Current criteria: The need for standardization of assays and reliability of aPL results (LA results APS ACTION)

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APS|ACTION

Background

- Detection of LA is challenging; especially in anticoagulated samples

- APS ACTION - 10 year international prospective study of disease course in aPL positive patients[1,2] (Patients included if tested at participating hospitals for aPL within one year prior to enrolment and fulfil International consensus criteria)[3]

- Five APS ACTION core laboratories worldwide [Padova (Italy), Sao Paulo (Brazil), Galveston (USA), Sydney (Australia), and London (UK)] are performing aPL tests using standard protocols and reagents to confirm the original hospital result

[1]Erkan D et al., *Lupus. 2012 Jun;21(7):695-8;*[2]Barbhaiya M et al., *Curr Rheumatol Rep. 2016 Oct;18(10):64;* [3]Miyakis S et al., *J Thromb Haemost 2006; 4: 295-306.*

Aims

1. **to validate the LA test performance between the five APS ACTION Core laboratories**

2. to examine the degree of agreement in LA status between Core laboratories and local/hospital laboratories contributing patients to the registry

LA Detection

a) based on two different tests with different assay principles

b) a three step procedure, consisting of:

- **Screening** (prolonged clotting time with a LA sensitive phospholipid)
- **Mixing** with normal plasma (failure to correct suggests an inhibitor)
- **Confirmation** (correction using modified phospholipid reagent shows phospholipid dependence)

(Based on recommendations from: ISTH[4,5], BCSH[6], CLSI[7])

[4]Pengo V et al., *J Thromb Haemost 2009; 7: 1737-40;* [5]Devreese KMJ et al., *J Thromb Haemost 2018; 16: 809–13;* [6]Keeling D et al., *Br J Haematol 2012;157:47-58;* [7]CLSI Guideline CLSI document H60-A, 2014. Clinical and Laboratory Standards Institute, Wayne, PA, USA;

Validation of Core Laboratory LA test performance

Five Core laboratories (anonymised A-E in no particular order)

- ACL TOP500 analyser
- Used the same Lot numbers of HemosIL DRVVT Screen/Confirm and HemosIL Silica Clotting Time (SCT) Screen/Confirm reagents

- **Samples:** a) Fresh vials of the 1st International Reference Panel for LA (LA negative (NLA), moderate positive (MLA), and strong positive (SLA) LA)

  b) HemosIL LA Negative (LA-) & LA Positive (LA+) Control plasmas

- Tested on each of three working days
- Results calculated as normalised ratios (using local or commercial pooled normal plasma (PNP), or where this was not available, IL LA negative control plasma)

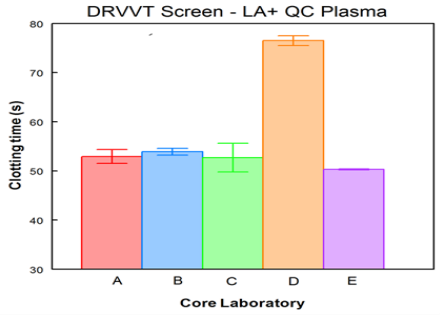
Results - Validation of Core Laboratory LA test performance

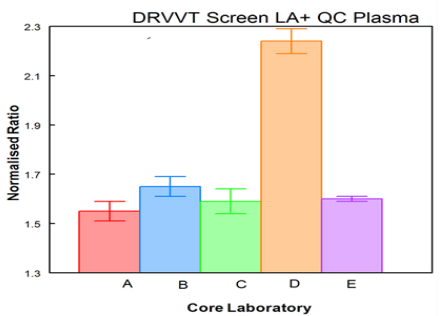
- Clotting times for the PNP used for normalised ratios was very similar between the five Core laboratories (CV <4%)

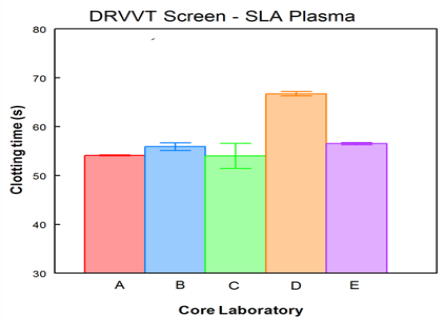
- DRVVT & SCT: Precision and agreement was generally good between all Core laboratories for LA negative and LA positive plasma (all CV ≤5%)

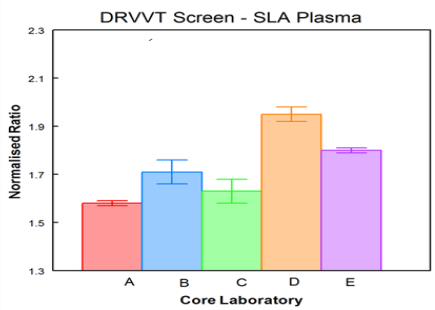
Results - Validation of Core Laboratory LA test performance

DRVVT Screen test: LA+ QC and SLA plasma

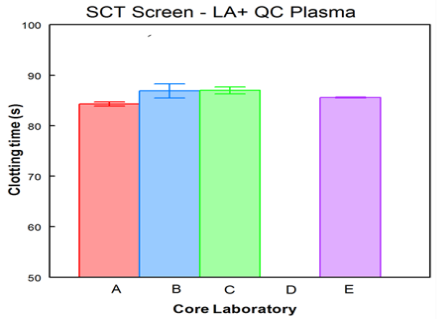


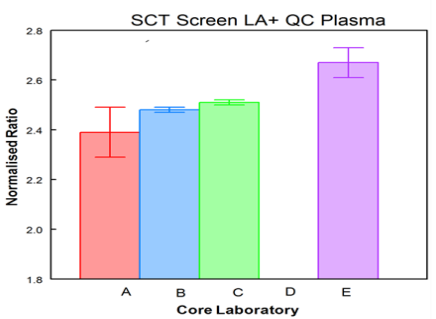


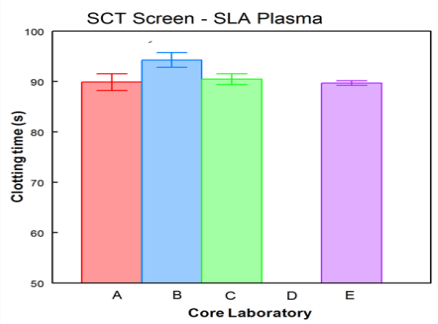


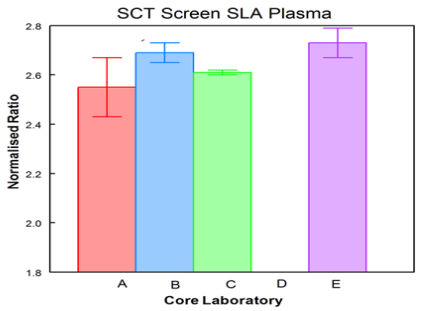


SCT screen test: LA+ QC and SLA plasma









Results - Validation of Core Laboratory LA test performance

DRVVT	LA+QC	NLA	MLA	SLA
Expected LA status:	Positive	Negative	Moderate	Strong
A	1.49	0.96	1.31	1.51
B	1.75	1.08	1.53	1.78
C	1.58	1.01	1.36	1.57
D	2.04	0.95	1.50	1.86
E	1.47	0.93	1.37	1.63

SCT	LA+QC	NLA	MLA	SLA
A	2.30	0.90	1.85	2.21
B	2.41	0.91	1.84	2.25
C	2.40	0.92	1.88	2.19
D	-	-	-	-
E	2.40	0.90	1.97	2.20

- Minor discrepancies disappeared when normalised screen/confirm ratios were calculated
- All laboratories correctly identified LA status, but owing to the lack of SCT data and markedly higher DRVVT ratios in LA positive samples, Core Laboratory D took no further part in the LA studies)

*Average values over three days shown
(Normal cut-off taken as 1.20)

Aims

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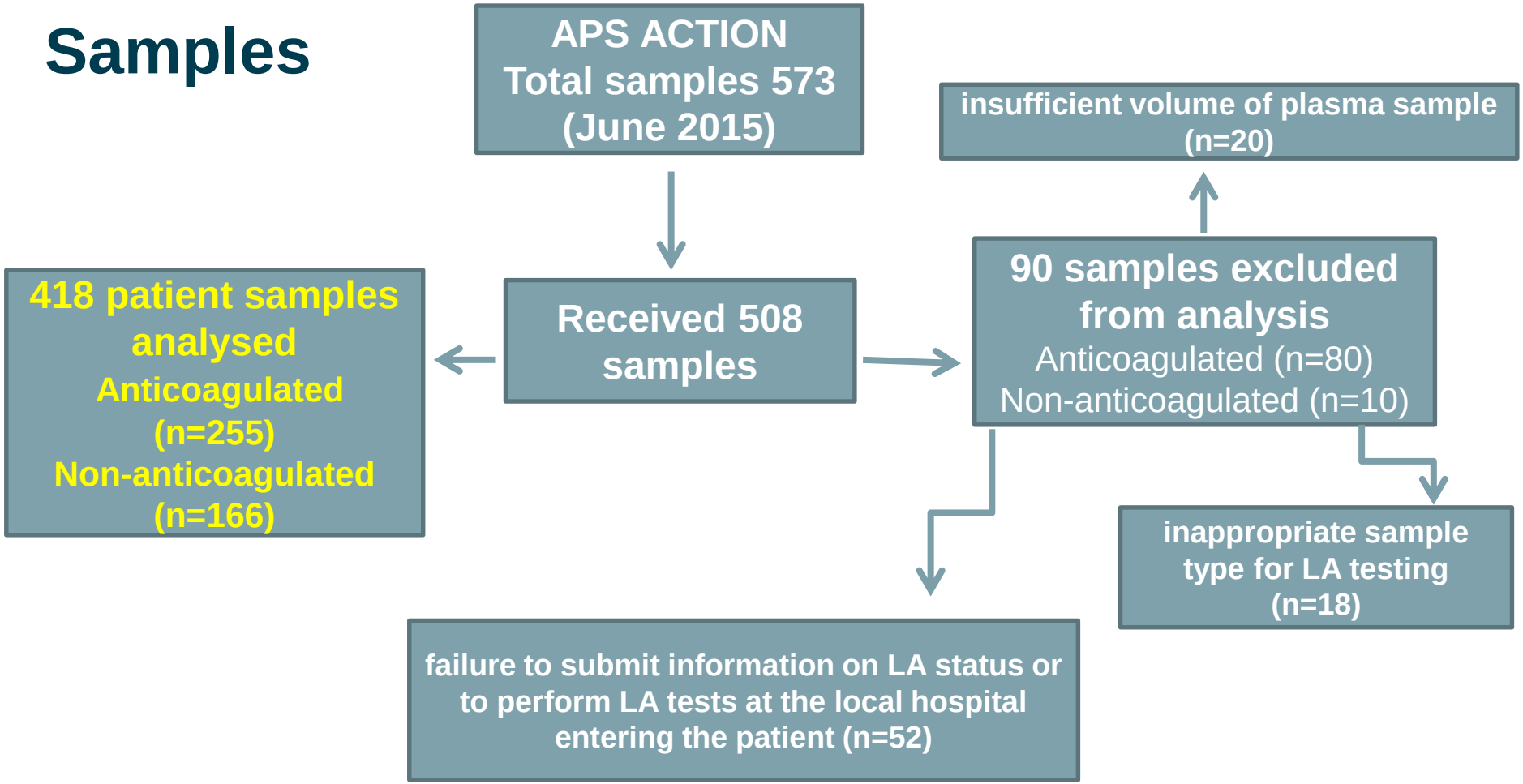
Samples


APS ACTION
Total samples 573
(June 2015)
insufficient volume of plasma sample
(n=20)
418 patient samples
analysed
Anticoagulated
(n=255)
Non-anticoagulated
(n=166)
Received 508
samples
90 samples excluded
from analysis
Anticoagulated (n=80)
Non-anticoagulated (n=10)
inappropriate sample
type for LA testing
(n=18)
failure to submit information on LA status or
to perform LA tests at the local hospital
entering the patient (n=52)

Agreement in LA status between Core and local labs

Non-anticoagulated patients

- 166 patient samples assessed at different Core laboratories
- HemosIL DRVVT and SCT Screen & Confirm reagents, ACL TOP500 analyser

- Equal volume mixtures of patient and normal plasma were tested to confirm the presence of an inhibitor

Agreement in LA status between Core and local labs

Anticoagulated Patients

252 samples from patients receiving anticoagulation

Assessed at a single Core laboratory (UK)

- **224 Vitamin K antagonists (VKA)**
  DRVVT (50:50 patient/normal plasma mixture) & Taipan/Ecarin time
- **6 Rivaroxaban (FXa inhibitor)**
  Taipan/Ecarin Time only
- **18 Low molecular weight heparin (LMWH)***
  DRVVT & Silica Clotting Time
- **4 both VKA and LMWH ***
  (Analysed as for VKA samples)

*prophylactic doses confirmed with anti-Xa assays

Analysis

LA status was considered positive if:

- DRVVT or SCT screen ratio >1.20 and normalised Screen/Confirm ratio >1.20

- TVT prolonged and the normalised TVT/ECT ratio >1.20

  In all tests, evidence of inhibition was provided by testing equal volume mixtures of patient and normal plasma

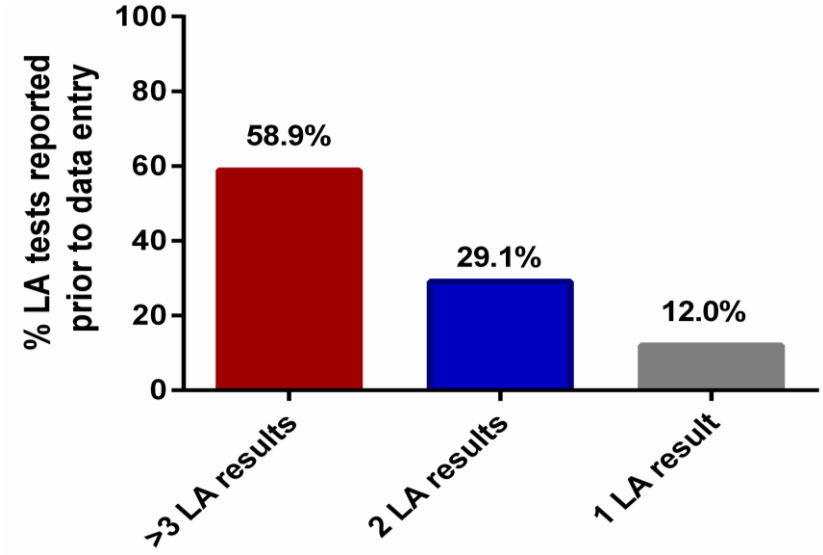
- Results were reported as **Positive**, **Negative** (“**not detected**” for anticoagulated patient samples), or **Equivocal** (where there was no evidence of an inhibitor or suspicion of an underlying coagulopathy)

- Agreement of categorical positive and negative LA status was assessed by κ-coefficients[8] (<0.20 poor; 0.21–0.40 fair; 0.41–0.60 moderate; 0.61–0.80 good; 0.81–1.00 very good) and the Holley and Gilford’s G test with a 99% confidence[9]

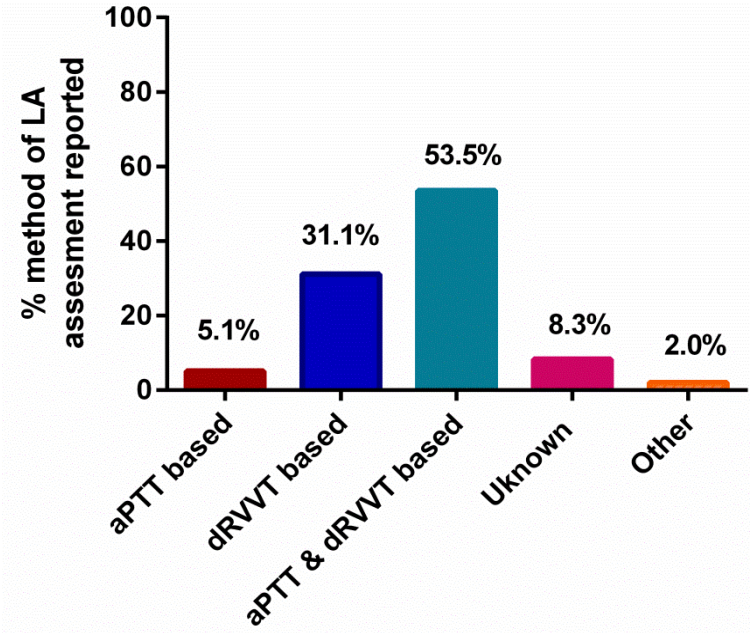
[8]Cohen J et al., Psychol Bull 1968; 70: 213–2; [9]Xu SJ et al.,Consult Clin Phychol. 2014 Dec; 82(6): 1219-27

Summary of results

- **Hospital LA results**



- **Method of LA assessment reported**
(out of 1039 tests recorded)



Summary of results- Non-anticoagulated

- 87.1% agreement between local/hospital and Core Laboratories

		Core Lab				
		POS	**NEG**	**Agreement**	**G-test**	**K Coeff**
Local Lab	**POS**	98	5	115/132	**32.6**	**0.589**
	NEG	12	17	87.1%	>99% confidence	p<0.001

- 17/132 (12.9%) samples discordant

- 34/166 samples (20.5%) were considered Equivocal by the Core Laboratory because an inhibitor could not be demonstrated (21 LAC negative and 13 LAC positive by local/hospital lab)

Summary of results- Non-anticoagulated

Possible reasons for discordant results

	Discordant Local vs Core lab	Equivocal in Core lab
Total number of samples	17	34
Samples tested on only one occasion at local lab	4	10
Tested more than once at local lab, but LA status varied	6	10
Method of LA assessment not specified/only one LA method performed	3	8
Tested ›1 at local lab, with consistent results on LA status	4?	6?

- Based on the above, determination of LA may not be reliable in the local/hospital laboratories in 13/17 discordant and 28/34 equivocal sample results (41/51, 80.4%) – i.e. in 41/166 (24.7%) of non-anticoagulated samples

Summary of results- Anticoagulated

- 77.2% Agreement between the local and Core laboratory

		Core Lab				
		POS	**Not Detected**	**Agreement**	**G-test**	**K Coeff**
Local Lab	**POS**	174	27	183/237 77.2%	**13.9** >99% confidence	**0.206** p<0.001
	NEG/Not Detected	27	9			

- 54/237 samples (22.7%) discordant

- 15/252 samples (6.0%) considered Equivocal by the Core Laboratory since they were negative by DRVVT and an inhibitor could not be demonstrated in the TVT

Summary of results- Anticoagulated

Possible reasons for discordant and equivocal results

	Discordant Local vs Core lab	Equivocal in Core lab
Total number of samples	54	15
Samples tested on only one occasion at local lab	15	4
Tested more than once at local lab, but LA status varied	20	3
Method of LA assessment not specified/only one LA method performed	11	5
Tested ›1 at local lab, with consistent results on LA status	8?	3?

- Determination of LA may not be reliable in the local laboratories in 46/54 discordant samples and 12/15 samples giving equivocal results (58/69, 84%) – i.e. in 58/252 (23%) anticoagulated patients

Conclusions

- Reduced variability and good agreement between laboratories can be achieved by use of same reagent, analyser type, and same protocols

- **87%** (non-anticoagulated samples) and **77%** (anticoagulated samples) agreement in LA status between local/hospital and Core laboratories (excluding equivocal samples)

- Local/hospital results in 80.4% of non-anticoagulated and 84% of anticoagulated discordant/equivocal samples may not be reliable. This accounts for 24.7% (of 166) non-anticoagulated and 23% (of 252) anticoagulated samples

- LA testing of longitudinal samples from annual follow up of patients in the APS Action is ongoing

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