Characterizations of fresh frozen controls plasmas CRYOCheck™ Lupus Negative (CCLN), weak positive (CCWLP) and positive (CCLP) for the detection and confirmation of the presence of lupus anticoagulant on the STA-R® (Stago) and CS-2400® (Sysmex) machines

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Introduction

Antiphospholipids syndrome is an autoimmune disease, that involves many symptoms; thrombocytopenia, thrombosis, stroke and obstetric accidents. This disease is due to the presence of heterogeneous immunoglobulins which binds phospholipids and/or proteins associated with the cell membrane which binds phospholipids in the plasma. These Immunoglobulins are named lupus anticoagulants (LA) and cause hypercoagulability state. The determination of the presence of LA is mainly based on coagulation assays which is dependent on phospholipids. These assays are the dRVVT (diluted Russell's viper venom time), the lupus sensitive activated partial thromboplastin time (aPTT), mixing- or integrated-assays, which include determination and confirmation assays of LA. Using controls to validate all results is a mandatory prerequisite, regardless of the method or the system used. For this purpose, that we have characterized 3 fresh frozen plasmas controls.

Materials and methods

Different batches of plasmas of Precision Biologic Inc society (Canada) with strong (CCLP), low (CCWLP) concentrations of LA or without LA (CCLN) are characterized on STA-R® Evolution (Stago, France) and CS-2400® (Sysmex, France) systems. All assays recommended by ISTH, have been tested on the positive plasmas and compared to the negative control.

CCLN : CRYOcheck™ Lupus Negative Control
CCWLP : CRYOcheck™ Weak Lupus Positive Control
CCLP : CRYOcheck™ Lupus Positive Control

Tests	Reagents	Manufacturers
aPTT	PTT-LA	Stago
	APTT-SP	Werfen
	SIRON-LS	Technoclone
	CEPHEN LS	Hyphen
dRVVT	DRVV Screen	Stago
	DRVV Confirm	Stago
	LA Check™	PBI
	LA Sure™	PBI
	LA-1 (determination)	Siemens
	LA-2 (confirmation)	Siemens
Platelet lysate	PNP	PBI
Pool of plasma	CCN-10	PBI
Buffer LS		Cryopep
Integrated assay	Hex LA™	PBI

Independent screening assays:
➢ aPTT lupus sensitive

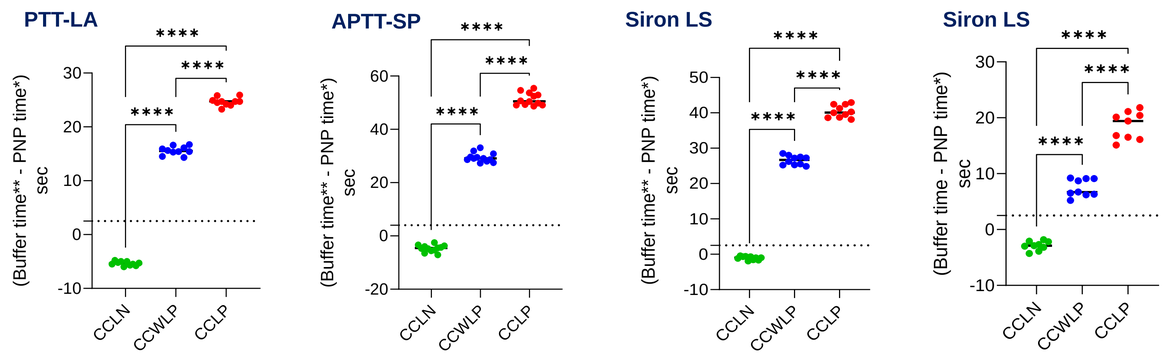
STA-R®		CCLN		CCWLP		CCLP		Positivity threshold (s)	P Value
Reagents	n	Average (s)	SD (s)	Average (s)	SD (s)	Average (s)	SD (s)		
PTT-LA	15	34.04	2.8	59.64	6.3	74.23	12.3	30.4 – 40.4	< 0.0001
APTT-SP	15	31.93	0.44	63.47	3.76	87.81	5.41	30.5 – 35.5	< 0.0001
Siron LS	15	25.92	1.52	48.04	4.26	62.31	5.81	25 - 38	< 0.0001

CS-2400®		CCLN		CCWLP		CCLP		Positivity threshold (s)	P Value
Reagents	n	Average (s)	SD (s)	Average (s)	SD (s)	Average (s)	SD (s)		
CEPHEN LS	9	30.39	0.17	64.29	0.84	83.83	1.69	27 - 36	< 0.0001
Siron LS	9	26.73	1.71	49.88	5.12	65.65	5.41	25 - 38	< 0.0001

Statistics: Ordinary one-way ANOVA: multiple comparisons

Independent confirmation assays:
➢ Platelet neutralization procedure (PNP*)

STA-R® CS-2400®



PNP*: platelet lysate
buffer**: buffer Cryopep LS, SP: Positivity threshold

Statistics: Ordinary one-way ANOVA: multiple comparisons. P < 0.0001: ****

Conclusion

Fresh frozen plasma controls with lupus in weak (CCWLP) or high (CCLP) concentrations of Precision Biologic Inc, have a very good discrimination between the positives controls and the negative control (CCLN) in independent assays on both systems and with all aPTT reagents tested. Similarly, the difference between negative and positive controls remains elevated in dRVVT assays and mixing assays on the STA-R® system. Similar observation has been obtained on the CS-2400® (data not shown). Furthermore, the Hex LA™ test, an integrated assay, is a fast ready to use test, very lupus sensitive and insensitive to the presence of heparin. It includes screening, confirmation and mixing assays in one kit, and shows an excellent discrimination between the three different controls. The results of three comercial controls labelled CE are very similar between both machines and with the different reagents tested. So, these are very good candidates for the validation of LA assay as a quality control on both systems, STA-R® and CS-2400®. The absence of reconstitution of CRYOCheck™ Lupus control plasmas, their immediately using after defrosting and the pertinence with results, add a significant gain for their using as controls in the validation of coagulation assays.

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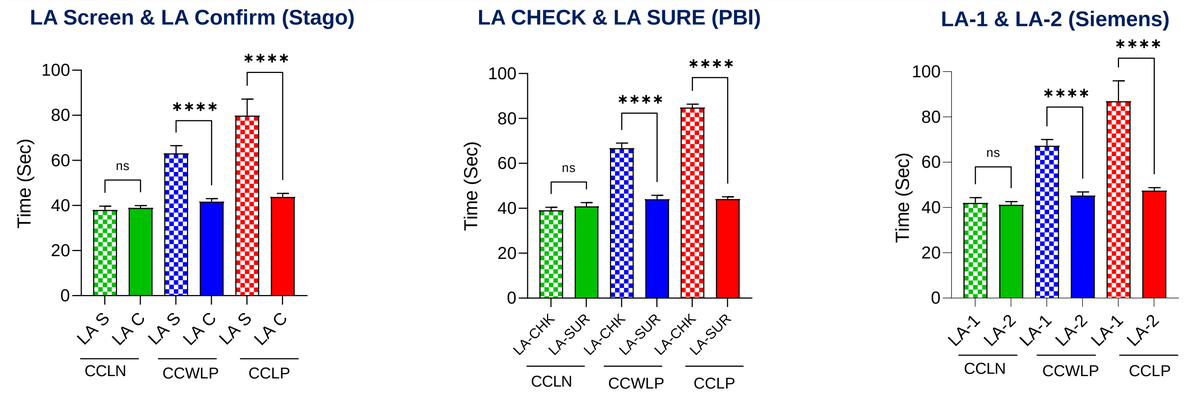
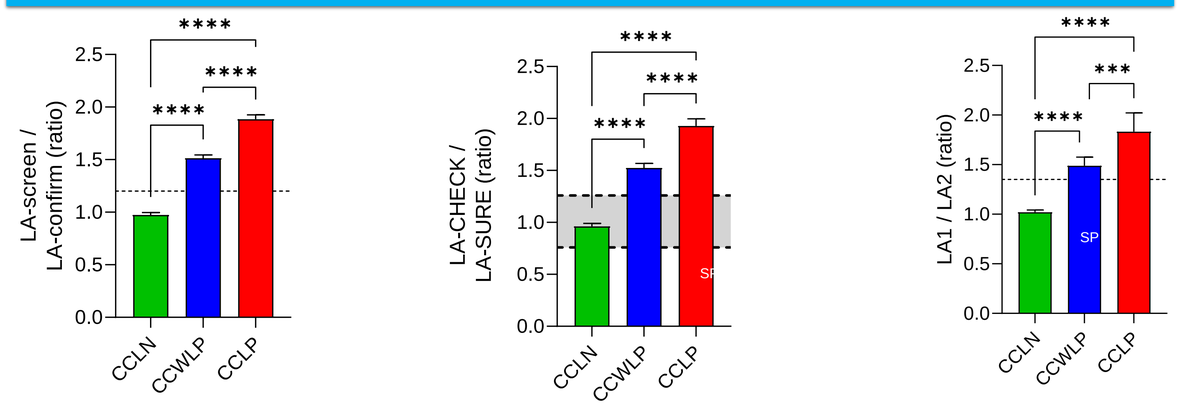
Lupus Anticoagulant tests:
➢ Diluted Russell Viper Venom Time (dRVVT)



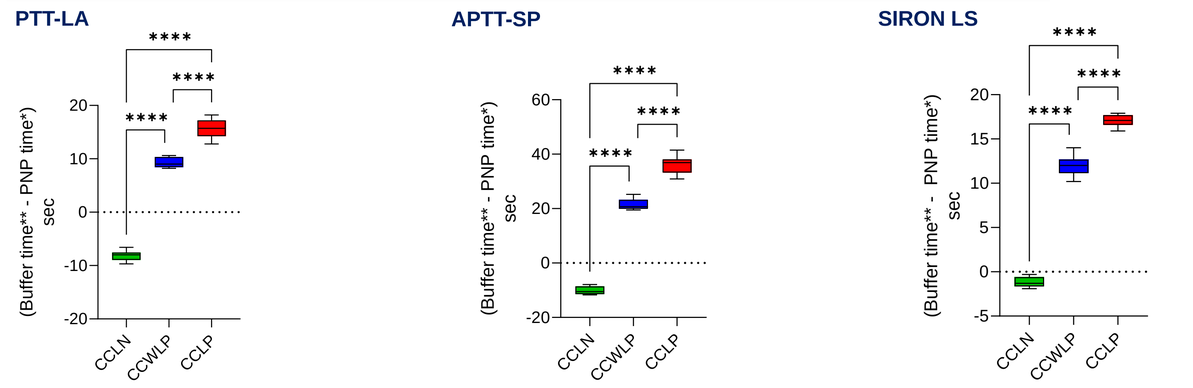
RATIO



Statistics: Ordinary one-way ANOVA: multiple comparisons
P: < 0.0001 : ****; P: 0.0005: ***; ns: non-significant, SP: Positivity threshold

All experiments has been realized on STA-R® system

Mixing tests:
➢ aPTT + PNP + normal pool plasma[1]



*PNP: platelet lysate, buffer**: buffer Cryopep LS
[1] normal pool plasma: 2 different batches of CCN-10 mix vol/vol

All experiments has been realized on STA-R® system

Mixing tests:
➢ dRVVT + normal pool plasma[1]

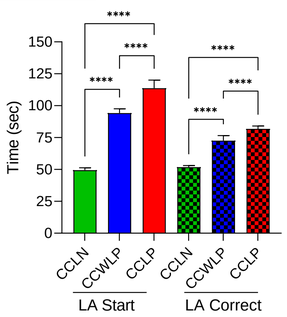
	Ratio Screen / Confirm			Ratio LA-1 / LA-2		
	Average	SD	P Value	Average	SD	P Value
CCLN	1.00	0.03		1.04	0.02	
CCWLP	1.32	0.06	< 0.0001	1.40	0.02	< 0.0001
CCLP	1.45	0.06	< 0.0001	1.54	0.03	< 0.0001

Statistics: Ordinary one-way ANOVA: multiple comparisons.
P: < 0.001: **** (compare to CCLN)

All experiments has been realized on STA-R® system

Integrated assays:
➢ Hexagonal phase phospholipid neutralization assay (HPPNA)

STA-R®		CCLN		CCWLP		CCLP		SP (s)
HEX-LA	n	Average (s)	SD (s)	Average (s)	SD (s)	Average (s)	SD (s)	
LA-START (LA-S)	18	49.46	1.91	94.15	3.40	113.7	6.37	
LA-CORRECT (LA-C)	18	51.84	1.24	72.58	3.97	81.84	2.19	
(LA-S) - (LA-C)	18	-2.39	2.02	31.59	2.1	31.83	7.5	6.66



Statistics: Ordinary one-way ANOVA: multiple comparisons.
P: < 0.0001: ****

SP: Positivity threshold