Laboratory Validation of a Novel Hexagonal Phase Phospholipid Neutralization Assay for Lupus Anticoagulant Detection

Abstract Presentation Number: PB0675

Colin Douglas, Rachel Clarke, Navya Kesavan, Derek Lamont, Ali Sadeghi-Khomami, Amanda Wood, Karen M. Black
Precision BioLogic Inc., Dartmouth, Nova Scotia, Canada

Presented at ISTH 2020
July 12–14, 2020

PrecisionBioLogic

Background

The hexagonal phase phospholipid neutralization test (HPNT) is an integrated and automatable coagulation assay for lupus anticoagulant (LA) detection. The assay measures shortened activated partial thromboplastin time (APTT) in the presence of excess hexagonal phase phospholipid and involves both a screening ("Start") and confirmatory ("Correct") assay.

Aim

The goal of this work is to characterize the performance of our HPNT (Hex LA) **on three different automated coagulation analyzers** manufactured by Diagnostica Stago (STA-R Evolution), Siemens Healthcare (BCS XP), and Instrumentation Laboratory (ACL TOP CTS).

Methods

Precision was determined through either a 20 x 2 run x 2 replicate or a 5 day x 2 run x 2 replicate study, performed on a single instrument by a single operator. Lupus anticoagulant negative (cryo*check*™ Lupus Negative), weak (cryo*check* Weak Lupus Positive) and strong (cryo*check* Lupus Positive) controls were measured repeatedly.

On board stability of the HPNT reagent set was measured by repeated testing of three levels of control (vide supra) using the same set of reagents over nine hours.

Interference from common coagulation interferents was tested by spiking an LA negative and LA positive plasma with either the substance (vide infra) or the substance's matrix. The spiked and blank samples were then tested (N=20) and compared for differences in correction.

Reference interval of the assay was measured by using three lots of reagent to test >120 ostensibly normal venipuncture plasma samples collected in 3.2% citrate. The normal range was calculated as the mean correction ± two standard deviations.

We compared our HPNT assay to a similar device on the STA-R Evolution in a **method comparison** study. Two hundred and twenty-six double spun plasma samples in 3.2% citrate were tested by both Hex LA and Staclot LA (Diagnostica Stago) at three distinct sites, and their results compared both qualitatively and quantitatively. To better understand how the assay adapts to optical analyzer platforms, we also measured an additional 50 samples by Hex LA once on each of the available analyzer platforms.

Conclusions

We observed excellent performance and stability of our newly developed HPNT on three common coagulation analyzers. The precision and reproducibility are excellent across lots and the assay results are stable enough to be used without replacement throughout a typical working day.

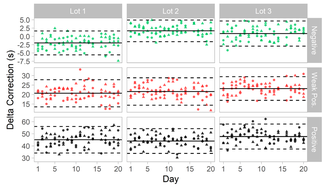
We observed interference in hemolytic LA positive samples, especially on platforms using optical endpoint detections. While the interference is quantifiable, it is not meaningful up to hemoglobin concentrations of 400 mg/dL. At this concentration and higher, patient samples would likely be rejected by visual inspection.

Our HPNT's convenient packaging and frozen format allows labs to cut down on preparation time and reagent spoilage without compromising performance.

Results

STA-R EVOLUTION

Precision



Control	N	Mean Delta	Repeatability SD	Repeatability %CV	Reproducibility SD	Reproducibility %CV
Lupus Negative	240	0.8	1.8	-	2.6	-
Weak Lupus Positive	240	23.2	3.3	14%	3.6	16%
Lupus Positive	250	47.7	5.0	10%	5.5	12%

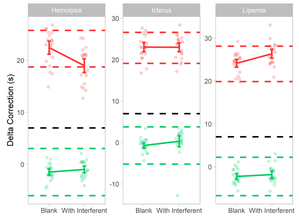
A multi-day single-site precision study was performed using our HPNT on the STA-R Evolution analyzer. An LA negative, weak LA positive, and strong LA positive control was repeatedly measured over 20 days, with 2 runs per day and 2 replicates per run. Means, repeatability and across lot reproducibility are reported in the table. The mean measured value is shown in the figures above as a solid black line, while the dashed lines represent plus or minus two standard deviations.

On Board Stability

	Mean Correction (N = 5)		
	Lupus Negative	Weak Lupus Positive	Lupus Positive
Beginning	0.8	20.7	43.0
2 hours	0.5	20.3	43.0
4 hours	0.1	19.5	41.0
6 hours	-0.1	20.2	43.4
7 hours	0.3	21.0	43.0
8 hours	0.2	21.2	44.7
9 hours	0.0	20.0	44.5

The stability of our HPNT reagents when loaded on the STA-R Evolution analyzer was tested by repeated measurement of three LA controls. The kit produced consistent results up to and beyond eight hours.

Interference



The impact of common coagulation interferents on our HPNT when used with the STA-R Evolution analyzer. **Hemolysis** (at 500 mg/dL hemoglobin), **icterus** (as 20 mg/dL unconjugated bilirubin) and **lipemia** (as 500 mg/dL Intralipid) were tested. Measurements of LA negative plasmas are shown in **green** and measurements of LA positive plasmas in **red**. The colored dashed lines represent the limits of acceptable interference, and the black dashed line denotes the lab- and platform-specific cut off.

Reference Interval

Unique Plasmas	Mean	St. Dev.	Normal Range (2σ)
120	-1.4	1.8	-5.0 – 2.2 seconds

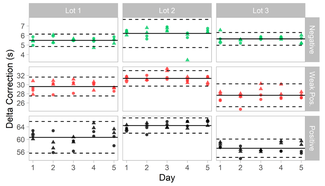
Method Comparison

Comparator Method	Staclot LA on STA-R Evolution
Lab-Specific Cutoff	7 seconds
Unique Plasmas Tested	226
Positive Agreement	122/130 (94%)
Negative Agreement	94/95 (99%)
Overall Agreement	217/226 (96%)
Pearson Correlation Coeff. (*r*)	0.93
Slope vs. Comparator (LA positive only)	1.15

Two hundred and twenty six plasma samples from a mix of LA positive and ostensibly healthy patients were tested by both Hex LA and Staclot LA. There was good agreement between the two assays, both qualitatively and quantitatively.

BCS XP

Precision



Control	N	Mean Delta	Repeatability SD	Repeatability %CV	Reproducibility SD	Reproducibility %CV
Lupus Negative	60	5.8	0.5	-	0.6	-
Weak Lupus Positive	60	29.6	0.9	3%	2.1	7%
Lupus Positive	60	60.7	1.5	2%	4.0	7%

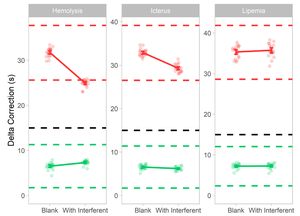
A multi-day single-site precision study was performed using our HPNT on a BCS XP analyzer. An LA negative, weak LA positive, and strong LA positive control was repeatedly measured over 5 days, with 2 runs per day and 2 replicates per run. Means, repeatability and across lot reproducibility are reported in the table. The mean measured value is shown in the figures above as a solid black line, while the dashed lines represent plus or minus two standard deviations.

On Board Stability

	Mean Correction (N = 5)		
	Lupus Negative	Weak Lupus Positive	Lupus Positive
Beginning	6.2	31.8	66.8
2 hours	6.7	32.5	67.0
4 hours	6.5	33.3	69.1
6 hours	6.8	33.3	69.6
7 hours	6.5	33.5	74.2
8 hours	6.8	34.8	73.7
9 hours	6.9	35.1	74.0

The stability of our HPNT reagents when loaded on the BCS XP analyzer was tested by repeated measurement of three LA controls. The kit produced consistent results up to and beyond eight hours.

Interference



The impact of common coagulation interferents on our HPNT when used with a BCS XP analyzer. **Hemolysis** (at 500 mg/dL hemoglobin), **icterus** (as 20 mg/dL unconjugated bilirubin) and **lipemia** (as 500 mg/dL Intralipid) were tested. Measurements of LA negative plasmas are shown in **green** and measurements of LA positive plasmas in **red**. The colored dashed lines represent the limits of acceptable interference, and the black dashed line denotes the lab- and platform-specific cut off.

Reference Interval

Unique Plasmas	Mean	St. Dev.	Normal Range (2σ)
130	4.2	2.3	-0.4 – 8.8 seconds

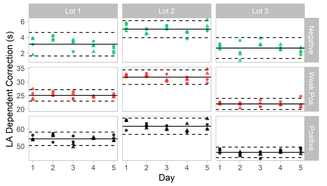
Method Comparison

Comparator Method	Hex LA on STA-R Evolution
Lab-Specific Cutoff	15 seconds
Unique Plasmas Tested	50
Positive Agreement	20/20 (100%)
Negative Agreement	30/30 (100%)
Overall Agreement	50/50 (100%)
Pearson Correlation Coeff. (*r*)	0.99
Slope vs. Comparator (LA positive only)	1.38

Fifty plasma samples from a mix of LA positive and ostensibly healthy patients were tested by Hex LA on both the STA-R Evolution and the BCS XP analyzer platforms. Both platforms produced the same qualitative results, and good correlation was observed between the measured corrections.

ACL TOP CTS

Precision



Control	N	Mean	Repeatability SD	Repeatability %CV	Reproducibility SD	Reproducibility %CV
Lupus Negative	60	3.6	0.5	-	1.4	-
Weak Lupus Positive	60	26.2	0.9	3%	5.2	19%
Lupus Positive	60	54.1	1.7	3%	7.9	15%

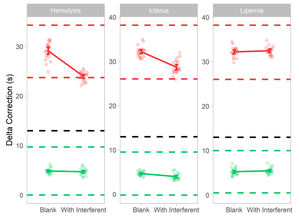
A multi-day single-site precision study was performed using our HPNT on the ACL TOP CTS analyzer. An LA negative, weak LA positive, and strong LA positive control was repeatedly measured over 5 days, with 2 runs per day and 2 replicates per run. Means, repeatability and across lot reproducibility are reported in the table. The mean measured value is shown in the figures above as a solid black line, while the dashed lines represent plus or minus two standard deviations.

On Board Stability

	Mean Correction (N = 5)		
	Lupus Negative	Weak Lupus Positive	Lupus Positive
Beginning	4.3	30.7	60.9
2 hours	6.5	30.2	60.4
4 hours	5.5	32.2	59.8
6 hours	5.1	32.7	63.8
7 hours	5.4	27.5	61.6
8 hours	5.2	30.0	59.1
9 hours	5.2	31.9	62.6

The stability of our HPNT reagents when loaded on the ACL TOP CTS analyzer was tested by repeated measurement of three LA controls. The kit produced consistent results up to and beyond eight hours.

Interference



The impact of common coagulation interferents on our HPNT when used with an ACL TOP CTS analyzer. **Hemolysis** (at 500 mg/dL hemoglobin), **icterus** (as 20 mg/dL unconjugated bilirubin) and **lipemia** (as 500 mg/dL Intralipid) were tested. Measurements of LA negative plasmas are shown in **green** and measurements of LA positive plasmas in **red**. The colored dashed lines represent the limits of acceptable interference, and the black dashed line denotes the lab- and platform-specific cut off.

Reference Interval

Unique Plasmas	Mean	St. Dev.	Normal Range (2σ)
122	4.3	1.3	1.8 – 7.0 seconds

Method Comparison

Comparator Method	Hex LA on STA-R Evolution
Lab-Specific Cutoff	13 seconds
Unique Plasmas Tested	50
Positive Agreement	20/20 (100%)
Negative Agreement	30/30 (100%)
Overall Agreement	50/50 (100%)
Pearson Correlation Coeff. (*r*)	0.99
Slope vs. Comparator (LA positive only)	1.40

Fifty plasma samples from a mix of LA positive and ostensibly healthy patients were tested by Hex LA on both the STA-R Evolution and the ACL TOP CTS analyzer platforms. Both platforms produced the same qualitative results, and good correlation was observed between the measured corrections.