Diagnostic algorithms for lupus anticoagulant detection

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CLSI is a global, not-for-profit, standards-developing organisation that promotes the development and use of voluntary consensus standards and guidelines within the health care community.

Its first guideline for LA testing is due for publication in early 2013.

As it is not yet published, content may be subject to change.

The British Committee for Standards in Haematology (BCSH) is a sub-committee of the British Society for Haematology (BSH).

The primary purpose of the BCSH is to provide haematologists with up to date advice on the diagnosis and treatment of haematological disease by the production of evidence based guidelines using a well defined BCSH process.

The recently published guidelines on antiphospholipid syndrome from the BCSH in 2012 update and replace the previous guideline published in 2000 based on relevant publications since then.

Main issues to be addressed

Pre-analytical	sample manipulation to generate PPP	centrifuge vs filter
	coagulation screen	LA responsive or unresponsive APTT?
LA assays	Screen:	how many tests?
		which ones?
	Mix:	ratio test:NPP
		? necessary
	Confirm:	which principle?
Numbers	clotting time vs ratio:	ratio via NPP or RI mean?
	reference intervals & cut-offs:	97.5th percentile vs 99th percentile
	interpretation of mix:	ICA vs mix specific range
	interpretation of confirm:	various calculations
Interferences	anticoagulant therapy	when CAN you test? How?
		when CAN'T you test?
		effects of new generation anticoagulants
	factor deficiency	how to exclude co-existence
	non-PL dependent inhibitors	

Pre-analytical

Preparation of plasma samples:

Collect blood into 0.109 mol/L trisodium citrate

(Double) centrifugation

Platelet count <10 x 10^9/L

Filtration through 0.2 µm filters or ultracentrifugation not recommended

Samples should not be repeatedly thawed and frozen

Pre-analytical

Preliminary coagulation screen:

Coagulation screen helpful to exclude undiagnosed coagulopathies and anticoagulant treatment

Prothrombin time
APTT
Thrombin time

Further suggests employing LA-unresponsive ‘routine’ APTT

- reduce serendipitous findings of LA in asymptomatic patients
- if normal, can interpret results from ‘LA-responsive’ APTT at face value

LA screening tests

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- 2 tests of different principles/pathways
- dRVVT & LA-responsive APTT preferred 1st line assays
- other assays not excluded as 1st or 2nd line assays

BSH

- dRVVT specifically recommended
- 2nd assay would normally be a suitable APTT
- other assays not excluded

ISTH

- dRVVT & LA-responsive APTT only
- other assays not recommended

dRVVT variation

Pengo V et al. Survey of lupus anticoagulant diagnosis by central evaluation of positive plasma samples. *J Thromb Haemost* 2007; 5: 925-930

Jennings I et al Potentially clinically important inaccuracies in testing for the lupus anticoagulant: an analysis of results from three surveys of the UK national external quality control scheme (NEQAS) for blood coagulation. *Thromb Haemost* 1997; 77(5): 934-37

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Moffat et al. Are laboratories following published recommendations for lupus anticoagulant testing? An international evaluation of practices. *Thromb Haemost* 2009; 101: 178-184

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APTT-based assays

LA-responsive (‘routine’) APTT
Dilute APTT
Kaolin Clotting Time
Silica Clotting Time

LA-responsive

Proven LA sensitivity

Silica activator
Low phospholipid content

Silica activator only?

Jacobsen EM, Barna-Cler L, Taylor JM, Triplett DA, Wisløff F. The lupus ratio test – an interlaboratory study on the detection of lupus anticoagulants by an APTT-based, integrated, and semi-quantitative test. *Thromb Haemost* 2000; 83: 704-708

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KCT not recommended:

poorer reproducibility compared with other available tests

problematic behaviour (of kaolin) in automated coagulometers

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low turbidity, slow settling reagents available

sensitive assay in experienced hands

Personal note:

1:4 dilution in normal plasma dilutes less potent antibodies: loss of sensitivity

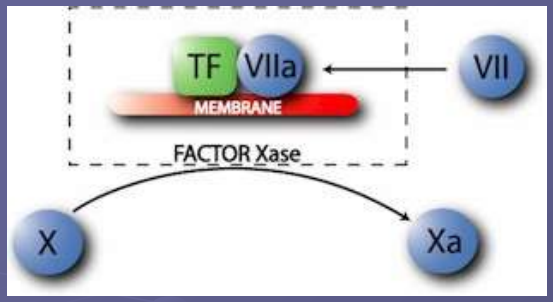
no commercially available PL-dependence confirmatory test: loss of specificity

Just as much variation in APTT reagents

Dilute prothrombin time not recommended because of thromboplastin variability

- UK NEQAS reports reveal that no two laboratories use the same dilutions/procedure
- High sensitivity with recombinant thromboplastin
- Poor specificity without a confirmatory test…….true to varying degrees for all tests

- Standardised kit with screen/confirm recently available & has been subjected to scrutiny
- Clinical experience suggests that dPT detects clinically significant antibodies



Evidence that some LA preferentially manifest in extrinsic pathway-based assays

Liestøl S, Jacobsen EM, Wisløff F. Dilute prothrombin-time based lupus ratio test. Integrated LA testing with recombinant tissue thromboplastin. *Thromb Res* 2002;105:177-182

Mackie IJ, Lawrie AS, Greenfield RS, Guinto ER, Machin SJ. A new lupus anticoagulant test based on dilute prothrombin time. *Thromb Res* 2004;114:673-674

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Martinuzzo M, Adamczuk M, Varela ML, Pombo G, Forastiero R. The activated seven lupus anticoagulant (ASLA) test has comparable sensitivity to classical assays for screening of lupus anticoagulant. *Thromb Haemost* 2005;93:1007-1009

Moore GW, Rangarajan S, Savidge GF. The activated seven lupus anticoagulant assay detects clinically significant antibodies. *Clin Appl Thromb/Haemost* 2008;14:332-337

Assays based on snake venoms fractions

Taipan, Textarin & Ecarin not recommended:

- no standardised commercial assays
- require further critical evaluation

Taipan, Textarin & Ecarin venoms

Triplett DA, Stocker KF, Unger GA, Barna LK. The Textarin/Ecarin ratio: a confirmatory test for lupus anticoagulants. *Thromb Haemost*. 1993; 70: 925-931

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Parmar K, Connor P, Hughes GRV, Hunt B J. Validation of the Taipan snake venom assay in routine practice to assess lupus anticoagulant status in patients being assessed for lupus anticoagulant and not receiving oral anticoagulant. *J Thromb Haemost* 2003;1 Suppl 1 July: abstract number PI553

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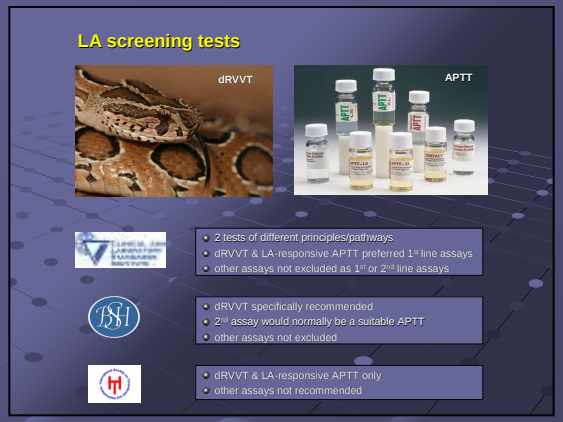
Numbers of screening tests



No single test is sensitive to all LA – use 2 tests of different principles

Risk of false-positive results increased to unacceptable level if >2 tests performed

>2 screening tests may well result in more positive individual screening test **results**

Application of the confirmatory test(s) will not lead to more positive overall **interpretations**

Instead genuine LA that were unreactive in first-line assays may be identified

Some labs perform 3 assays, covering intrinsic, extrinsic & common pathways to minimise this problem

CLSI supports limiting to 2 while cognisant that LA heterogeneity may necessitate additional screening tests

Mixing test

Mixing test unnecessary only if:

(i) LA screen elevated
(ii) Associated confirm test corrects mathematically AND into reference range
(iii) No evidence of other causes of elevated clotting times

Mixing test improves specificity but introduces dilution factor that can mask weak LA

If screen & confirm on undiluted plasma appear positive and no evidence of other causes of elevated clotting times, consider LA positive even if mixing test negative

In principle, integrated tests do not require performance of the mixing test

Evaluate with Index of Circulating Anticoagulant (ICA) or mixing test-specific cut-off

Paradigm shift

Even 1:1 mixing studies can dilute LA to give clotting time/ratio below cut-off

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Male C, Lechner K, Speiser W, Pabinger I. Transient lupus anticoagulants in children: stepwise disappearance of diagnostic features. *Thromb Haemost* 2000; 83: 174-175

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Screen – Confirm - Mix

Confirmatory test for phospholipid dependence

Screen and confirm must be based on the same test principle

Screen and confirm must be based on the same test principle

Not explicit in SSC 2009 but this is an update and it is explicit in SSC 1995

% correction of ratio	$\frac{\text{(screen ratio – confirm ratio)}}{\text{screen ratio}} \times 100\%$
Normalised test/confirm ratio	$\frac{\text{screen normalised ratio}}{\text{confirm normalised ratio}}$

Ratio calculations

Screen & confirm ratios calculated using normal pool plasma clotting time as the denominator

Screen & confirm ratios calculated using RI mean clotting time as the denominator

- Not all NPP generate the same clotting times with different reagents for the same test type

Gardiner C et al. The importance of locally derived reference ranges and standardized calculation of dilute Russell's viper venom time results in screening for lupus anticoagulant. *Br J Haematol.* 2000;111:1230-1235

Jennings I et al. UK National External Quality Assessment Scheme for Blood Coagulation. Lupus anticoagulant testing: improvements in performance in a UK NEQAS proficiency testing exercise after dissemination of national guidelines on laboratory methods. *Br J Haematol.* 2002;119:364-369

- Results from NPPs taken into different sample tubes &/or lyophilised may not correlate with local patient samples

Hirst CF, Poller L. The cause of turbidity in lyophilised plasmas and its effects on coagulation tests. *J Clin Pathol* 1992; 45: 701-703

De Laat B et al. An international multicentre-laboratory evaluation of a new assay to detect specifically lupus anticoagulants dependent on the presence of anti-beta2-glycoprotein autoantibodies. *Thromb Haemost* 2011;9:149–53

- Local RI mean negates variability between different NPP preparations or different batches of a given NPP

Moore GW et al. Lupus anticoagulant detection: out of control? *Int Journal Lab Haematol* 2012: Sep 17. doi: 10.1111/ijlh.12006. [Epub ahead of print]

Comparison of NPP mean clotting times

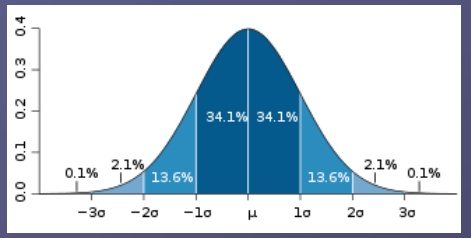
	DRVVT screen	DRVVT confirm	DAPTT screen	DAPTT confirm
CRYO*check*™ frozen normal pool mean (s)	44.0	37.8	36.0	42.8
Locally prepared normal pool mean (s)	44.8	34.8	38.1	40.3
Technoclone lyophilised platelet poor plasma mean (s)	47.4	35.9	42.8	46.8
Reference interval mean (s)	43.8	37.6	41.4	45.9

CRYO*check*™ frozen normal pool virtually identical to RI means for DRVVTs

Technoclone lyophilised platelet poor plasma closest to RI means for DAPTTs

Moore GW et al. Lupus anticoagulant detection: out of control? *Int Journal Lab Haematol* 2012: Sep 17. [Epub ahead of print]

Cut-off values



Aligns with CLSI C28-A3 How to define and determine reference intervals in the clinical laboratory

Clotting assays, including APTT, dRVVT & dPT have Gaussian distributions (parametric appropriate)

≥40 donors & calculate mean ±2SD

Will generate 2.5% tails but composite LA testing not just screen result reveals whether LA present or not

Reference intervals can be established by transference

Cut-off values should be specific for reagent/analyser pairing

Cut-offs may be available from manufacturer but local validation advised

Historically: mean + 2SD (97.5th centile)

99th centile (mean + 2.3SD) would improve specificity (but reduce sensitivity)

Large numbers of suitably prepared normal donors needed to estimate 97.5th or 99th with accuracy

Previously established cut-offs (manufacturer or different analyser); validate with fewer donors(20 – 60)

Cut-off values should be specific for reagent/analyser pairing

Do not use cut-offs from elsewhere

99th centile from at least 40 donors

LA testing during therapeutic anticoagulation

Most patients can wait for LA testing until the period of anticoagulation is complete

VKAs

Utility of testing undiluted plasma is disputed

Perform screen & confirm on 1:1 mixtures with NPP

Positive result is diagnostic but negative result does not exclude a weak LA

TSVT + Ecarin time or platelet neutralisation procedure useful secondary testing

No limits placed on INR values

Result interpretation is difficult because of prolonged basal clotting times

Recommend testing 1 - 2 weeks after discontinuation of treatment or when INR <1.5

If INR 1.5 - 3.0, consider 1:1 mixing studies; interpretation may be difficult & titre diluted 2-fold

Textarin / Taipan / Ecarin testing not recommended as they require further critical evaluation

UFH

CLSI gives examples of when LA can be detected

BCSH recommends not to perform LA testing

Summary

	CLSI	BSH
Sample preparation	Double centrifugation	
Testing order	Screen –Confirm-Mix	Screen –Confirm-Mix (implied)
Assays	DRVVT & APTT &/or others	DRVVT plus APTT or others
Ratios	RI mean denominator	NPP denominator
Reference interval/cut-offs	2SD of the mean	
PL-dependence calculations	% correction of screen ratio by confirm ratio or Screen normalised ratio/confirm normalised ratio	
Testing on VKAs	Screen & confirm on 1:1 mixture with NPP TSVT + ET or PNP	
Testing on UFH	Can detect LA in some cases	Not recommended
Interpretive reporting	Recommended	

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