

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

A. 510(k) Number:

k122584

B. Purpose for Submission:

New device

C. Measurand:

Prothrombin Time (PT) and Fibrinogen PT derived

D. Type of Test:

Quantitative

E. Applicant:

Instrumentation Laboratory Company

F. Proprietary and Established Names:

HemosIL® ReadiplasTin

G. Regulatory Information:

1. Regulation section:

21 CFR §864.7750, Prothrombin time test
21 CFR §864.7340, Fibrinogen determination system

2. Classification:

Class II

3. Product code:

GJS, Test, Time, Prothrombin
GIS, Test, Fibrinogen

4. Panel:

81 Hematology

H. Intended Use:

1. Intended use(s):
HemosIL® ReadiPlasTin is an in vitro diagnostic thromboplastin reagent, based on recombinant human tissue factor, for the quantitative determination, in human citrated plasma, of Prothrombin Time (PT) and Fibrinogen, on the ACL TOP® Family of analyzers. The product is intended to be used for the evaluation of the extrinsic coagulation pathway and the monitoring of Oral Vitamin K Antagonist Therapy.
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 700, ACL TOP 700 CTS, and ACL TOP 700 LAS, ACL TOP 500 CTS.

I. Device Description:

The ReadiPlasTin kit includes a liquid liposomal thromboplastin reagent preparation that contains recombinant human tissue factor (RTF) in a synthetic phospholipid blend combined with calcium chloride, buffer and a preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):
HemosIL RecombiPlasTin 2G
2. Predicate K number(s):
k070005
3. Comparison with predicate:

| Similarities | | |
|---------------------|--|---|
| Item | Device: (k122584) HemosIL ReadiPlasTin | Predicate: (k070005) HemosIL RecombiPlasTin 2G |
| Manufacturer | Instrumentation Laboratory Co. | Same |
| Indications for Use | HemosIL® ReadiPlasTin is an in vitro diagnostic thromboplastin reagent, based on recombinant human tissue factor, for the quantitative determination, in human citrated plasma, of Prothrombin Time (PT) and Fibrinogen, on the ACL TOP® | Same |

| Similarities | | |
|---------------------|--|---|
| Item | Device: (k122584) HemosIL ReadiPlasTin | Predicate: (k070005) HemosIL RecombiPlasTin 2G |
| | Family of analyzers. The product is intended to be used for the evaluation of the extrinsic coagulation pathway and the monitoring of Oral Vitamin K Antagonist Therapy. | |
| Test Principle | In the PT test, the addition of the tissue thromboplastin (ReadiPlasTin reagent) to the patient plasma in the presence of calcium ions initiates the activation of the extrinsic pathway. This results ultimately in the conversion of fibrinogen to fibrin, with formation of a solid gel. The Fibrinogen is quantitated (PT-based method) by relating the absorbance or light scatter during clotting to a calibrator. | Same |
| Closed vial storage | 2 - 8°C | Same |

| Differences | | |
|---------------------|---|--|
| Item | Device: (k122584) HemosIL ReadiPlasTin | Predicate: (k070005) HemosIL RecombiPlasTin 2G |
| Kit Composition | <u>Reagent</u> : 5 x 0.5 or 1 mL vials of <i>liquid</i> recombinant human tissue factor, synthetic phospholipids with stabilizers, preservative, and buffer. <u>Diluent</u> : 5 x 9.5 or 19 mL of an aqueous solution of calcium chloride, polybrene and preservative. | <u>Reagent</u> : 5 x 8 or 20 mL vials of <i>lyophilized</i> recombinant human tissue factor, synthetic phospholipids with stabilizers, preservative and buffer. <u>Diluent</u> : 5 x 8 or 20 mL vials of an aqueous solution of calcium chloride, polybrene and a preservative. |
| Open vial stability | 10 days at 2-8°C in original vial or at 15°C on-board the instrument | 10 days at 2-8°C, 5 days at 15-25°C in the original vial or 10 days at 15°C on-board the instrument |
| Sample type | Citrated plasma; 3.2% or 3.8% standardized for a single concentration | Same |

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

CLSI EP5-A2: Evaluation of Precision of Quantitative Measurement Methods; Approved Guidelines-Second Edition

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI EP07-A2: Interference Testing in Clinical Chemistry

CLSI EP9-A2: Method Comparison and Bias Estimation Using Patient Samples

CLSI EP17-A: Protocols for the Determination of Limits of Detection and Limits of Quantitation

CLSI H21-A5, Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays

CLSI H47-A2: One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test

CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

L. Test Principle:

Prothrombin Time- the thromboplastin reagent included in the ReadiPlasTin kit, after mixing with the ReadiPlasTin Diluent, is a liposomal preparation that contains recombinant human tissue factor (RTF), relipidated in a synthetic phospholipid blend. In the Prothrombin Time (PT) test, the addition of the tissue thromboplastin (ReadiPlasTin reagent) to the patient plasma in the presence of calcium ions initiates the activation of the extrinsic pathway. This results ultimately in the conversion of fibrinogen to fibrin, with formation of a solid gel.

Fibrinogen-derived- fibrinogen is quantitated (PT-based method) by relating the absorbance during clotting to a calibrator.

M. Performance Characteristics (if/when applicable):

The following associated controls and calibrators were used in the ReadiPlasTin performance testing:

- HemosIL Normal Control k021023
- HemosIL Low Abnormal Control k021022
- HemosIL High Abnormal Control k021024
- HemosIL Low Fibrinogen Control k033414
- HemosIL Routine Control Levels 1, 2, & 3 k082859
- HemosIL Calibration Plasma k041905
- HemosIL ISI Calibrate k090563
- HemosIL INR Validate k090563

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility studies were performed with three lots of HemosIL ReadiPlasTin on one ACL TOP 500 CTS and two ACL TOP 700 analyzers 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 reproducibility data and acceptance criteria are listed in the following tables.

Table 1- Prothrombin Time

| Sample | Range of sample means (Prothrombin Time sec) | Within-run CV% | Between-run CV% | Within-device CV% | Acceptance Criteria |
|-------------------|--|----------------|-----------------|-------------------|---------------------|
| Normal Control | 11.4 – 12.1 | 0.6 | 0.7-0.9 | 1.1-1.4 | ≤ 3% |
| Low Abn Control | 21.6 – 22.1 | 0.6-1.1 | 0.0-2.7 | 1.2-2.9 | ≤ 4% |
| High Abn Control | 34.8 – 36.2 | 0.5-0.9 | 0.9-1.8 | 1.4-1.9 | ≤ 5% |
| Routine Control 1 | 11.6 – 12.3 | 0.7 | 0.0-1.1 | 0.9-1.6 | ≤ 3% |
| Routine Control 2 | 31.5 – 34.1 | 0.6-1.1 | 0.5-1.9 | 1.4-2.1 | ≤ 4% |
| Routine Control 3 | 48.9 – 57.1 | 0.5-1.1 | 0.8-1.8 | 1.4-2.0 | ≤ 5% |

Table 2- Fibrinogen PT-derived

| Sample | Range of sample means (Fibrinogen-derived mg/dL) | Within-run CV% | Between-run CV% | Within-device CV% | Acceptance Criteria |
|-------------------|--|----------------|-----------------|-------------------|---------------------|
| Normal Control | 285-298 | 1.1-1.2 | 1.4-1.8 | 2.0-2.2 | ≤ 15% |
| Routine Control 1 | 288-298 | 1.0-1.1 | 1.3-2.0 | 2.2-2.5 | ≤ 15% |
| Low Abn Control | 115-122 | 1.4-1.8 | 1.0-1.6 | 1.8-2.4 | ≤ 15% |
| Low Fib Control | 135-136 | 2.9-3.4 | 0.0 | 3.1-3.5 | ≤ 15% |

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

Repeatability for HemosIL ReadiplasTin was performed by testing a three lots of the applicable controls and calibrators with one lot of HemosIL ReadiplasTin on a representative member of the ACL TOP instrument family (ACL TOP 700), for 5 days, 1 run/day, and 4 replicates per run for each sample level (N=20 per level/ instrument/ lot). The within-run CV% for PT seconds were ≤5% and for Fibrinogen PT-derived were ≤15% employing the controls and calibrators. All result met acceptance criteria.

b. Linearity/assay reportable range:

Fibrinogen linearity testing was performed in accordance with CLSI EP06, using one lot of HemosIL ReadiplasTin reagent, on a representative member of the ACL TOP Family (ACL TOP 700). Two samples, a high-fibrinogen donor sample (~800 mg/dL) and a fibrinogen-deficient donor plasma sample (< 60 mg/dL), were used to prepare 10 samples ranging from ~40 mg/dL to ~800 mg/dL. Fibrinogen concentrations were determined by HemosIL Fibrinogen C and were tested against ReadiplasTin in quadruplicate. Theoretical fibrinogen values were calculated as the weighted average of the highest and lowest fibrinogen in the series. Acceptance criteria were as follows: slope, 0.85 – 1.15; $R^2 \geq 0.95$. The results of the study fell within acceptable limits and demonstrated that HemosIL ReadiplasTin is linear within the range of 60 to 700 mg/dL.

Extrinsic factor linearity testing was performed with factor deficient plasma for FII, FV, FVII and FX, using one lot of ReadiplasTin on a representative

member of the ACL TOP Family (ACL TOP 700). For each extrinsic factor, 14 levels of theoretical extrinsic factor % were created from a high patient plasma pool. Results met acceptance criteria (AC):

Table 3- Acceptance Criteria Extrinsic Factor Linearity

| Factor | Range | Slope | AC | R ² | AC |
|--------|----------|--------|----------|----------------|----------------------|
| II | 0.1-166% | 0.9251 | 0.90-1.1 | 0.9990 | R ² >0.90 |
| V | 0.2-158% | 0.9512 | 0.90-1.1 | 0.9993 | R ² >0.90 |
| VII | 0.1-160% | 0.9454 | 0.90-1.1 | 0.9993 | R ² >0.90 |
| X | 0.3-163% | 0.9328 | 0.90-1.1 | 0.9954 | R ² >0.90 |

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

Real Time Stability (closed vial):

The general storage condition for the ReadPlasTin kit is at 2-8°C. Three lots were stored and at periodic times sample vials were removed for stability testing. The lots were tested on an ACL TOP 700 and ACL TOP 500 CTS for the recovery of ReadPlasTin for the plasma controls. Using the mean of the ReadPlasTin values for each control plasma sample at month zero as a baseline, the change (%) for each time point was calculated for PT and PT-based Fibrinogen. Real time stability was tested for 18 months for the first and second lot, and 15 months for the third lot. All the real time stability tests performed were within the established acceptance range. Results for real time stability support a 15-month shelf-life claim of the ReadPlasTin kit when stored at 2-8 °C. Ongoing real-time stability testing will be used to update the shelf-life.

On-board Stability (open vial):

On-board stability testing was performed on 2 different lots of HemosIL ReadPlasTin on a representative member of the ACL TOP Family (ACL TOP 500 CTS). Two lots of ReadPlasTin reagent were placed in the reagent block of an ACL TOP 500 CTS. Each of the control levels listed above was tested for PT and PT-based Fib at 0, 3, 7, 10 & 11 day time points. Each control plasma sample was tested in quadruplicate. Using the mean of the ReadPlasTin values of each control plasma sample on day zero as a base line, the change (%) for each time point was calculated for PT and PT-based Fibrinogen. Acceptable performance was determined if the percentage change in the recovery, relative to the initial baseline value, met the following specifications:

Table 4- Stability Specifications

| Sample | PT(sec) | Fib PT-based (mg/dL) |
|-------------------------------|-------------------------|-------------------------|
| HemosIL Normal Control | baseline mean \pm 10% | baseline mean \pm 15% |
| HemosIL Low Abnormal Control | baseline mean \pm 15% | baseline mean \pm 15% |
| HemosIL High Abnormal Control | baseline mean \pm 15% | NA |
| Low Fibrinogen Control | NA | baseline mean \pm 15% |

The data from both HemosIL ReadiPlasTin lots support an on-board stability of 10 days at 15°C.

Reconstituted stability at 2-8° C:

Vials of two lots of ReadiPlasTin reagent were prepared according to the insert, placed at 2-8°C and taken out periodically to be tested on an ACL TOP 500 CTS. Each of the control levels listed above was tested in quadruplicate for PT and PT-based Fib at 0, 3, 10, 11, 14 and 21 day time points. After testing, the vials of reagent were returned to 2-8°C. Using the mean of the ReadiPlasTin values of each control plasma sample on day zero as a baseline, the change (%) for each time point was calculated for PT and PT-based Fib. Performance of the reagent was judged to be acceptable if the percentage change in the recovery, relative to the initial baseline value met the specification listed in Table 3. The data supports the claim of reconstituted stability for 10 days at 2-8°C.

d. *Detection limit:*
Not applicable

e. *Analytical specificity:*

Interference limits were established for the following substances: Unfractionated Heparin (UHF), Low Molecular Weight Heparin (LMWH), Hemoglobin, Bilirubin, Triglycerides and the antibiotic Daptomycin. An interference study was performed with one lot of ReadiPlasTin on a representative member of the ACL TOP® Family (ACL TOP (base)). Two levels of plasma were used: 1.) a commercially available normal platelet poor plasma pool from 20 or more male and female donors; 2.) a commercially available pool of oral anticoagulant patient samples with an INR 2.0-3.0. The two sample levels were spiked with multiple levels of the indicated interferent and tested in quadruplicate. The data was then compared to the unspiked control result. Performance of the reagent was judged to be acceptable if the ReadiPlasTin reagent met the following recovery specifications:

Table 5- Recovery Specifications

| Assay | Acceptance Limit |
|----------------------------|-------------------------------------|
| Prothrombin Time (PT) | < \pm 10% of the unspiked control |
| Fibrinogen (mg/dL) derived | < \pm 15% of the unspiked control |

The data support the following interference limit claims regarding HemosIL

ReadiPlasTin:

Table 5- Interference Limits

| Assay | Heparin | Hemoglobin | Triglyceride | Bilirubin | Daptomycin |
|--------------------|------------|------------|--------------|-----------|------------|
| PT | ≤1.0 IU/mL | ≤500 mg/dL | ≤1000 mg/dL | ≤50 mg/dL | ≤100 µg/mL |
| Fibrinogen derived | ≤1.5 IU/mL | ≤500 mg/dL | ≤600 mg/dL | ≤50 mg/dL | ≤100 µg/mL |

- f. *Assay cut-off:*
Not applicable

2. Comparison studies:

- a. *Method comparison with predicate device:*

An in-house method comparison study was performed to compare the performance of the new HemosIL ReadPlasTin assay versus its predicate, the RecombiPlasTin 2G assay, on two (2) representative members of the ACL TOP Family (ACL TOP 700 and ACL TOP 500 CTS). Samples outside the linear range or the range for the assay were removed from the data analysis. A breakdown of the 248 samples is detailed in the table below:

Table 6- In-House Method Comparison Sample Distribution

| Sample Type | |
|--|-----|
| Total Samples | 248 |
| Normal | 125 |
| Abnormal | 123 |
| – Oral Anticoagulation Therapy (OAT) | 65 |
| – Factor Deficiency | 25 |
| FII-Deficiency | 7 |
| FV-Deficiency | 6 |
| FVII-Deficiency | 6 |
| FX-Deficiency | 6 |
| – Liver Disease | 5 |
| – Lupus Anticoagulant positive | 5 |
| – Antiphospholipid Syndrome (APS) | 3 |
| – Disseminated Intravascular Coagulation | 4 |
| – Vitamin K disease | 6 |
| – Fibrinogen | 2 |
| Potential Interferents | |
| Unfractionated Heparin (UFH) | 3 |
| Low Molecular Weight Heparin | 4 |
| – Daptomycin Therapy | 0 |

Acceptance criteria were as follows:

PT-INR- slope must fall between 0.90 - 1.10 and $r \geq 0.95$.

Fibrinogen derived- slope must fall between 0.85 - 1.15 and $r \geq 0.95$.

Results and analysis of the in-house study are as follows:

Table 7- In-House Method Comparison Prothrombin Time (sec) – All samples

| | ACL TOP 700 | ACL TOP 500 CTS |
|-----------------------------|---------------------|------------------------|
| Slope (95% CI) Deming | 0.913 (0.907-0.919) | 0.903 (0.896-0.910) |
| Intercept (95% CI) Deming | 0.948 (0.835-1.062) | 1.091 (0.945–1.236) |
| Correlation coefficient (r) | 0.9989 | 0.9981 |
| Total samples | 248 | 248 |
| Outliers | 2 | 1 |
| No clot detected | 1 | 1 |
| Outside test range | 13 | 7 |
| n | 232 | 239 |

Table 8- In-House Method Comparison PT INR - OAT

| | ACL TOP 700 | ACL TOP 500 CTS |
|-----------------------------|------------------------|------------------------|
| Slope (95% CI) Deming | 0.979 (0.961 - 0.996) | 0.937 (0.914 - 0.959) |
| Intercept (95% CI) Deming | 0.026 (-0.020 - 0.072) | 0.166 (0.100 - 0.232) |
| Correlation coefficient (r) | 0.9975 | 0.9955 |
| Total OAT samples | 65 | 65 |
| Outliers | 0 | 0 |
| Outside linear range | 0 | 0 |
| n | 65 | 65 |

Table 9- In-House Method Comparison Fibrinogen PT derived

| Fibrinogen (mg/dL) | ACL TOP 700 | ACL TOP 500 CTS |
|-----------------------------|---------------------|------------------------|
| Slope (95% CI) Deming | 0.946 (0.935-0.958) | 0.975(0.962-0.988) |
| Intercept (95% CI) Deming | -4.3 (-9.1-0.5) | -7.6 (-12.9- -2.2) |
| Correlation coefficient (r) | 0.9955 | 0.9945 |
| Total samples | 248 | 248 |
| No clot detected | 1 | 1 |
| Outside linear range | 6 | 5 |
| n | 241 | 242 |

Additional method comparison studies were conducted between HemosIL ReadiPlasTin and its predicate (HemosIL RecombiPlasTin 2G) at 3 US field sites, using a total of 715 patient samples and consisting of both normal (n = 310) and abnormal samples (n = 405). Of the abnormal specimens, 249 include patients on Oral Anticoagulant Therapy (OAT) and 156 are obtained from patients with various disease states (Table 10). Both normal and abnormal samples were tested contemporaneously using a representative member of the ACL TOP Family (ACL TOP 700).

Table 10- Method Comparison Sample Distribution by Site

| | Site 1 | Site 2 | Site 3 | Total |
|-------------------------------|--------|--------|--------|-------|
| Patient Samples | 255 | 325 | 135 | 715 |
| Normal | 125 | 153 | 32 | 310 |
| Abnormal | 130 | 172 | 103 | 405 |
| Lupus | 8 | 5 | 7 | 20 |
| Liver Disease | 4 | 6 | 2 | 12 |
| DIC | 1 | 3 | 0 | 4 |
| OAT (Warfarin/ Coumadin) | 76 | 90 | 71 | 249 |
| Extrinsic Factor Deficiencies | 27 | 8 | 2 | 37 |
| FII-Deficiency | 6 | 2 | 0 | 8 |
| FV-Deficiency | 7 | 2 | 1 | 10 |
| FVII-Deficiency | 8 | 2 | 1 | 11 |
| FX-Deficiency | 6 | 2 | 0 | 8 |
| Potential Interferents: | | | | |
| Heparins | | | | |
| UFH | 7 | 16 | 14 | 37 |
| UFH+ Coumadin | 0 | 14 | 0 | 14 |
| LMWH | 7 | 9 | 1 | 17 |
| Daptomycin Therapy | 0 | 9 | 6 | 15 |

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

Table 11- Method Comparison Prothrombin Time (sec)

| | Site 1 | Site 2 | Site 3 |
|------------------------|-----------------------|-----------------------|-----------------------|
| Slope, Deming (95% CI) | 0.909 (0.900 - 0.918) | 0.970 (0.962 - 0.978) | 0.889 (0.874 - 0.903) |
| Intercept (95% CI) | 0.45 (0.24 to 0.66) | 0.51 (0.34 to 0.68) | 1.76 (1.40 to 2.12) |
| r | 0.9966 | 0.9972 | 0.9955 |
| Total samples | 255 | 313 | 135 |
| Outlier | 1 | 1 | 0 |
| n | 254 | 312 | 135 |

Table 12- Method Comparison PT INR

| | Site 1 | Site 2 | Site 3 |
|------------------------|------------------------|------------------------|-----------------------|
| Slope, Deming (95% CI) | 0.938 (0.916 to 0.961) | 0.928 (0.892 to 0.963) | 0.914 (0.891 - 0.937) |
| Intercept (95% CI) | 0.138 (0.079 - 0.197) | 0.119 (0.033 - 0.205) | 0.120 (0.060 - 0.181) |
| r | 0.9947 | 0.9838 | 0.9945 |
| Total samples | 76 | 90 | 71 |
| Outlier | 0 | 1 | 0 |
| n | 76 | 89 | 71 |

Table 13- Method Comparison Fibrinogen PT-derived

| | Site 1 | Site 2 | Site 3 |
|------------------------|---------------------|---------------------|-----------------------|
| Slope, Deming (95% CI) | 0.947 (0.936-0.957) | 0.971 (0.961-0.982) | 0.998 (0.978 - 1.019) |
| Intercept (95% CI) | 4.5 (-0.3 - 9.3) | 0.56 (-4.4 - 5.5) | -4.2 (-13.6 - 5.2) |
| r | 0.9964 | 0.9956 | 0.9931 |
| Total samples | 255 | 313 | 135 |
| Outside linear | 18 | 29 | 6 |
| Outlier | 0 | 0 | 0 |
| n | 237 | 284 | 129 |

b. Matrix comparison:

Fresh vs. Once Thawed Samples- Plasma samples (n=245) were collected in accordance with CLSI H21-A5. Samples included both normal (n=140) and abnormal (n=105) samples. Abnormal samples included patients receiving Daptomycin, heparin (LMWH and UHF) and oral anticoagulation therapy (OAT), as well as patients with liver disease. Testing was performed on a representative member of the ACL TOP Family (ACL TOP 700), with the new HemosIL ReadiPlasTin assay. After testing the fresh samples, the samples were stored at or below -65°C for at least 24 hours, prior to being thawed and re-tested. The HemosIL ReadiPlasTin results obtained from once-thawed when compared to those from fresh samples, must meet the following acceptance criteria:

Table 14- Acceptance Criteria Fresh vs. Once Thawed Plasma Samples

| | Slope | r |
|--------------|-----------|--------|
| PT-based FIB | 0.85-1.15 | ≥ 0.95 |
| PT/INR | 0.90-1.10 | ≥ 0.95 |

The regression analysis was performed by plotting the results from once-thawed patient samples against those from freshly drawn patient samples. The results for PT seconds and PT-based Fibrinogen are reported below:

Table 15- Fresh vs. Once Thawed Plasma Samples

| Item | PT (sec) | Fib-PT derived (mg/dL) |
|------------------------|-----------------------|------------------------|
| Slope, Deming (95% CI) | 0.969 (0.961 - 0.977) | 1.023 (1.011 - 1.035) |
| Intercept (95% CI) | 0.39 (0.24 - 0.54) | -8.5 (-14.0 - -2.9) |
| r | 0.9979 | 0.9960 |
| Total | 245 | 245 |
| Outlier | 1 | 2 |
| Out of range | 0 | 19 |
| n | 244 | 224 |

The results demonstrate that HemosIL ReadiPlasTin delivers comparable results on fresh samples and once-thawed samples.

Comparison of 3.2% and 3.8% sodium citrate plasma- Two parallel blood samples were drawn, from a total of 30 ostensibly normal donors, using both a 3.2% and 3.8% sodium citrate sample tube. Plasma from each donor was promptly recovered after centrifugation. A portion of donor plasmas was pooled and depleted of vitamin-K dependent proteins. This pool was used to create a set of abnormal samples with a wide range of PT values. Samples were run in duplicate on an ACL TOP 500 CTS. Acceptance criteria were as follows:

Table 16- 3.2% and 3.8% sodium citrate comparison

| | Slope | Correlation coefficient (r) |
|-----------------------|-------------|-----------------------------|
| Prothrombin Time | 0.90 – 1.10 | ≥ 0.95 |
| Fibrinogen PT-derived | 0.85 – 1.15 | ≥ 0.90 |

The method comparison between samples collected with 3.2% citrate tubes and the same samples collected into 3.8% citrate tubes demonstrated acceptable correlation for PT with a Deming regression slope, intercept and correlation coefficient of 1.071, 0, and 0.9983 respectively. The Deming regression slope, intercept and correlation coefficient for Fibrinogen PT derived were 0.902, 1.4, and 0.9326 respectively.

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):

Extrinsic Factor Sensitivity: To demonstrate extrinsic factor sensitivity, a study was performed to evaluate the performance of the HemosIL® ReadiPlasTin assay and determine the factor level at which the Prothrombin Time rises above the upper limit of the reference interval. Samples were prepared by mixing HemosIL factor deficient plasma with normal pool plasma of known assayed factors to create 11 dilutions. Concentrations ranged from 7% to 110% factor activity. Prothrombin time was determined on each plasma dilution in duplicate on a representative member of the ACL TOP Family (ACL TOP 700). “Factor sensitivity” is defined as the factor level at which PT rises above the upper limit of the reference interval. PT values in seconds (Y-axis) were plotted against % Factor value (X-axis). The cut-off for each factor was determined as the point where the % Factor intersects the upper limit of the reference interval. The assay is sensitive to factors II, V,

VII and X at the following levels: Factor II: 29%, Factor V: 49%, Factor VII: 50% and Factor X: 56%.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

A reference interval study was performed using one lot of HemosIL ReadiplasTin reagent kit on a representative member of the ACL TOP Family (ACL TOP 700). One hundred ninety nine (199) clinical samples (104 male and 95 female) from ostensibly “normal patients” were used in this study. Normal patient samples were tested for Prothrombin time and derived Fibrinogen values. The results were statistically analyzed for their distribution in order to verify the normal range for ReadiplasTin:

Table 17- Reference Range Verification

| Reference Intervals | Lower Limit | Upper Limit |
|--------------------------------|--------------------|--------------------|
| PT (seconds) | 10.2 | 12.9 |
| Fibrinogen -PT derived (mg/dL) | 282 | 553 |

The sponsor labeling indicates ReadiplasTin results were obtained using a specific lot of reagent. Due to many variables which may affect clotting times, each laboratory should establish its own normal range.

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.