

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

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

k110031

**B. Purpose for Submission:**

Clearance of a new device

**C. Measurand:**

Lupus Anticoagulant

**D. Type of Test:**

Dilute venom clotting assay

**E. Applicant:**

Instrument Laboratory Co

**F. Proprietary and Established Names:**

HemosIL® dRVVT Screen

HemosIL® dRVVT Confirm

**G. Regulatory Information:**

1. Regulation section:

21 CFR §864.8950, Russell viper venom reagent

21 CFR §864.5425, Multipurpose system for in vitro coagulation studies

2. Classification:

Class II

3. Product code:

GIR, Reagent, Russell Viper Venom

GGC, Multipurpose system for in vitro coagulation studies

4. Panel:

81 Hematology

**H. Intended Use:**

1. Intended use(s):

The HemosIL dRVVT Screen and HemosIL dRVVT Confirm assays are qualitative in-vitro diagnostic products to aid in the detection of lupus anticoagulants in human citrated plasma by the diluted Russell's Viper Venom method, on the ACL TOP® Family. The HemosIL dRVVT Screen and HemosIL dRVVT Confirm assays are intended to evaluate patients who have unexplained prolonged APTT test results. The HemosIL dRVVT Screen and HemosIL dRVVT Confirm assays should be used in parallel as an integrated test for Lupus Anticoagulant detection.

2. Indication(s) for use:

Same as above

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

For use on IL Coagulation systems ACL TOP Family (ACL TOP Base and ACL TOP 500 CTS).

**I. Device Description:**

HemosIL® dRVVT Screen and Confirm assay uses diluted Russell viper venom to detect lupus anticoagulant. HemosIL dRVVT Screen assays are a lyophilized reagent containing a low concentration of phospholipids, anti-heparin agents, calcium, buffer, stabilizers and a dye. The HemosIL® dRVVT Confirm assay has a higher concentration of phospholipids than the HemosIL® Screen assay. Both assays together are to be used as part of profile to detect lupus anticoagulants. HemosIL dRVVT Screen and Confirm assays are packaged as 10- 2ml vials. HemosIL® LAC assays are the parent assay to the HemosIL dRVVT Screen and Confirm. The assays differ based on current in-house manufacturing and prolonged assay stability claims. dRVVT Screen and Confirm are an integrated test as referenced in the ISTH 2009 committee recommendation for Lupus Anticoagulant Detection.

**J. Substantial Equivalence Information:**

1. Predicate device name(s) and Predicate 510k number(s):

HemosIL® LAC Screen and HemosIL® LAC Confirm (k990302)

2. Comparison with predicate:

<b>Similarities</b>		
Item	Device	Predicate
	HemosIL® dRVVT Screen and Confirm (k110031)	HemosIL® LA Screen and Confirm (k990302)
Intended Use	The HemosIL dRVVT Screen and HemosIL dRVVT Confirm assays are qualitative in-vitro diagnostic products to aid in the detection of lupus anticoagulants in human citrated plasma by the diluted Russell’s Viper Venom method, on the ACL TOP® Family. The HemosIL dRVVT Screen and HemosIL dRVVT Confirm assays are intended to evaluate patients who have unexplained prolonged APTT test results. The HemosIL dRVVT Screen and HemosIL dRVVT Confirm assays should be used in parallel as an integrated test for Lupus Anticoagulant detection.	IL Test LAC Screen and IL Test LAC Confirm are in vitro diagnostic products for the Detection of lupus anticoagulants (a type of phospholipid interfering antibody) in human citrated plasma on IL Coagulation Systems. These tests are indicated for use with patients who have prolonged APTT test of undetermined origin.
Sample Type	Citrated plasma	Same
Test Principle	HemosIL® dRVVT screen is a diluted Russell viper venom method performed in low phospholipids concentrations designed as a screening reagent. HemosIL® dRVVT confirm is a diluted Russell viper venom method performed at high phospholipids	Same

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	concentrations designed to neutralize the LA and correct the clotting time.  Functional Clotting Assay	
Shelf Life (unopened)	2 years	Same

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	HemosIL® dRVVT Screen and Confirm (k110031)	HemosIL® LA Screen and Confirm (k990302)
Reconstituted Stability	15 days @2-8°C	2 days @2-8°C
On Board Stability	3 days @ 15°C	24 hours at 15°C
Manufacturer	DSRV Inc. USA	Instrument Laboratory Co., USA
Test Systems	ACL TOP Family (ACL TOP Base and ACL 500 CTS)	IL coagulation system: [ACL TOP Family, ACL ELITE®/ELITE PRO/8/9/1000, ACL Futura/ACL Advance/ACL Advance, ACL Classic (100-7000)].

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

- CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods, 2nd Edition, 08/20/2004.
- CLSI EP06-A2: Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach, Approved Guidelines, 01/01/2003
- CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline. 2nd Edition, 01/01/2005
- CLSI EP09-A2: Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline. 2nd Edition, 01/01/2002
- CLSI H21-A5: Collection, Transport and Processing Of Blood Specimens for Testing Plasma Based Coagulation and Molecular Haemostasis Assays; Approved Guidelines, 01/01/2008
- CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents: Approved Guideline, 01/01/2009
- CLSI EP28-A3: Reference Range Data: Defining. Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, 09/28/2009

## **L. Test Principle:**

Lupus Anticoagulants (LA) belongs to the group of antiphospholipid antibodies which are directed against negatively-charged phospholipids or complexes between phospholipids and proteins (either  $\beta$ -2-glycoprotein I or clotting factors such as prothrombin). When determined by their ability to prolong phospholipid-dependent tests (APTT, SCT, dRVVT), these antibodies are referred as LA. Patients with LA are at increased risk of clinical complications such as thrombosis and recurrent miscarriages.

The dRVVT Screen and dRVVT Confirm assays are improved dRVVT reagents intended to simplify and standardize the detection of LA in clinical evaluations<sup>1, 3</sup>. The dRVVT Screen assay is poor in phospholipid, making it sensitive to LA. The additional amount of phospholipid (bi-layers) in dRVVT Confirm neutralizes LA to give shorter clotting times.

Russell's Viper Venom, in the presence of calcium, directly activates factor X (in the test sample). The dRVVT Screen and dRVVT Confirm assays are therefore unaffected by contact factor abnormalities, factor VII, VIII and IX deficiencies or inhibitors. Heparin interference up to 1 U/mL is neutralized by polybrene. As a result, dRVVT Screen and dRVVT Confirm are more specific tests for the evaluation of LA than APTT.

dRVVT Screen and Confirm are being used as an integrated test as referenced in the ISTH 2009 committee recommendation for Lupus Anticoagulant Detection.

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

### 1. Analytical performance:

#### *a. Precision/Reproducibility:*

Precision studies were performed on three reagent lots of HemosIL® dRVVT Screen and Confirm analyzing three lots of HemosIL® LA Negative, Weak Pos and Positive controls. Results (clotting time in seconds) were determined from testing two runs per day over 20 days on three ACP TOP family (ACL TOP base and ACL TOP 500CTS) analyzers according to CLSI EP5-A2. The mean, SD and 95% CI were calculated for each analyte. The %CV of the average clotting times was < 5% and was within the acceptance criteria of  $\leq 6\%$  for repeatability and  $\leq 6\%$  for reproducibility. The acceptance criteria are based on the normalized ratio value:

Level 1: HemosIL® Negative control:  $NR \leq 1.10$  (k102552)

Level 2: HemosIL® LA Weak Positive:  $NR \geq 1.25$  and  $< 1.40$  (In-house)

Level 3: HemosIL LA Positive control:  $\geq 1.40$  (k102252)

Summary results of the precision study are as follows:

ACL TOP Family	Mean(Normalized dRVVT Ratio)
LA Negative Control	1.00
Weakly LA Positive	1.35
LA Positive Control	1.77

HemosIL® dRVVT NR

Samples	Within Run %CV			Between Run % CV		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
LA Negative	1.2	2.0	0.8	1.7	2.8	1.9
LA Weak Positive	1.1	0.9	0.6	2.7	2.1	2.0
LA Positive	1.5	0.9	1.1	4.4	3.4	2.5

b. *Linearity/assay reportable range:*

Not Applicable

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

The reconstituted stability of the HemosIL® dRVVT Screen and HemosIL® dRVVT Confirm reagents were assessed by longitudinal studies. With criteria of an age-related trend and a maximum shift of 6% of the zero point clotting time, the predicted reconstituted stability of the HemosIL® dRVVT Screen and HemosIL® dRVVT Confirm reagents are 15 days when reconstituted and stored at 2-8°C, 3 days on board stability when stored at 15°C and up to a 2 year lyophilized shelf life at 2-8°C. (based on accelerated study). Real time stability testing is on-going for the 2 yr shelf life.

d. *Detection limit:*

Not Applicable

e. *Analytical specificity:*

Interference studies were determined on the ACL TOP instrument family line (ACL TOP, ACL TOP 500CTS) for HemosIL® dRVVT Screen and Confirm. Interferents were spiked into 3 levels of pooled normal plasma, weak LA positive plasma and high LA positive plasma at various concentrations with acceptance criteria of  $\leq 15\%$  compared to the un-spiked control results. The maximum concentrations of the study are:

<b>Interferents</b>	<b>Max tolerated concentration</b>
Unfractionated Heparin (UFH)	≤ 1.0 IU/mL
Low molecular weight Heparin (LMWH)	≤ 1.0 IU/mL
Hemoglobin	≤ 200 mg/dL
Bilirubin	≤ 10 mg/dL
Triglyceride	≤500 mg/dL

Clinical samples of patients with known LA and other miscellaneous clinical conditions were analyzed with the following results:

	<b>ACL TOP</b>	<b>ACL 500 CTS</b>
LA Positive	100% 35/35	100% 35/35
Oral Anticoagulant Therapy (OAT)	40% 2/5	40% 2/5
UFH	20% 1/5	20% 1/5
LMWH	0% 0/5	0% 0/5
Disseminated Intravascular Coagulation (DIC )	0% 0/5	0% 0/5
Factor Deficiency	0% 0/6	0% 0/5

*f. Assay cut-off:*

A normalized ratio cut-off was established in-house using 40 normal patients and validated by random selection of 115 samples and calculating the mean +3SD, with the following results:

<u>System</u>	<u>dRVVT NR</u>
ACLTOP Base	>1.2
TOP 500CTS	>1.2

2. Comparison studies:

*a. Method comparison with predicate device:*

Method comparison study was conducted internally on 115 samples (80 normal/35 LA positive) and over 3 three field sites on 409 patient samples (271 normal, 138 positive) using HemosIL® dRVVT Screen HemosIL® dRVVT Confirm and HemosIL® LAC Screen, HemosIL LA Confirm on ACL TOP Family analyzers (ACL TOP and ACL TOP 500) .

Each sample was classified as being positive or negative for LA for each of the reagents (assays) by calculating the normalized ratio. Percent agreement with corresponding 95% confidence intervals is reported as:

**Internal in-house study:**

<b>Instrument</b>	<b>PPA</b>	<b>NPA</b>	<b>95% CI</b>
ACL Base	100% (35/35)	100% (80/80)	90.1-100.0%
ACL 500 CTS	100% (35/35)	100% (80/80)	95.4-100.0%

**Over 3 Field Sites:**

	<b>Site 1</b>	<b>Site 2</b>	<b>Site 3</b>	<b>Specifications</b>
PPA	92.7% (38/41)	90.2% (46/51)	98.1%(52/53)	≥80%
Confidence Interval	80.6 - 97.5%	79.0 - 95.7%	90.1 - 99.7%	
NPA	98.9% (91/92)	98.9% (91/92)	100% (80/80)	≥80%
Confidence Interval	94.1 - 99.8%	94.1 - 99.8%	95.4 - 100%	
Overall Agreement	97% (129/133)	95.8% (137/143)	99.2% (132/133)	≥80%

*b. Matrix comparison:*

A study comparing 3.2% versus 3.8% citrated plasma was performed. Two blood samples were drawn, from 26 donors, using both a 3.2% and 3.8% sodium citrate sample tubes respectively. The plasma from each donor was promptly recovered from the sample by centrifugation, in accordance with the updated 2009 ISTH guideline for Lupus anticoagulant detection. Two pools, with different sodium citrate concentrations, were prepared using the same volume of citrated plasma from each donor. Artificial LA-Positive samples were prepared by spiking with different amounts of  $\beta$ 2gPI antibodies to produce a range of concentrations. A total of 24 pairs of samples were prepared in this manner. The results of the study based on statistical analysis meeting the outlined specifications are as follows:

<i>3.8 v. 3.2% Na Citrate</i>	<i>PPA</i>	<i>CI 95%</i>	<i>NPA</i>	<i>CI 95%</i>
ACL TOP	19/19 -100%	83.2-100%	24/26 (92%)	75.9-97.9%

A fresh vs. frozen study was conducted in which 26 sample from normal patients were spiked with different concentration of  $\beta$ 2gPL antibodies. A set of the 26 samples were maintained at room temperature while another set of the 26 samples were frozen at -64°C for 24 hours and thawed at room temperature. Both sets of samples were tested on the ACL TOP and the test results calculated based the established normalized ratio cutoff. The compared normalized ratio for both sets of frozen ad room temperature samples were expressed as positive and negative result agreement (PPA and NPA) as the following:

<i>Fresh vs. Frozen</i>	<i>PPA</i>	<i>CI 95%</i>	<i>NPA</i>	<i>CI 95%</i>
ACL TOP	28/28 (100%)	87.9% -100%	26/26 (100%)	87.1% -100%

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 normal range study was performed on 120 healthy volunteers determined by screening with a PT and APTT test and analyzed on both ACL TOP Family analyzers (ACL TOP and ACL TOP 500). Results showed the normalized ratio correlates closely within the instrument family:

<b>Instruments</b>	<b>Normalized Ratio</b>
ACL Base TOP	0.92 -- 1.11
ACL TOP 500 CTS	0.91 – 1.13

**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.