Hex LA Lupus Anticoagulant Test Performance Analysis on Optical Analyzer

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#248

INTRODUCTION

Lupus anticoagulants (LA) result in prolongation and inhibition of phospholipid based clotting time and shortens with additional phospholipid. The hexagonal phase phospholipid neutralization test (HPNT), a silica-based assay, is one of four possible confirmatory tests recommended for LA testing.

OBJECTIVES

We evaluated a new, FDA approved kit for usability and performance against the predicate method on an optical based analyzer.

METHODS

Hex LA (CRYO*check*™ Hex LA™, Precision Biologic, Dartmouth, NS Canada) assays were performed on ACL TOP® Family 700/750 hemostasis instrumentation (Werfen, Bedford, MA).

HPNT Delta (Delta) is calculated by comparing the clot times of patient plasma before (Start) and after addition of hexagonal phase phospholipid (Correct) at optical measurement of 671nm.

Precision studies were conducted on negative, weak, and positive lupus controls.

Accuracy was assessed on waste samples submitted for LA panel testing to our laboratory (n=52) and compared to results of Staclot LA (Staclot® LA 20, Diagnostica Stago, Asnières-sur-Seine, France)(predicate method) performed on ACL TOP® Family 700/750 hemostasis instrumentation (Werfen, Bedford, MA).

Interpretative result reporting:

- Hex LA delta(s): negative: <13.0, positive: ≥13.0
- Staclot LA delta(s) negative: <9.0, positive: ≥9.0

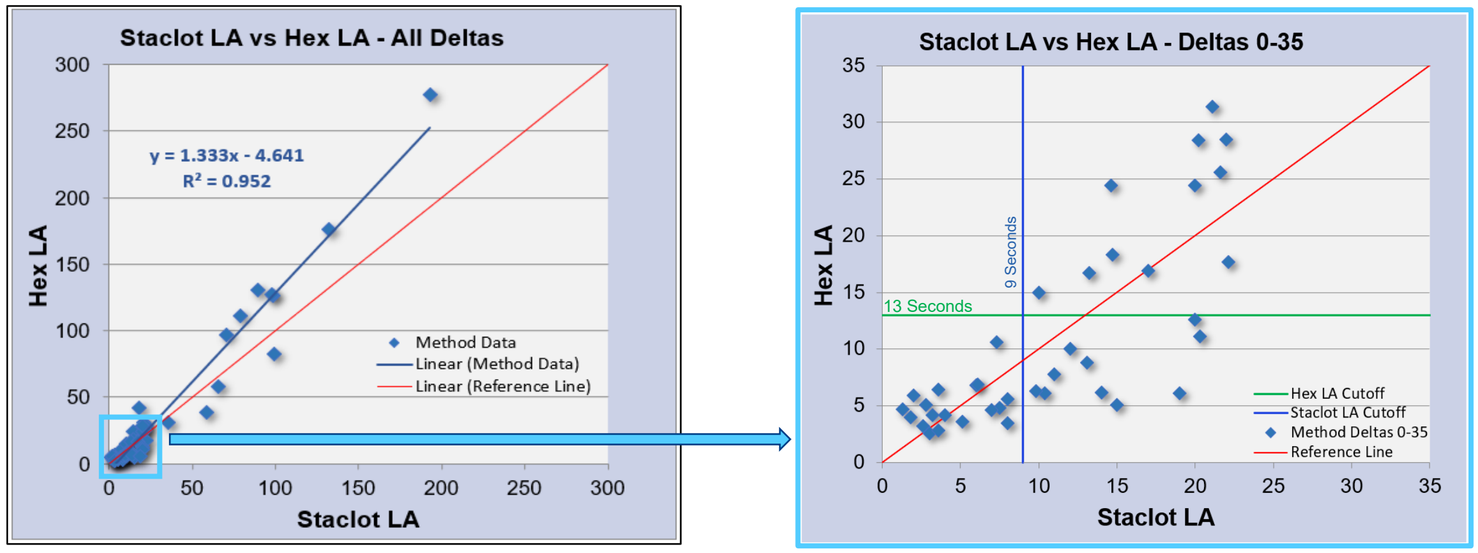
RESULTS

Precision data: Three levels of control material across two lots of Hex LA demonstrated %CV ranging from 2.4 to 6.6 for Start/Correct, SD ranging from 1.2 to 5.6 for Delta.

		Hex LA Lot 010					Hex LA Lot 012		
		(n=80)	Intra (Repeatability)		Inter (Within Lab)		(n=10)	Inter Lot (Within Lab)	
Test	Control	Mean	SD	%CV	SD	%CV	Mean	SD	%CV
Hex LA Start	LN 002	44.9	1.1	**2.5**	1.3	**2.8**	46.3	1.2	**2.5**
	WL 030	93.6	3.0	**3.2**	3.3	**3.5**	104.6	3.8	**3.7**
	LP 6255	124.4	3.8	**3.1**	4.1	**3.3**	138.7	5.3	**3.8**
Hex LA Correct	LN 002	42.2	1.0	**2.4**	1.3	**3.1**	42.5	1.4	**3.3**
	WL 030	57.4	2.7	**4.7**	3.0	**5.2**	67.6	4.5	**6.6**
	LP 6255	65.5	2.4	**3.7**	2.9	**4.4**	78.8	3.8	**4.8**
Delta	LN 002	2.7	**1.2**	*45.8	**1.2**	*46.0	3.9	**1.5**	39.2
	WL 030	36.1	**2.6**	7.1	**2.8**	7.9	37.0	**5.6**	15.1
	LP 6255	58.9	**3.1**	5.3	**3.4**	5.9	59.9	**5.5**	9.2

TABLE 1: Precision of CRYO*check*™ Hex LA™ (LN: lupus negative, WL: weak lupus, LP: lupus positive) *Note: High %CV observed on low numerical delta range of -1.8 – 5.9

Method Comparison: 52 Samples were tested on the TOP Analyzer for Staclot LA and Hex LA.



Figures: Method Correlation (above) and summary of LA interpretative results (below)

	Hex LA Pos	Hex LA Neg	Total
Staclot LA Pos	23	10	33
Staclot LA Neg	0	19	19
Total	23	29	52

Interpretative correlation using delta cut off for ACL TOP yielded:
100% Positive Agreement
66% Negative Agreement
81% Overall Agreement

DISCUSSION

Ten samples had positive Staclot LA and negative Hex LA

- 8/10 were not suggestive of LA based on aggregate interpretation of the LA panel.
- 2/10 had borderline/positive IgM, and undetectable IgG anticardiolipin antibody, undetectable IgM and IgG anti-beta 2 glycoprotein I antibodies, one consistent with LA by two other paired test systems, the other not. Both had Hex LA deltas above manufacture normal range, but not elevated above laboratory verified interpretative cutoff.
- Lack of a gold standard test for LAC poses a challenge in evaluation for LAC.

CONCLUSIONS

In our assessment, the Precision Biologic Hex LA kit was user friendly with preparation expediated by thawing instead of reconstituting of products.

There are fewer kit components, with normal pool plasma added to the start and correct reagents.

The kit demonstrated acceptable precision within and across lots. Accuracy assessments demonstrate good positive predictive value to the predicate method. Sensitivity and specificity was favorable, based on the panel of LA results.

More observations and cautious interpretation are required in weakly positive LA.