510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K042576

B. Purpose for Submission:

To obtain clearance for the Coatest SP FVIII assay, a photometric assay for the determination of factor VIII activity.

C. Measurand:

Factor VIII

D. Type of Test:

Photometric qualitative and quantitative factor determination

E. Applicant:

Instrumentation Laboratory Company

F. Proprietary and Established Names:

Coatest SP FVIII

G. Regulatory Information:

1. Regulation section:

   864.7290

2. Classification:

   II

3. Product code:

   GGP

4. Panel:

81 Hematology

H. Intended Use:

1. Intended use(s):

   Coatest SP FVIII is intended for the photometric determination of factor VIII activity in citrated plasma.

2. Indication(s) for use:

   Coatest SP FVIII is intended for the photometric determination of factor VIII activity in citrated plasma, such as when identifying factor VIII deficiency or monitoring patients on replacement therapy, as well as for potency estimation of FVIII concentrates. For *in vitro* diagnostic use.

3. Special conditions for use statement(s):

   Not applicable

4. Special instrument requirements:

   Not applicable

I. Device Description:

Coatest SP FVIII is a modified version of Coatest Factor VIII (K833892) reformulated to European Pharmacopoeia Standards. Coatest SP FVIII is a photometric assay containing a chromogenic substrate, S-2765, with EDTA added as a preservative, lyophilized bovine factors IXa and X with bovine albumin added as a stabilizing agent. The device also contains calcium chloride, Tris buffer stock solution containing sodium chloride, bovine serum albumin with added antimicrobial in addition to a mixture of highly purified synthetic phospholipids.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Coatest Factor VIII

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

   K833892

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Same as predicate and identify factor VIII deficiency, or monitoring patients on replacement therapy as well as for potency estimation for FVIII concentrates	Photometric determination of FVIII activity in citrated plasma
Reagent: Factor IXa + X & CaCl2	Same as predicate	Bovine factors IXa & X with bovine albumin added as stabilizer
Storage	Same as predicate	2-8° C until expiration
Linearity	Same as predicate	0-150%
Detection Limit	Same as predicate	1% factor VIII

Differences		
Item	Device	Predicate
Reagent: Chromogenic substrate	S-2765 + I-2581 with mannitol and EDTA as a preservative	S-2222 + I2581 with mannitol
Buffer Stock Solution	Increased BSA concentration from 2% to 10% and added antimicrobial (Ciproflaxin) in buffer reagent	Tris buffer containing NaCl and BSA
Phospholipid	Synthetic phospholipids	Porcine brain emulsion

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

L. Test Principle:

In the presence of calcium and phospholipids, factor X is activated to factor Xa by factor IXa. This generation is greatly stimulated by factor VIII, which may be considered as a cofactor in this reaction. By using optimal amounts of Ca^{2+} and phospholipids and an excess of factors IXa and X, the rate of activation of factor X is solely dependent on the amount of factor VIII. Factor Xa hydrolyses the chromogenic substrate S-2765 thus liberating the chromophoric group, pNA. The

color is then read photometrically at 405 nm. The generated factor Xa and thus the intensity of color are proportional to the factor VIII activity in the sample. Hydrolysis of S-2765 by thrombin formed is prevented by the addition of the synthetic thrombin inhibitor, I-2581, together with the substrate.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Manual Method: A precision study was performed with the manual method using HemosIL Normal Control (K021023) for the normal range and HemosIL High Abnormal Control (K021024) diluted with factor diluent (saline) for the low range. Each control level was run in duplicate twice a day over twenty days (n=80).

Statistics calculated according to NCCLS Document EP5-T2

Acceptance Criteria:

	Within Run %CV	Total %CV
HemosIL Normal Control	< 6%	< 9%
HemosIL High Abnormal Control	< 6%	< 9%

Control	n	Mean % FVIII	Within run % CV	Between run % CV	Total % CV
Abnormal	80	14.4	4.3	3.7	5.6
Normal	80	83	3.4	3.8	5.3

Instrument Application: An additional precision study was performed on an ACL 9000 using HemosIL Normal Control (K021023) for the normal range and HemosIL High Abnormal Control (K021024) diluted with factor diluent (saline) for the low range. Each control level was run in duplicate twice a day over twenty days (n=80).

Statistics calculated according to NCCLS Document EP5-T2

Acceptance Criteria:	Within Run %CV	Total %CV
HemosIL Normal Control	< 6%	< 9%
HemosIL High Abnormal Control	< 6%	< 9%

Control	n	Mean % FVIII	Within run % CV	Between run % CV	Total % CV
Abnormal	80	17.3	5.7	1.0	6.3
Normal	80	102	4.7	3.7	7.1

b. Linearity/assay reportable range:

Manual Method: A linearity study was performed using a high FVIII sample (150%) diluted in factor VIII deficient plasma to prepare seven concentrations. Each level was tested in quadruplicate with the manual method.

Acceptance criteria: $R^2 \geq 0.99$

A graph of the results with the manual method show linearity throughout the claimed range in the product insert of 0-150% factor VIII ($R^2 > 0.9911$).

Instrument Application: An additional linearity study was performed using a high FVIII sample (150%) diluted in factor VIII deficient plasma to prepare six concentrations. Each level was tested in quadruplicate on an ACL 9000.

Acceptance criteria: $R^2 \geq 0.99$

A graph of the results from the ACL 9000 show linearity throughout the claimed range in the instrument application sheet of 0-150% factor VIII ($R^2 > 0.9984$).

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

Value assignments for the controls and calibrator were determined in multiple runs using specific lots of reagents and against a House Standard, which is traceable to the 4th International Standard for FVIII/vWF (NIBSC Code: 97/586).

d. Detection limit:

Manual Method: Detection limit testing was performed using factor VIII deficient plasma run in replicates of five with the manual method using both the normal and low ranges. The mean value (n=5) plus three standard deviations were calculated.

NOTE: The low range results support the insert claim of a 1% detection limit given that FVIII deficient plasma runs at 0.0% in the low range.

Mean % FVIII = 0.04 SD = 0.0 Mean + 3SD = 0.04

Instrument Application: Additional detection limit testing was performed using factor VIII deficient plasma run in replicates of five on the ACL 9000 using both the normal and low ranges. The mean value (n=5) plus three standard deviations were calculated.

NOTE: The low range results support the insert claim of a 1% detection limit given that FVIII deficient plasma runs at 0.0% in the low range.

Mean % FVIII = -0.5 SD = 0.1 Mean + 3SD = -0.2

e. Analytical specificity:

Manual Method: Interference testing was performed using the manual method by spiking levels of each interferent into two different factor VIII plasma sample levels and comparing the results against the unspiked sample results. The two factor VIII levels were prepared using: 1) fresh frozen pooled plasma (FFP) for the normal level and 2) FFP diluted with factor VIII deficient plasma for the 30% level. All sample were tested in triplicate (n=3) with a single lot of Coatest SP FVIII reagents.

Acceptance criteria: Recovery of ± 10% of the unspiked sample result

The data support the interference claims in the Coatest SP FVIII product insert for no significant interference by:

- Triglycerides up to 700 mg/dl
- Bilirubin up to 20 mg/dl
- Hemoglobin up to 100 mg/dl
- Unfractionated heparin up to 1.0 IU/ml

The Coatest SP FVIII product insert includes the limitation that hemolyzed samples in the low range should not be analyzed.

Instrument Application: Additional interference testing was performed on an ACL 9000 by spiking levels of each interferent into two different factor VIII plasma sample levels and comparing the results against the unspiked sample results. The two factor VIII levels were prepared using: 1) HemosIL Normal Control (K021023) for the normal level and 2) HemosIL Normal Control (K021023) diluted with factor VIII deficient plasma for the 30% level. All samples were tested in triplicate (n=3) with a single lot of Coatest SP FVIII reagents.

Acceptance criteria: Recovery of ± 10% of the unspiked sample result

The data support the interference claims in the ACL 8000/9000/10000 instrument application sheet for no significant interference by:

- Triglycerides up to 900 mg/dl
- Bilirubin up to 20 mg/dl
- Hemoglobin up to 50 mg/dl
- Unfractionated heparin up to 1.0 IU/ml

The ACL instrument application sheet (low range) for Coatest SP FVIII includes the limitation that hemolyzed samples should not be analyzed.

Lupus anticoagulant: The product insert for the predicate device, Coatest Factor VIII (K833892), states that due to the high dilutions used, there is no underestimation of FVIII activity in samples containing lupus anticoagulant. To verify this claim for the new Coatest SP FVIII, the results were compared from testing 10 lupus anticoagulant samples in duplicate with the new Coatest SP FVIII versus the predicate Coatest Factor VIII using the manual method. The two tests gave statistically equivalent results. The mean %FVIII of the predicate device was 104 with a SD of 23.2 and the mean %FVIII if Coatest SP was 105 with a SD of 21.6.

System Sensitivity:

Manual Method: System sensitivity for the low and normal ranges was calculated with the manual method as the absorbance change for 1% of factor VIII activity using the slope of the standard curve.

ΔA_{405} per 1% of FVIII activity: Low Range = 0.043 & Normal Range = 0.010.

Instrument Application: System sensitivity for the low and normal ranges was calculated on the ACL 9000 as the absorbance change for 1% of factor VIII activity using the slope of the standard curve.

ΔA_{405} per 1% of FVIII activity: Low Range = 0.024 & Normal Range = 0.005.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:
In-House Study-

Manual Method: An in-house method comparison study was performed at Instrumentation Laboratory's facility in Orangeburg, New York, to compare the performance of the new Coatest SP FVIII versus the predicate Coatest Factor VIII (K833892) using the manual method for both tests. The study used 181 citrated plasma samples (106 normal and 75 abnormal), each run in

duplicate.

Samples reporting outside the tests' ranges were diluted and reanalyzed according to manufacturers' instructions. No artificially prepared samples were used in the study. The samples ranged in value from 1.2 to 588% factor VIII.

The clinical breakdown of the abnormal patient samples (obtained from CliniSys Associates in Atlanta, Georgia) is as follows:

Abnormal Sample Type	Quantity
Low Factor VIII	17
vWF Disease	10
Heparin Therapy	10
Oral Anticoagulant Therapy	10
Lupus Anticoagulant	10
Liver Disease	9
High Factor VIII	9

Acceptance criteria: Slope: 0.90-1.10 r: > 0.95

The results of running Coatest SP FVIII versus the predicate, Coatest Factor VIII, show a correlation of 0.987 and a slope of 1.09, indicating that the performance of the factor assays is statistically similar.

Instrument Application: An additional in-house method comparison study was performed at Instrumentation Laboratory's facility in Milan, Italy, to compare the performance of the new Coatest SP FVIII on an ACL 9000 versus Coamatic Factor VIII (K981038) on an ACL 10000. The study used 90 citrated plasma samples (41 normal and 49 abnormal), each run in duplicate.

Samples reporting outside the tests' ranges were diluted and reanalyzed according to manufacturers' instructions. The samples in the low range were obtained by dilution with Factor Diluent (saline). No spiked samples were used in the study. The samples ranged in value from 1.6 to 217% factor VIII.

The clinical breakdown of the abnormal patient samples (obtained from Sacco Hospital in Milan, Italy) is as follows:

Abnormal Sample Type	Quantity
Low Factor VIII	28
Oral Anticoagulant Therapy	10
Liver Disease	5

High Factor VIII	6

Acceptance criteria: Slope: 0.90-1.10 r: > 0.95

The results of running Coatest SP FVIII versus the predicate, Coatest Factor VIII, show a correlation of 0.992 and a slope of 1.00, indicating that the performance of the two tests is statistically similar.

Field Site Study-

At A. Bianchi Bonomi, Haemofilia Centre (hemophilia center in Milan, Italy), a method comparison study of the new Coatest SP FVIII performed on an ACL 9000 versus Coamatic FVIII (K981038) performed on an ELECTRA 1600C was conducted using 336 citrated plasma samples (152 normal and 184 abnormal), each run in duplicate.

Samples reporting outside the tests' ranges were diluted and reanalyzed according to manufacturers' instructions. Of the 336 patient samples, 4 were artificially prepared by spiking normal patient samples to heparin concentrations of 0.1, 0.2, 0.4, and 1.9 IU/ml. The samples ranged in value from 1.0 to 373% factor VIII.

The clinical breakdown of the abnormal patient samples is as follows:

Abnormal Sample Type	Quantity
Hemophilia	57
vWF Disease	33
Oral Anticoagulant Therapy	31
Lupus Anticoagulant	22
Heparin Therapy	10
Liver Disease	13
DVT	10
Normal Sample Spiked with Heparin	4
Factor VII Deficient	2
Factor IX Deficient	2

Acceptance criteria: Slope: 0.90-1.10 r: > 0.95

The results of running Coatest SP FVIII on an ACL 9000 versus Coamatic FVIII (K981098) performed on an ELECTRA show a correlation of 0.990 and a slope of 0.98, indicating that the performance of the two tests is statistically similar.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

An in-house study of 181 citrated plasmas (106 normal and 75 abnormal) were performed by manual method. The samples ranged in value from 1.2% to 588% factor VIII.

An additional in-house study of 90 citrated plasmas (41 normal and 49 abnormal) were performed on an ACL 9000. The samples ranged in value from 1.6% to 217% factor VIII.

A field-site study of 336 citrated plasmas (152 normal and 184 abnormal), each run in duplicate were performed on an ACL 9000. The samples ranged in value from 1.0% to 373% factor VIII.

b. Clinical specificity:

Not applicable

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

Stability:

Reconstituted/open vial stability testing at 2-8° C was performed to support the following product insert claims:

- S-2765 + I-2581: Reconstituted substrate is stable 3 months at 2-8° C
- Cacl2: Opened vial is stable 3 months at 2-8° C
- Buffer, stock solution: Opened vial is stable 3 months at 2-8° C
- Phospholipid: Opened vial is stable for 3 months at 2-8° C
- Factor Reagent (IXa + X): Aliquotted for -20° C for 3 months

For three different lots of Coatest SP FVIII reagents, vials of each component (Buffer, Phospholipids, Calcium, Factor Reagent and Substrate) were pooled, tested at time zero and stored at 2-8° C (-20° C for Factor IXa + X) for the duration of testing.

HemosIL Normal Control and HemosIL High Abnormal Control were tested in triplicate using the manual test at the following time intervals (days): 0, 7, 14, 21, 28, 42, 56, 70, 90, and 120. The data indicate that the reagents are stable for the times specified in the product insert.

Working Factor Reagent Stability:

The Working Factor Reagent is a mixture of Phospholipid Reagent and Factor Reagent (phospholipids + factor IXa + factor X reagent). Stability testing of the Working Factor Reagent was performed to support the new claim added to the product insert for 12 hours on ice.

Using three different lots of Coatest SP FVIII reagents, the Working Factor Reagent was prepared per the insert instructions and placed on ice for the duration of testing. At each time interval (hours: 0, 2, 4, 8, 12, 24, 48), HemosIL Normal Control and HemosIL High Abnormal Control were tested in triplicate using the manual test. The data indicate that the Working Factor Reagent is stable on ice for a minimum of 12 hours.

Shelf-life Stability:

A shelf-life stability study is ongoing at 2-8° C using three different lots of Coatest SP FVIII reagents. At each time interval (Day 0, 3 months, 6, 9, and 12 months), HemosIL Normal Control and HemosIL High Abnormal Control were tested in duplicate using the manual method. The results to date support a shelf-life stability of 12 months.

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

121 citrated plasmas samples from healthy donors were tested in duplicate with Coatest SP FVIII using the manual method. The 95% reference intervals were calculated as recommended by NCCLS Document C28-A.

152 citrated plasmas samples from healthy donors were tested in duplicate with Coatest SP FVIII on the ACL 9000. The 95% reference intervals were calculated as recommended by NCCLS Document C28-A.

	Manual Method	Instrument Application
Mean	87.3	102.2
SD	19.3	23.4
95% Reference Interval	48.6 to 126.0	55.4 to 148.9

N. Proposed Labeling:

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

O. Conclusion:

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