)
Correlation coefficient (r)	0.9885	0.9884
total samples (n)	98	88
ACL TOP 500 CTS	Reagent	
	SynthASil	APTT-SP
Slope (95% CI)	1.112 (1.083 to 1.141)	0.913 (0.889 to 0.936)
Intercept (95% CI)	-3.57 (-5.73 to -1.40)	+0.56 (-1.19 to +2.31)
Correlation coefficient (r)	0.9923	0.9926
total samples (n)	90	90

Additional method comparisons between HemosIL® Factor VIII Deficient Plasma (test device) and Factor VIII deficient plasma (predicate) were conducted at three sites using a total of 336 patient samples. Both normal and

abnormal samples were tested contemporaneously using an ACL TOP analyzer. Results were calculated by comparing first replicate values. The clinical breakdown of the samples is detailed in the table below.

	Site 1	Site 2	Site 3
Total Samples (n)	109	125	102
Normal samples	31	40	34
Abnormal:	78	85	68
- OAT	20	20	11
- Liver Disease	1	20	4
- Hemophilia	34	34	14
- Other	23	11	39

Acceptance criteria were identical to the in-house method comparison study previously stated. Results and analysis are as follows:

	Site 1	Site 2	Site 3
Slope (95% CI)	1.074 (1.036 to 1.112)	0.871 (0.853 to 0.888)	1.138 (1.098 to 1.178)
Intercept (95% CI)	- 4.83 ( - 8.15 to 1.52)	- 0.25 ( - 1.78 to 1.28)	- 9.31 ( - 12.63 to 5.99)
Correlation coefficient (r)	0.9829	0.9935	0.9842
Total samples (n)	109	125	102

b. *Matrix comparison:*

*Fresh vs. Once Thawed Samples* – Plasma samples were collected in tubes containing 3.2% sodium citrate as an anticoagulant and processed. Testing was performed on the ACL TOP with the HemosIL Factor VIII Deficient Plasma. One hundred nineteen (119) patient samples (37 normal and 82 abnormal) were used for the comparison. After testing the fresh samples they were stored at or below -65°C for at least 24 hours before being thawed and re-tested. Acceptance criteria were as follows: slope 0.85 to 1.15 and  $r \geq 0.95$ . The regression analysis produced a slope = 1.011 and a correlation coefficient (r) = 0.9880.

*Comparison of 3.2% and 3.8% sodium citrate plasma* - Two blood samples were drawn, from a total of 37 normal donors, using both a 3.2% and 3.8% sodium citrate sample tube respectively. Plasma samples were obtained from prompt centrifugation and Factor VIII was depleted from the donor plasmas with different sodium citrate concentrations using antibody resin. The donor samples then were diluted with the appropriate citrated Factor VIII depleted plasma to produce a range of concentrations. A total of 29 pairs of artificial samples were prepared and tested with SynthASil and APTT SP APTT reagents. Acceptance criteria were as follows: slope 0.85 to 1.15 and  $r \geq 0.95$ . The regression slope and correlation coefficient were 0.885 and 0.987 for SynthASil respectively. The slope and correlation coefficient were 0.907 and 0.9870 respectively for APTT SP.

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:  
Verification of the normal range for Factor VIII activity was performed. Apparently normal healthy donors were tested for Factor VIII activity during field studies at two US sites with the ACL TOP using SynthASil and APTT SP reagents. The results for 20 normals from each site were statistically analyzed for their distribution in order to verify the normal range for Factor VIII activity. The results obtained at each site verified the normal range Factor VIII activity of 50 - 150% when using the HemosIL® Factor VIII deficient plasma.

**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.