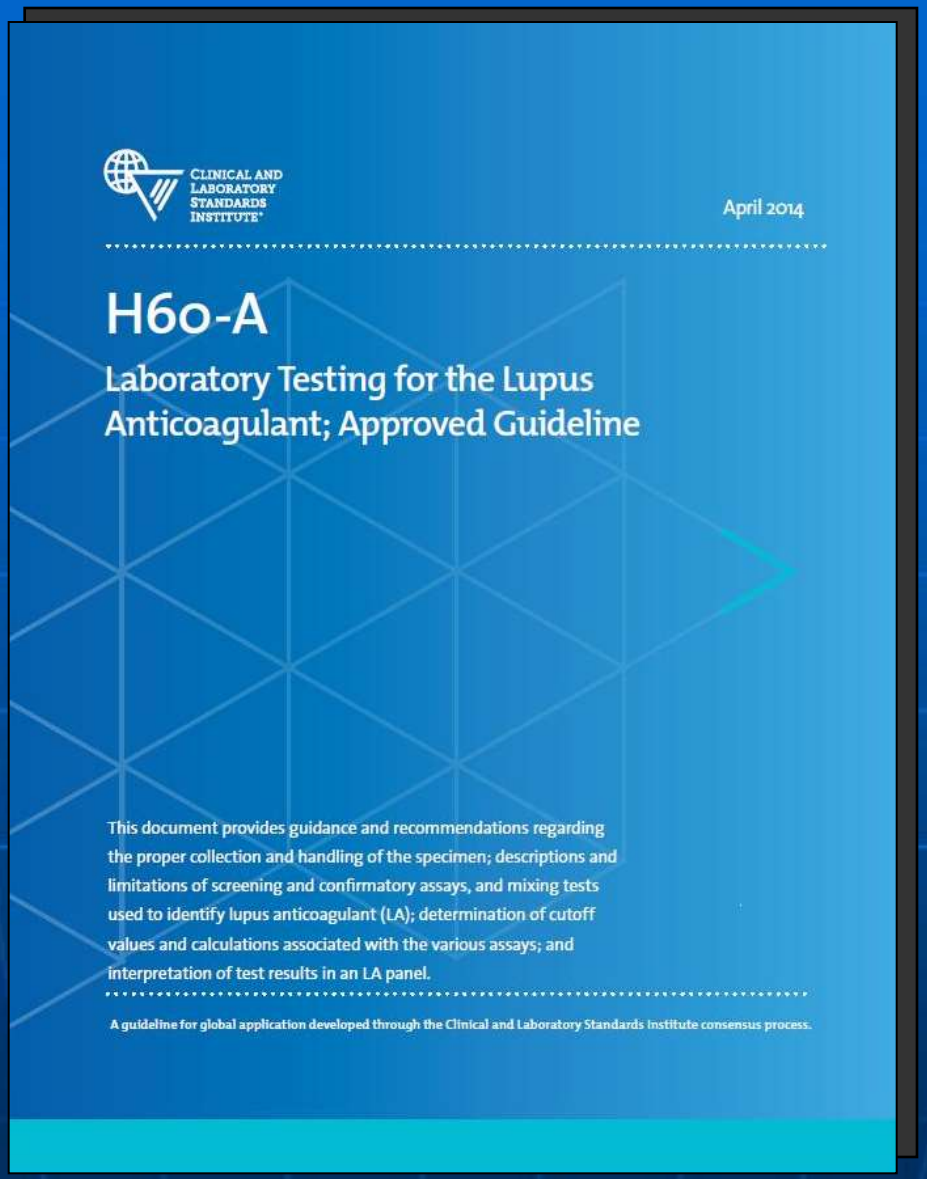
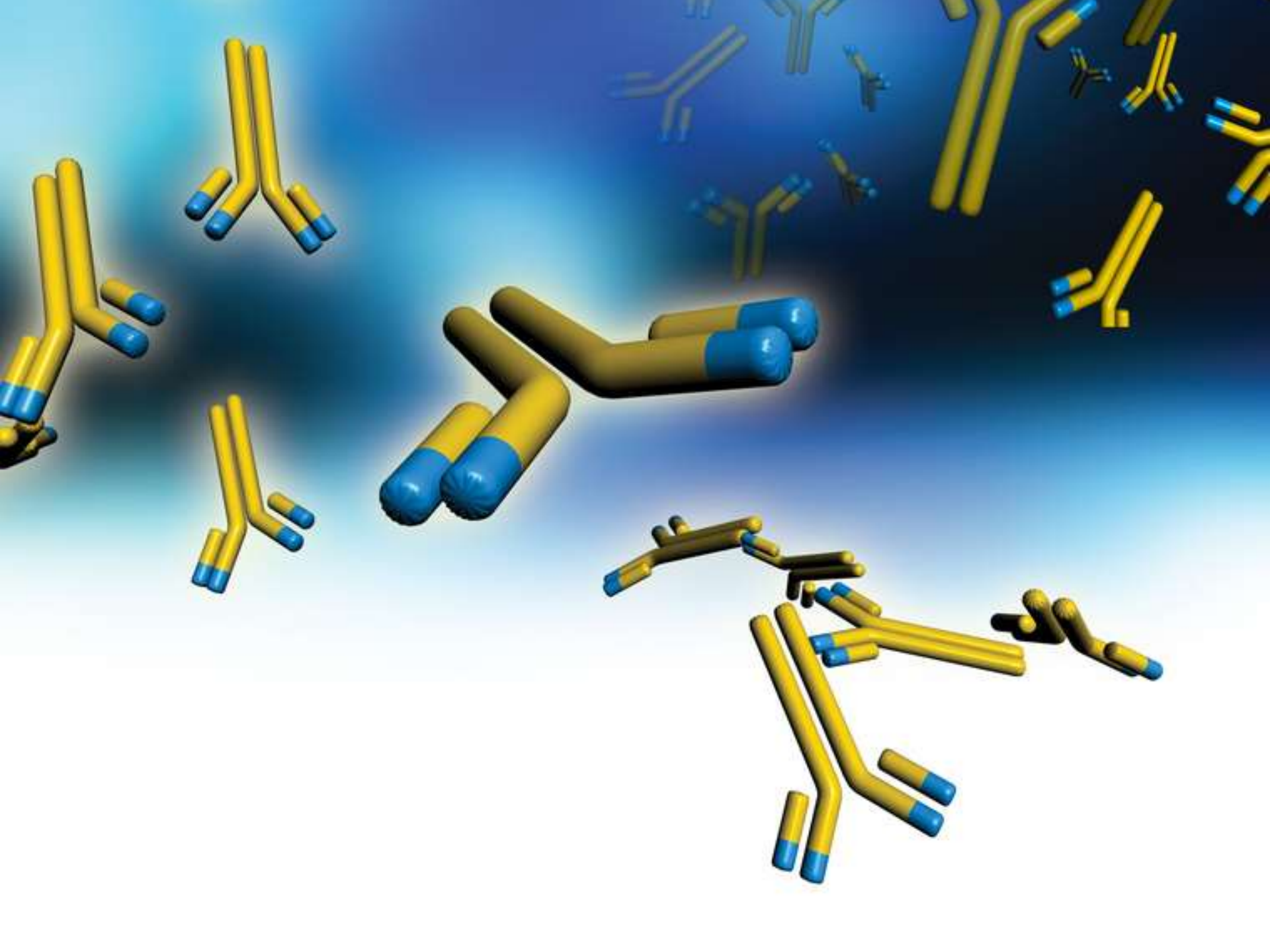
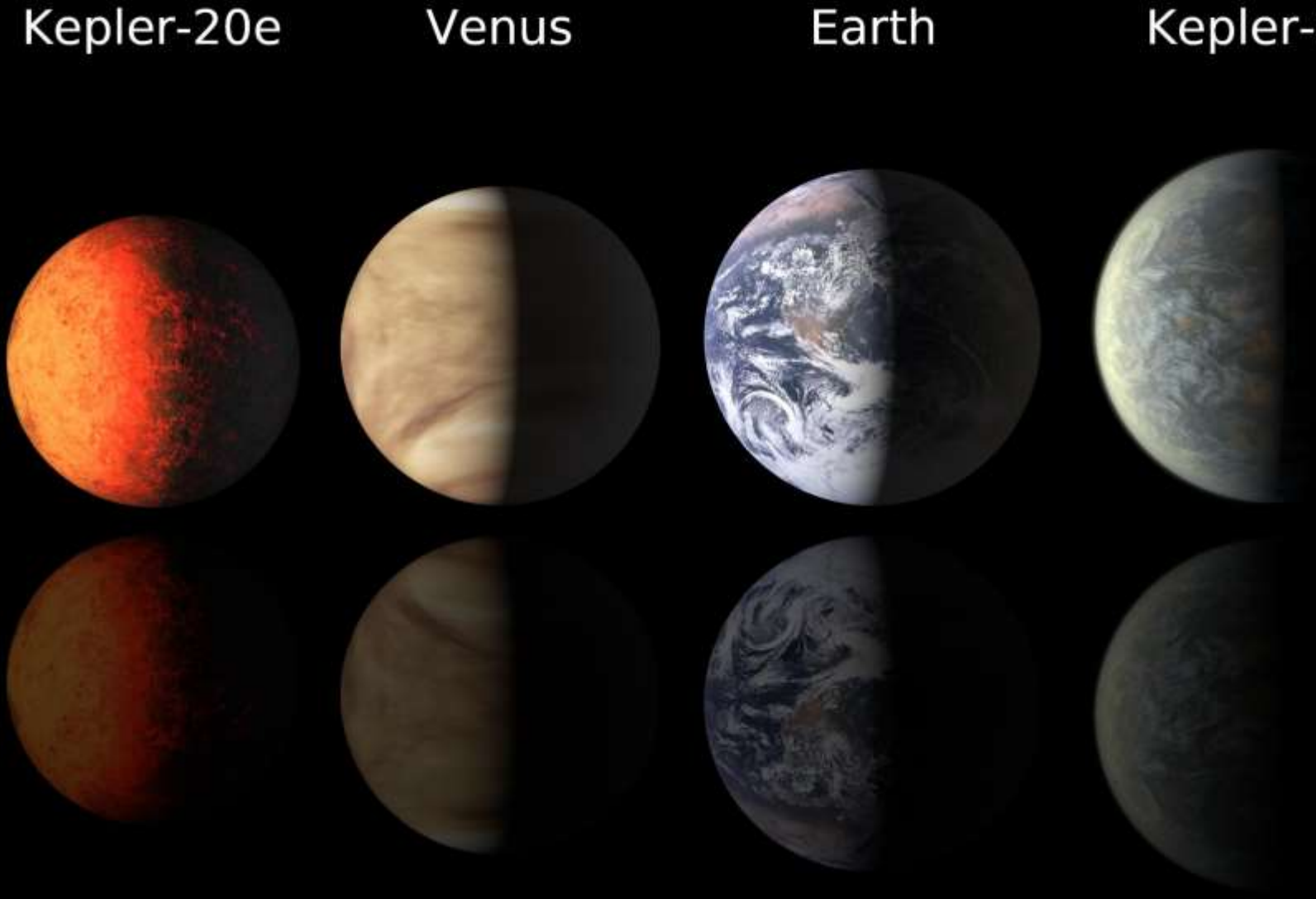



viapath

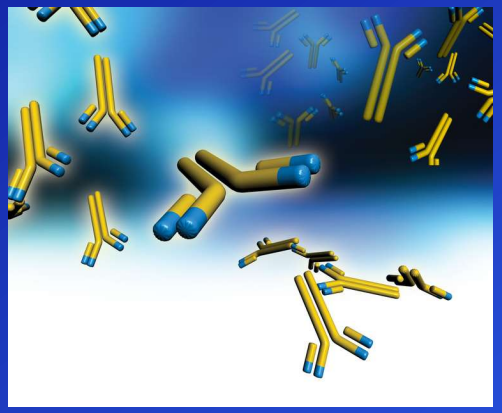
Dr Gary Moore

Guy's and St Thomas' NHS




Kepler-20e
Venus
Earth
Kepler-20f

Sub-atomic particles



Lupus anticoagulants


Extra-solar planets

Detected by inference

Based on exclusion of other possible causes of our findings

## The problem with detection by inference is specificity…..

All LA assays are ‘global’ tests designed to detect the antibodies based on the assumption (hope?) that everything else about the patient’s coagulation is normal

| **Intrinsic pathway-based assays** | | → | **Non-LA causes of screening test elevation** |
|---|---|---|---|
| APTTs | LA-responsive APTT | | Deficiencies of factors I, II, V, VIII, IX, X, XI, XII, PK, HMWK |
| | dAPTT | | Anticoagulation with VKA, UFH, (LMWH), Direct-FXa, DTI |
| | KCT | | Non-phospholipid-dependent inhibitor |
| | SCT | | Aprotinin |
| | | | **Shortening of screening test** |
| | | | Elevated FVIII, FIX |
| | | | Elevated fibrinogen |

| **Extrinsic pathway-based assays** | | → | **Non-LA causes of screening test elevation** |
|---|---|---|---|
| | dPT | | Deficiencies of factors I, II, V, X (dPT only FVII, VIII, IX) |
| | ASLA | | Anticoagulation with VKA, Direct-FXa, DTI, (UFH) |
| | | | Non-phospholipid-dependent inhibitor |

| **Common pathway-based assays** | | → | **Non-LA causes of screening test elevation** |
|---|---|---|---|
| FX activation | dRVVT | | Deficiencies of factors I, II, V, X |
| | VLVT | | Anticoagulation with VKA, Direct-FXa, DTI, (UFH) |
| | | | Non-phospholipid-dependent inhibitor |

| **Common pathway-based assays** | | → | **Non-LA causes of screening test elevation** |
|---|---|---|---|
| FII activation | Textarin time | | Deficiencies of factors I, II |
| | TSVT | | Anticoagulation with UFH, DTI |
| | | | Non-phospholipid-dependent inhibitor |

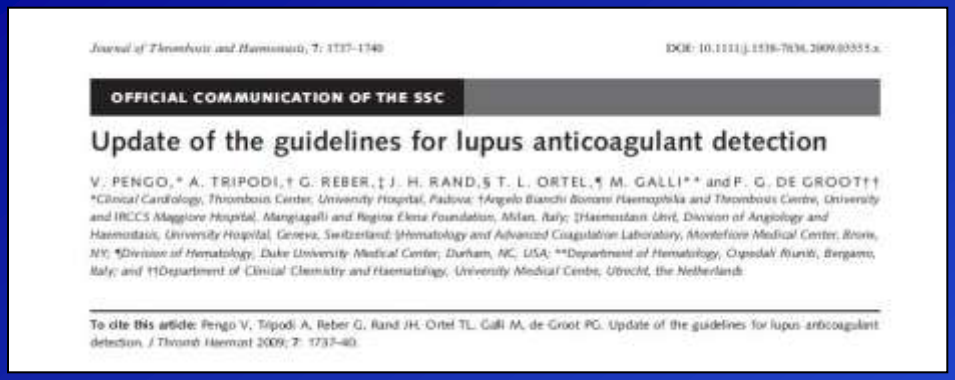
# ‘Traditional’ diagnostic criteria

- Prolongation of at least one phospholipid-dependent coagulation test — Screen
- Evidence of inhibitory activity demonstrated by the effect of test plasma on NPP — Mix
- Confirmation of the phospholipid-dependent nature of the inhibitor — Confirm
- Exclusion of other causes of elevated clotting times that can mask, mimic or co-exist with LA

Routine PT & APTT
Thrombin & Reptilase time
Factor assays
anti-Xa or DTI assay
Telephone call

# Why do we need guidelines?

| | |
|---|---|
| **Antibody heterogeneity** | epitope specificity<br>concentration / avidity / affinity |
| **Reagent variation** | activators<br>phospholipid |
| **Analyser end-point detection** | tilt-tube<br>mechanical<br>photo-optical |
| **No gold standard assay** | no such thing as a LA assay |
| **No reference preparation** | what do you compare with? |
| **Different interpretation strategies** | clotting times<br>normalised ratios<br>calculations for PL-dependence<br>mixing test interpretation |



*Journal of Thrombosis and Haemostasis*, 7: 1737–1740 DOI: 10.1111/j.1538-7836.2009.03555.x

**OFFICIAL COMMUNICATION OF THE SSC**

## Update of the guidelines for lupus anticoagulant detection

V. PENGO,* A. TRIPODI,† G. REBER,‡ J. H. RAND,§ T. L. ORTEL,¶ M. GALLI** and F. G. DE GROOT††
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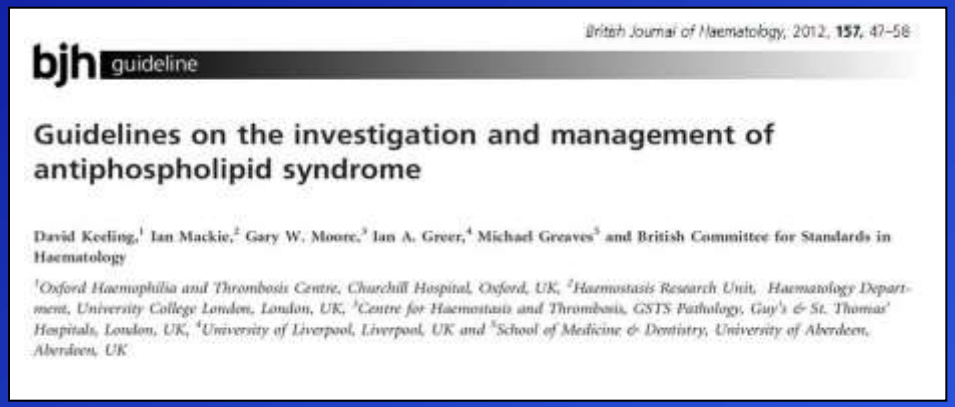
International Society on Thrombosis and Haemostasis

2009



*British Journal of Haematology*, 2012, **157**, 47–58

**bjh** guideline

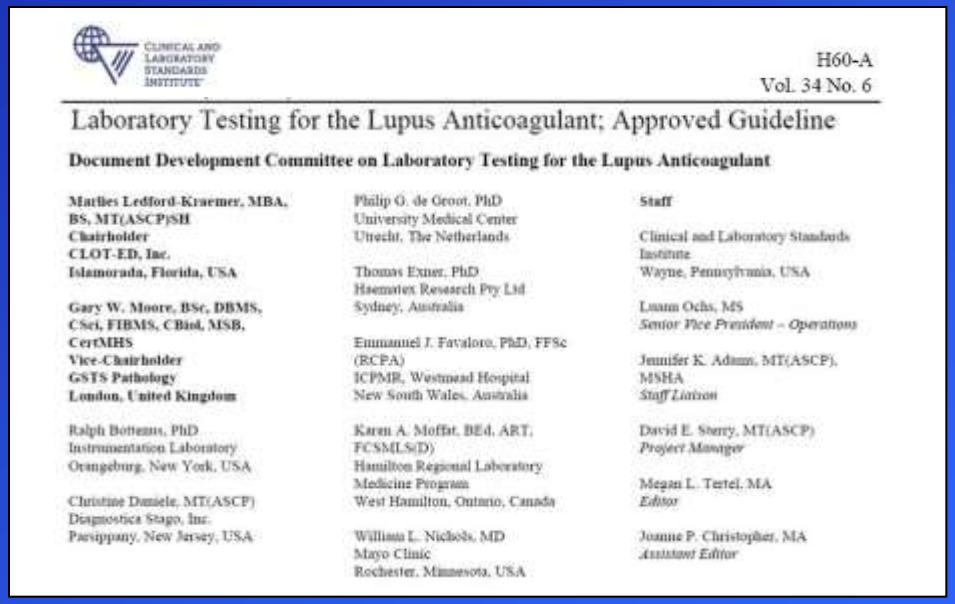
## Guidelines on the investigation and management of antiphospholipid syndrome

**David Keeling,[1] Ian Mackie,[2] Gary W. Moore,[3] Ian A. Greer,[4] Michael Greaves[5] and British Committee for Standards in Haematology**

[1]*Oxford Haemophilia and Thrombosis Centre, Churchill Hospital, Oxford, UK,* [2]*Haemostasis Research Unit, Haematology Department, University College London, London, UK,* [3]*Centre for Haemostasis and Thrombosis, GSTS Pathology, Guy's & St. Thomas' Hospitals, London, UK,* [4]*University of Liverpool, Liverpool, UK and* [5]*School of Medicine & Dentistry, University of Aberdeen, Aberdeen, UK*

BSH

2012



CLINICAL AND LABORATORY STANDARDS INSTITUTE

H60-A
Vol. 34 No. 6

## Laboratory Testing for the Lupus Anticoagulant; Approved Guideline

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2014

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# Laboratory Testing for the Lupus Anticoagulant; Approved Guideline

Volume 34 Number 6

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## Abstract

Identification of the lupus anticoagulant (LA) by laboratory testing is critical for diagnosing the antiphospholipid syndrome and investigating unexpectedly prolonged activated partial thromboplastin time values. The "anticoagulant" effect of LA is restricted to the prolongation of clotting times when using *in vitro*, clot-based coagulation assays that are used as surrogates for identifying LA. Clinical and Laboratory Standards Institute document H60—*Laboratory Testing for the Lupus Anticoagulant; Approved Guideline* provides guidance and recommendations regarding the proper collection and handling of the specimen; descriptions and limitations of screening and confirmatory assays, and mixing tests used to identify LA; determination of cutoff values and calculations associated with the various assays; and interpretation of test results in an LA panel. The guideline is provided for use by laboratorians, physician stakeholders, manufacturers of LA assays, researchers, external quality assessment programs, and accrediting and regulatory agencies. The intent of this guideline is to present information in a practical and easily understandable format; thereby facilitating a standardized approach to LA testing, gaining acceptance in practice, and improving testing quality.

Committee comprised of 24 members from 7 countries representing academia, reference & hospital laboratories, EQA programs, industry, and government. Includes past & present ISTH-SSC & BCSH guideline authors.

# Pre-examination issues

**Blood collection**

105 – 109 mmol/L tri-sodium citrate

**Preparation of plasma samples**

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

Store at -70°C

# Preliminary coagulation screen

Prothrombin time, APTT, thrombin time

- Exclude undiagnosed coagulopathies or undisclosed anticoagulation
- Assess severity of known coagulopathy or degree of anticoagulation
- Assess which subsequent LA assays may be affected
- May suggest presence of a LA
- Assess sample integrity

Employ LA-unresponsive ‘routine’ APTT

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

Metjian & Lim. *Hematology Am Soc Hematol Educ Program.* 2009:247-249; Erkan D et al. *Arthritis Rheum*. 2007;56(7):2382-2391

# Which tests to use and how many?

**Table 1. Historical Perspective of Laboratory Tests Used for the Detection of LA**

| Test | Year | LA Test Type | Plasma Type | Reference |
|---|---|---|---|---|
| PT | 1935 | NA | Neat | 155 |
| PTT | 1953 | NA | Neat | 146 |
| KCT | 1958 | NA | Neat | 147 |
| APTT | 1961 | Independent Screening | Neat | 186 |
| TTI | 1976 | Paired | Neat | 181 |
| KCT | 1978 | Independent Screening | Diluted | 187 |
| PNP | 1983 | Independent Confirmatory | Neat | 190 |
| dAPTT | 1985 | Independent Screening | Neat | 183 |
| dRVVT | 1986 | Paired | Neat | 207 |
| SCT | 1992 | Paired | Neat | 185 |
| HPNT | 1993 | Integrated | Diluted | 197 |
| Textarin/Ecarin | 1993 | Paired | Neat | 211 |
| APTT lupus ratio test | 1993 | Integrated | Diluted | 204 |
| TSVT | 1994 | Independent Screening | Neat | 213 |
| dPT | 1994 | Paired | Neat | 91 |
| ASLA | 2002 | Paired | Neat | 234 |
| dPT lupus ratio test | 2002 | Integrated | Diluted | 221 |

Abbreviations: APTT, activated partial thromboplastin time; ASLA, activated seven lupus anticoagulant; dAPTT, dilute activated partial thromboplastin time; dPT, dilute prothrombin time; dRVVT, dilute Russell's viper venom time; HPNT, hexagonal phase phospholipid neutralization test; KCT, kaolin clotting time; LA, lupus anticoagulant(s); NA, not applicable; PNP, platelet neutralization procedure; PT, prothrombin time; PTT, partial thromboplastin time; SCT, silica clotting time; TTI, tissue thromboplastin inhibition; TSVT, Taipan snake venom time.

# Numbers of screening tests

No single test is sensitive to all LA – use (at least) 2 tests of different principles

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

dRVVT & APTT - potential inconsistency between techniques used for additional test methods

Some patients will generate an elevated screening test with at least one test/reagent type

Chances of this occurring increase as more tests performed

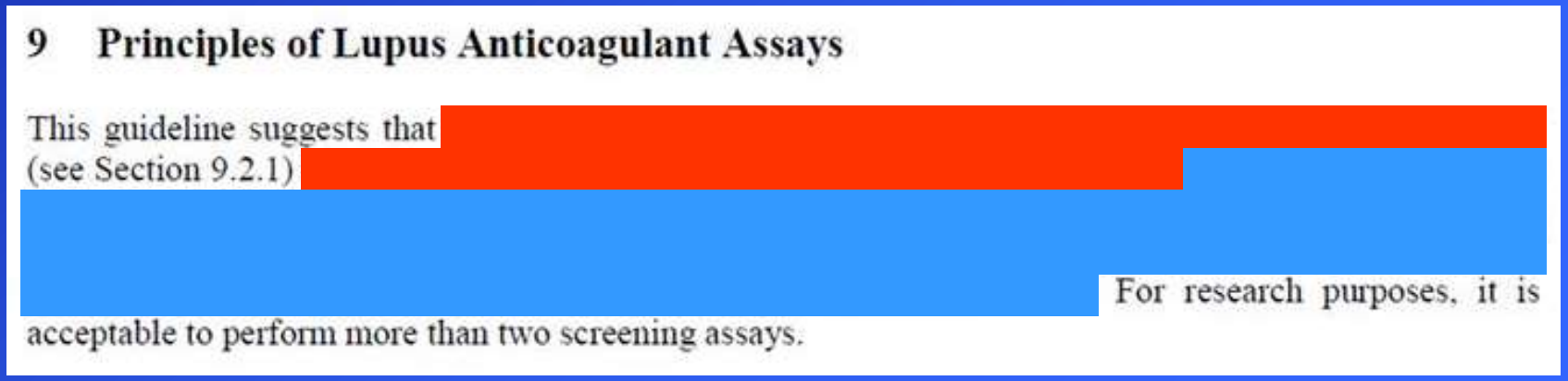
- genuine LA unreactive in other reagents
- 'weak' LA
- discrete analytical error
- merely because the patient is a natural statistical outlier for that reagent/analyser pairing
- ethnic differences

# Numbers of screening tests

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

Applying confirmatory test(s) will not lead to more positive overall **interpretations**

Outliers and non-PL dependent abnormalities will commonly generate concordant screen and confirm results



9 Principles of Lupus Anticoagulant Assays

This guideline suggests that (see Section 9.2.1) For research purposes, it is acceptable to perform more than two screening assays.



dRVVT & LA-responsive APTT is a sensitive & specific pairing that will detect **most** LAs

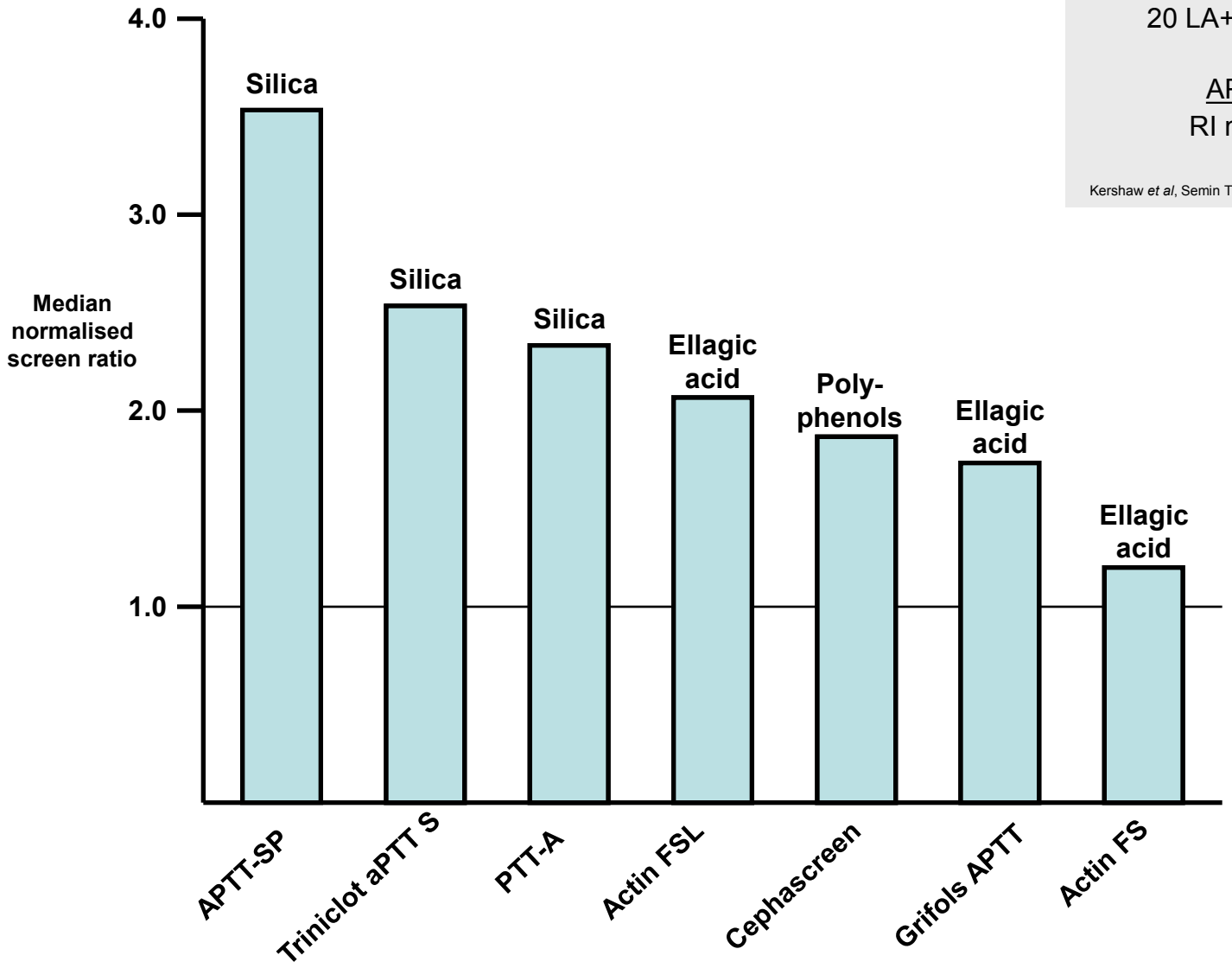
APTT less specific than dRVVT

dRVVT sensitive to $\beta_2$GPI-dependent antibodies & correlates well with APS/thrombosis

Between-reagent variability exists for both dRVVT & APTT with respect to LA detection

Russell's viper
(Daboia russelli)

# APTT-based assays – only employ silica activator?



*Journal of Thrombosis and Haemostasis*, **10**: 2338–2343 DOI: 10.1111/j.1538-7836.2012.04906.x

**ORIGINAL ARTICLE**

# APTT reagent with ellagic acid as activator shows adequate lupus anticoagulant sensitivity in comparison to silica-based reagent

O. KUMANO,* M. IEKO,*† S. NAITO,† M. YOSHIDA† and N. TAKAHASHI*

*Department of *Internal Medicine and †Clinical Laboratory, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido, Japan*

**Table 3** Sensitivity to and specificity for LA

| | In-house reagent | Commercial reagent | | | |
|---|---|---|---|---|---|
| Abbreviation | SL | EA | SLA | FSL | SP | PTT |
| Activator | Silica | Ellagic acid | Ellagic acid | Ellagic acid | Silica | Silica |
| ICA cut-off | 12.9 | 11.5 | 13.2 | 15.6 | 14.3 | 14.0 |
| Sensitivity | | | | | | |
| Specificity | 100% | 98% | 98% | 98% | 100% | 95% |

Sensitivity and specificity for LA-positive samples were calculated using the ICA cut-off value for each reagent.

## Kaolin Clotting Time

Poor reproducibility compared with other assays

Low turbidity, slow settling reagents available

Sensitive assay in experienced hands

## Dilute Prothrombin Time

Thromboplastin variability - although high sensitivity with recombinant thromboplastin

Clinical experience indicating detection of clinically significant antibodies

Standardised kit

- suggestion that LA detection improved when dRVVT & APTT accompanied by dPT

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

Liestøl S et al.. *Thromb Res* 2002;105:177-182; Mackie IJ et al. *Thromb Res* 2004;114:673-674; Devreese KMJ. *Thromb Res* 2008;123:404-411; Lawrie AS et al. *J Thromb Haemost* 2005;3 (Suppl 1) P1817; Galli M et al. *Blood*. 2007;110:1178-1183; Moore GW et al. *Blood Coagul Fibrinolysis* 2002;13:261-269; Martinuzzo M et al. *Thromb Haemost* 2005;93:1007-1009; Moore GW et al. *Clin Appl Thromb/Haemost* 2008;14:332-337

## The Textarin/Ecarin Ratio: A Confirmatory Test for Lupus Anticoagulants

Douglas A. Triplett, Kurt F. Stocker, Gail A. Unger, and Linda K. Barna

**Thrombosis and Haemostasis** 70 (6) 925–931 (1993)

## The Taipan snake venom time: a new test for lupus anticoagulant

A M Rooney, T McNally, I J Mackie, S J Machin

*J Clin Pathol* 1994;47:497–501

## The Ecarin time is an improved confirmatory test for the Taipan snake venom time in warfarinized patients with lupus anticoagulants

Gary W. Moore, Mark P. Smith and Geoffrey F. Savidge

**Blood Coagulation and Fibrinolysis** 2003, 14:307–312

## Detection of lupus anticoagulant in the presence of rivaroxaban using Taipan snake venom time

G. M. A. VAN OS,*† B. DE LAAT,*‡§ P. W. KAMPHUISEN,¶ J. C. M. MEIJERS†¶ and PH. G. DE GROOT*

*J Thromb Haemost* 2011; 9: 1657–9.


Group D prothrombin activator
Textarin time


Group A prothrombin activator
Ecarin/Echis time


Group C prothrombin activator
Taipan snake venom time (TSVT)

CE

# Taipan/Echis venom clotting time Ratios for the detection of Lupus Anticoagulants

For in vitro diagnostic use only.

**Intended Use**
Diagen Taipan and Echis venom clotting times are suitable for use in the *in vitro* detection of lupus anticoagulants (LA) and particularly useful in patients receiving vitamin K antagonist (VKA) therapy.

**Summary and Principle**
LAs comprise part of the heterogeneous spectrum of acquired autoantibodies named antiphospholipid antibodies (APA) [1]. The occurrence and persistence of APA can be associated with a wide range of clinical signs and symptoms, most commonly arterial and venous thrombosis and pregnancy morbidity.
When in the **presence of phospholipid** and calcium ions, Taipan (*Oxyuranus s. scutellatus*) venom is a direct activator of both native prothrombin [2], and that produced when a patient is anticoagulated with VKA (des-carboxy-prothrombin). This is also true in the absence of clotting factors V, VII and X, which makes it of value in the detection of the presence of LAs in patients receiving oral anticoagulant therapy (OAT), where factors II, VII and X are reduced. The diluted Prothrombin Time (DPT) is profoundly affected by reduction in factors II, VII and X, whereas the Dilute Russell's Viper Venom Time (DRVVT) is affected by a reduction in factors II and X. The Taipan snake venom time (TSVT) however, is only affected by a reduction of factor II. Echis (*Echis Carinatus*) venom similarly activates prothrombin & des-carboxy-prothrombin in the absence of clotting factors V, VII and X but importantly, **without the requirement of Phospholipid or Calcium ions**. Taking all of these points into consideration, the TSVT performed in parallel with the Echis clotting time (ECT) [3,4] can be considered a useful **additional** assay in the diagnosis of LA [5] along with the most frequently used assays, the DRVVT and a variety of Activated Partial Thromboplastin Time (APTT) based tests.

Samples are considered positive for LA if the TSVT is prolonged but the ECT is normal – see interpretation.

**Reagent**
**Taipan Snake Venom - Catalogue Number TAVX320**
A lyophilised dilution of Taipan venom extract in calcium chloride, stabilised with albumin, and buffered. For reconstitution remove metal cap and rubber bung, and then add 5.0 mL of distilled water to the contents of the vial. Allow 10 - 15 minutes for complete solution.

**Echis Snake Venom – Catalogue Number ECTT330**
A lyophilised dilution of Echis venom extract, stabilised with albumin, and buffered. For reconstitution remove metal cap and rubber bung, and then add 2.0 mL of distilled water to the contents of the vial. Allow 10 - 15 minutes for complete solution.

**Warnings and Precautions**
Both Diagen Taipan and *Echis carinatus* venom are for *in vitro* diagnostic use only. The reagents contain snake venoms, which are poisons and may be fatal if they enter the bloodstream. Normal precautions should therefore be taken when handling. Please refer to the MSDS (available on request) for further information. All waste must be disposed of whilst observing all local and national laws.

**Collection of Blood Samples**
Blood (9 parts) is collected into 1 part of 0.106 M tri-sodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma is aspirated carefully to avoid cellular contamination and re-centrifuged in a separate, capped container for a further 15 minutes at 2500 g to produce Platelet Poor Plasma (PPP). The plasma should be stored in stoppered tubes.





**Procedure**
The following section details the products required and procedure used for the Taipan Snake Venom Time (TSVT) & Echis Clotting Time (ECT).

**Materials Required**
Cat. No.
TAVX320 - Taipan Snake Venom (6 x 5.0 mL vials).
ECTT330 - Echis Snake Venom (6 x 2.0 mL vials).

**Materials and equipment required, but not provided:**
1. General routine laboratory coagulation equipment.
2. Reaction cups or test tubes (12 x 75 mm).
3. Pipette delivering between 100 µL, 200 µL, 2 mL and 5 mL.
4. Bell and Alton Platelet Substitute (BAPS040).
5. Imidazole Buffer (IMBX600).
6. 12.5 mM (M/80) $CaCl_2$.
7. Distilled water.

**Manual Technique**
**Preparation**
1. Dilute the Taipan venom* a further 1/2 to 1/6 in 12.5mM $CaCl_2$ (M/80).
2. Dilute the Bell and Alton platelet substitute* 1/2 to 1/8 in Imidazole buffer, or alternatively use washed platelets.
***See Taipan venom instructions for use for results using typical reagent batch dilutions.**

**Taipan Snake Venom time (TSVT) - manual method**
1. Add 100 µL of test plasma to 100 µL of platelet substitute and incubate at 37°C for 60 seconds.
2. Add 200 µL of diluted Taipan venom and record the clotting time.
3. Repeat steps 1 & 2 using platelet poor normal control plasma pool.
4. Once both clotting times have been recorded the TSVT ratio of test plasma / normal control plasma pool can be calculated.
**Please note that normal control plasma pool must be tested in parallel with the patient sample.**

**Echis Clotting time (ECT) - manual method**
1. Add 100 µL of test plasma to test tube and incubate at 37°C for 60 seconds.
2. Add 200 µL of Echis venom and record the clotting time.
3. Repeat steps 1 & 2 using normal control plasma pool.
4. Once both clotting times have been recorded the ECT ratio of test plasma / normal control can be calculated.
**Please note that normal control plasma pool must be tested in parallel with the patient sample.**

**Notes:**
1. Tubes should be new and scrupulously clean.
2. Water bath temperature should be 37°C.
3. **For photo-optical and mechanical instruments, follow the manufacturer's instructions.**

**Interpretation**
In our hands, the normal **TSVT** ratio is defined as 0.90 – 1.12 **ratios greater than 1.12 suggests the presence of LA**. The cut off ratio of 1.12 is dependent, in part, by the sensitivity of the test system and the choice of Taipan Venom & Phospholipid (Platelet substitute or washed Platelets) dilutions. Our in house method aims to achieve a normal plasma clotting time of approximately 30 seconds. However, it is **most important that each laboratory determines appropriate dilutions and cut off value for each lot of reagents.**

In our hands, a normal **ECT** ratio is defined as 0.90 – 1.11.

Samples are considered positive for a LA if the **TSVT ratio is greater than 1.12 which is corrected by ≥10% by the ECT ratio.** [5,6]

**Quality Control**
All laboratories should have in place a quality control system that uses normal and abnormal controls to evaluate instrument, reagent and user performance. LA negative and positive controls should be tested alongside patient samples. The controls must be platelet poor, with fewer than $10^9$ platelets/µL. If the controls do not perform within their defined reference ranges, patient results should be considered invalid.

# 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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Forastiero RR, Cerrato GS, Carreras LO. Evaluation of recently described tests for detection of the lupus anticoagulant. *Thromb Haemost* 1994;72:728-783

Luddington R, Scales C, Baglin T. Lupus anticoagulant testing with optical end point automation. *Thromb Res* 1999; 96:197-203

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# Reference interval mean clotting time for calculating ratios

| Normal Pooled Plasma | dRVVT screen (s) | dRVVT confirm (s) | dAPTT screen (s) | dAPTT confirm (s) |
|---|---|---|---|---|
| CRYO*check*™ frozen normal pool mean | 44.0 | 37.8 | 36.0 | 42.8 |
| Locally prepared frozen normal pool mean | 44.8 | 34.8 | 38.1 | 40.3 |
| Technoclone lyophilised platelet poor plasma mean | 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 J Lab Haematol* 2013;35:128-136

# False positive or negative results with unsuitable NPP

| Ref. interval | dRVVT screen | dRVVT confirm | dAPTT screen | dAPTT confirm |
|---|---|---|---|---|
| Clotting times (s) | 37.1 – 51.1 | 33.8 – 41.4 | 33.1 – 49.7 | 37.6 – 54.2 |
| Ratios | 0.85 – 1.17 | 0.90 – 1.10 | 0.80 – 1.20 | 0.82 – 1.18 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **False negative dRVVT screen:** | 54.7 s / 47.4 s | = | 1.15 | 54.7 s / 43.8 s | = | 1.25 |
| | Technoclone NPP | | | RI mean | | |
| **False positive dAPTT screen:** | 47.0 s / 36.0 s | = | 1.31 | 47.0 s / 41.4 s | = | 1.14 |
| | CRYO*check* NPP | | | RI mean | | |
| **False negative dAPTT interpretation:** | 51.6 s / 38.1 s | = | 1.35 | 51.6 s / 41.4 s | = | 1.25 |
| | Local NPP | | | RI mean | | |
| **Confirmatory tests** | 50.5 s / 40.3 s | = | 1.25 | 50.5 s / 45.9 s | = | 1.10 |
| | Local NPP | | | RI mean | | |
| **% correction (<10)** | 7.4 | | | 12.0 | | |

# Mixing test

Perform on 1:1 mixture with NPP

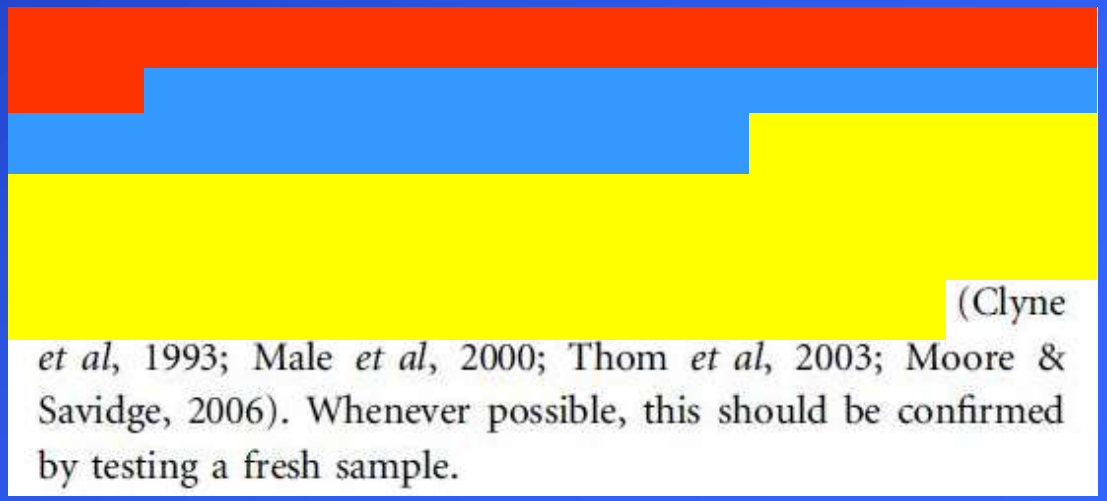
Evaluate with Index of Circulating Anticoagulant (ICA) or mixing test-specific cut-off

**Dilution effect can obscure 'weak' LA**

ISTH-SSC 1995 & 2009

BCSH 1991, 2000 & 2012

**2012**



(Clyne *et al*, 1993; Male *et al*, 2000; Thom *et al*, 2003; Moore & Savidge, 2006). Whenever possible, this should be confirmed by testing a fresh sample.

# Paradigm shift in test order

Order of testing algorithm: **screening, confirmatory, mixing**

H-60 assigns a lower priority to a mixing test because of its limitations

Prioritises the demonstration of PL dependence of the antibody over showing inhibitory action of LA in an assay principle known to compromise detection

# When to omit the mixing test

Mixing test can be omitted **only if**:

(i) LA screening test elevated

(ii) Associated confirm test corrects mathematically AND into reference interval

(iii) No evidence of other causes of elevated clotting times

| **Test** | | **Result** | **RI** |
|---|---|---|---|
| PT (s) | (DXa-sensitive) | **11** | (10 -12) |
| APTT (s) | (LA-unresponsive) | **27** | (22 – 30) |
| TT (s) | | **13** | (12 – 15) |
| dRVVT screen ratio | | **1.42** | (0.84 – 1.18) |
| dRVVT confirm ratio**0.98** | | (0.88 – 1.12) | |
| % correction | | **31.0** | (<10) |
| Screen/confirm ratio | | **1.45** | (<1.15) |
| dRVVT 1:1 mix ratio | | 1.08 | (0.90 – 1.10) |

# When to use mixing tests

Confirm result in LA +ve patients does not always shorten to within the RI

| | | |
|---|---|---|
| dRVVT screen ratio | 1.98 | (0.84 – 1.18) |
| dRVVT confirm ratio | 1.35 | (0.88 – 1.12) |
| % correction | 31.8 | (<10) |
| Screen/confirm ratio | 1.47 | (<1.15) |

Elevated confirm: potent/avid LA

co-existing abnormality

| | | |
|---|---|---|
| dRVVT screen ratio | 1.98 | |
| dRVVT confirm ratio | 1.85 | |
| % correction | 6.6 | |
| Screen/confirm ratio | 1.07 | |

| | | |
|---|---|---|
| Mixing test screen ratio | 1.59 | (0.90 – 1.10) |
| Mixing test confirm ratio | 1.54 | (0.89 – 1.10) |

**Non-phospholipid dependent inhibitor**

| | |
|---|---|
| Mixing test screen ratio | 1.01 |
| Mixing test confirm ratio | 1.02 |

**Factor deficiency**

| | |
|---|---|
| Mixing test screen ratio | 1.42 |
| Mixing test confirm ratio | 1.08 |

**Lupus anticoagulant**

Lupus anticoagulant co-factor effect

| | |
|---|---|
| dRVVT screen ratio | 1.29 |
| dRVVT confirm ratio | 1.12 |
| Mixing test screen ratio | 1.98 |
| Mixing test confirm ratio | 1.08 |

# Algorithm

| | |
|---|---|
| PT | Normal |
| APTT | Normal |
| TT | Normal |
| Fibrinogen | Normal |

| | |
|---|---|
| PT | Normal |
| APTT | Elevated |
| APTT 50:50 | Elevated |
| TT | Normal |
| Fibrinogen | Normal |

| | |
|---|---|
| PT | Normal |
| APTT | Elevated |
| APTT 50:50 | Normal |
| TT | Normal |
| Fibrinogen | Normal |



Above RI

Above RI

1. Correction
2. Confirm ratio above RI

dRVVT confirm on neat plasma

dAPTT confirm on neat plasma

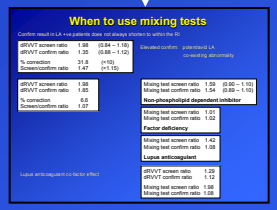
1. Correction
2. Confirm ratio above RI

dRVVT screen & confirm on 50:50 mix

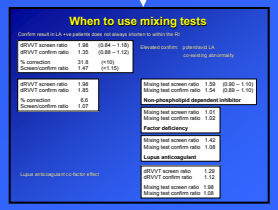
1. Correction
2. Confirm ratio within RI

1. Correction
2. Confirm ratio within RI

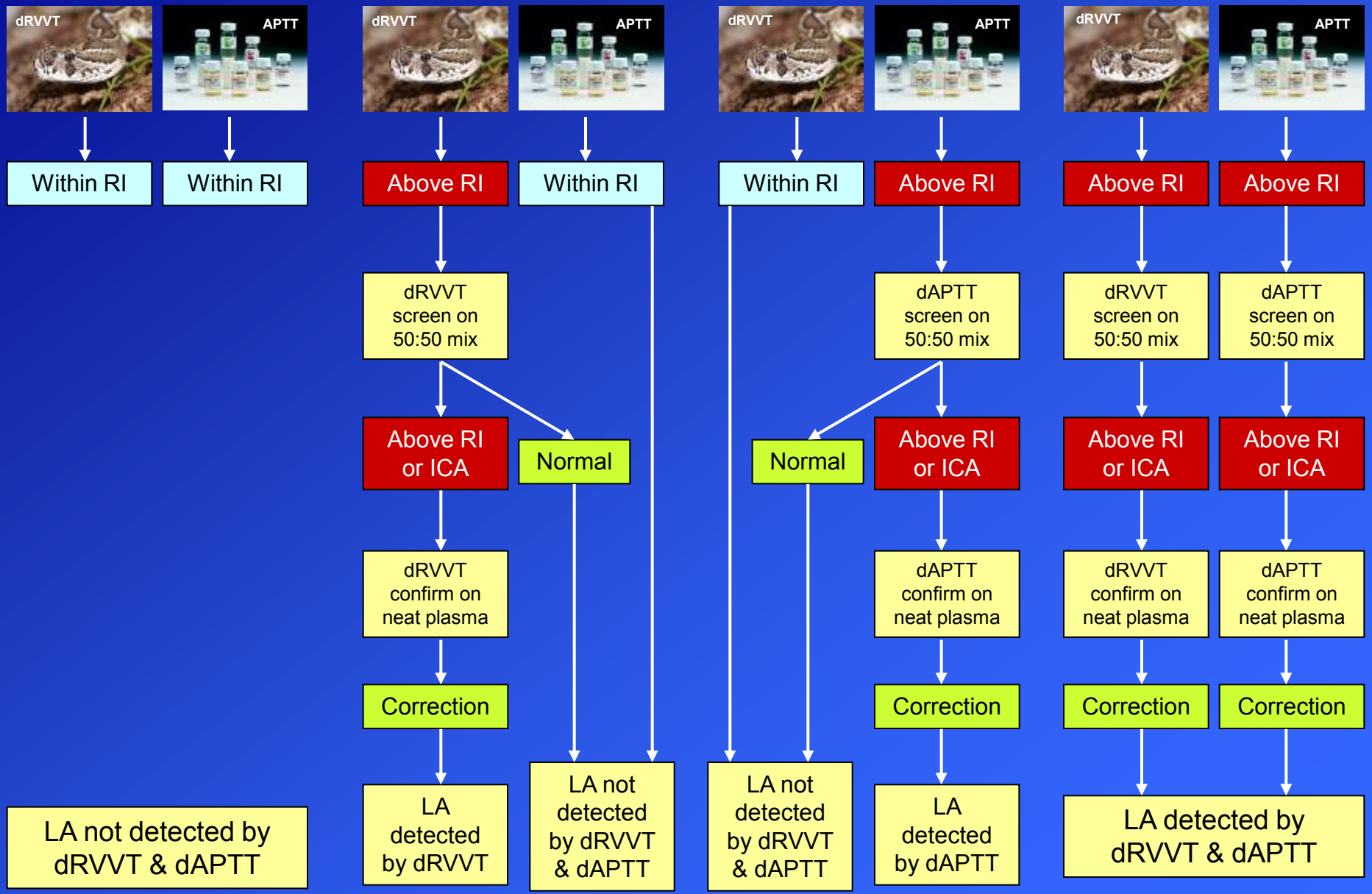
dAPTT screen & confirm on 50:50 mix




LA detected by dRVVT & dAPTT



# Standard algorithm



# Confirmatory test for phospholipid dependence

Screen & confirm must be based on the same test principle

**Paired tests**

Screen (low PL) and confirm (high PL) e.g. dRVVT, SCT, dPT

(i) Normalised test/confirm ratio

$$\frac{\text{screen normalised ratio}}{\text{confirm normalised ratio}}$$

(ii) % correction of ratio

$$\frac{(\text{screen ratio} - \text{confirm ratio})}{\text{screen ratio}} \times 100\%$$

**Independent tests**

APTT + platelet neutralisation procedure (delta)

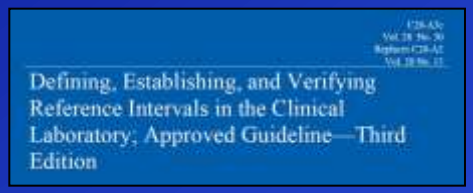
**Integrated tests**

APTT-based hexagonal phase neutralisation test (delta)

# Cut-off values

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

Aligns with CLSI C28-A3




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

Historically: mean + 2SD (97.5th percentile)

99th percentile (mean + 2.3SD if Gaussian) would improve specificity but reduce sensitivity

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

# LA testing during VKA anticoagulation

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

| INR | dRVVT | Confirm |
|---|---|---|
| 2.0 – 4.5 | 0.84 – 1.18 | 0.88 – 1.12 |
| **3.8** | **2.87** | **2.39** |

| 1:1 screen | 1:1 confirm | Screen/confirm ratio | % correction |
|---|---|---|---|
| 0.90 – 1.10 | 0.89 – 1.10 | < 1.15 | <10% |
| **1.46** | **1.07** | **1.36** | **26.7** |

| 1:1 screen | 1:1 confirm |
|---|---|
| 0.90 – 1.10 | 0.89 – 1.10 |
| **1.07** | **1.00** |

Table of interferences, including anticoagulant therapy

Guide to interpreting composites, including detection of LA during anticoagulant therapy

## Appendix E. Table of Interferences and Limitations for Specific Lupus Anticoagulant Tests

| | VKA | UFH | LMWH | DTI | | | FXa Inhibitors | | FVIII Inhibitors | FV Inhibitors | Factor Deficiencies | Acute Phase Response (Elevated FVIII, Fibrinogen, Platelet Fragments, CRP) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Argatroban | Bivalirudin | Dabigatran | Hirudin | Indirect: Fondaparinux | Direct: Rivaroxaban | | | | |
| **Preliminary Examination Assays** | | | | | | | | | | | | | |
| APTT | Y | Y | Y[a] | Y | Y | Y[10] | Y | Y[10], N[11] | Y[1,4,9,12,13] | Y | Y | All but VII | Y[17] |
| PT | Y | Y[a] | N[a] | Y | Y | Y[10] | Y | Y[10] | Y[1,4,9,12,13] | N | Y | I, II, V, VII, X | N |
| TT | N | Y | N[a] | Y | Y | Y[10] | Y | N | N[12] | N | N | I | N |
| **Intrinsic Pathway Assays** | | | | | | | | | | | | | |
| APTT Screening | Y | Y | Y[a] | Y | Y | Y[10] | Y | Y[10], N[11] | Y[1,4,9,12,13] | Y | Y | All but VII | Y[17] |
| SCT Screening | Y | Y[11] | Y | Y | Y | Y[10] | Y | Y, N[11] | Y[10] | Y | Y | All but VII | Y |
| SCT Confirmatory | Y | Y[a] | N[a] | | | Y[10] | | | Y[10] | Y[9] | | All but VII | |
| RCT (Screening Only) | Y | Y | Y[a] | Y | Y | | Y | Y | | Y | Y | All but VII | Y |
| PNP (Confirmatory Only) | Y[8] | Y[11] | | | | | | | | Y[8,12,13] | Y[12] | | |
| HPNT (Integrated) | Y, N[8,11] | Y, N[8,11] | N[8,14] | Y | Y | Y | Y | | N[12] | Y[8], N[12] | Y[12], N[8] | Y, N[a] | Y[17] |
| **Common Pathway Assay** | | | | | | | | | | | | | |
| dRVVT Screening | Y[15] | N[a] | N[a] | Y[15] | Y[15] | Y[10] | Y[12,16] | Y, N[10] | Y[9,12,16] | N[12] | Y[12] | I, II, V, X | Y[a] |
| dRVVT Confirmatory | Y[11] | N[a] | N[a] | Y[12], N[15] | Y[15] | Y[10] | Y[12], N[10] | N | Y[9,12,16] | N | N | I, II, V, X[12] | Y[a] |
| TSVT (Screening Only) | N[11] | Y[a] | | | Y[10] | | | N | N[9] | N | Y | I, II | |
| **Extrinsic Pathway Assay** | | | | | | | | | | | | | |
| dPT (Screening Only) | Y | N[a] | | Y | Y | Y[10] | Y | Y | Y[9,14] | N | Y | I, II, V, VII, X[12] | N |
| NOTE | Y=YES, listed therapeutic agents, inhibitors, factor deficiencies, and/or acute phase response will affect an LA assay; N=NO, listed therapeutic agents, inhibitors, factor deficiencies, and/or acute phase response will not affect an LA assay; Y* =Usually YES, but may depend on the reagent or the concentration/level of interfering substance; N* =Not usually, but may depend on the reagent or the concentration/level of the interfering substance. Blank Space=no published information available. | | | | | | | | | | | | |
| Abbreviations: | APTT, activated partial thromboplastin time; CRP, C reactive protein; dPT, dilute prothrombin time; dRVVT, dilute Russell's viper venom time; DTI, direct thrombin inhibitor(s); FV, factor V; FVIII, factor VIII; FXa, activated factor X; HPNT, hexagonal phase phospholipid test; I, fibrinogen; II, prothrombin; RCT, kaolin clotting time; LA, lupus anticoagulant(s); LMWH, low molecular weight heparin(s); PNP, platelet neutralization procedure; PT, prothrombin time; SCT, silica clotting time; TT, thrombin time; TSVT, Taipan snake venom time; UFH, unfractionated heparin(s); VKA, vitamin K antagonist(s). | | | | | | | | | | | | |

## Appendix G1. Interpretive Comments and Rationale for Comments Based on Patient Examples (Data)

| | Example | LA Testing Panel Examples | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Preliminary Examination Assays | | | Intrinsic Pathway Assay: Independent Tests (APTT and PNP) | | | | | | | Common Pathway Assay: Paired Test (dRVVT)* | | | | | | | | | |
| | | | | | APTT Screen | | PNP Confirm | | | APTT 1:1 Mix Test | | dRVVT Screen | | dRVVT Confirm | | Paired Test Calculations | | dRVVT Screen 1:1 Mix | | dRVVT Confirm 1:1 Mix | |
| | | PT (s) or PT-INR | APTT (s) | TT (s) | APTT Screen (s) | APTT Screen N Ratio | PNP #1 Plasma / Saline (s) | PNP #2 Plasma / PL (s) | PNP delta (s) (#1 – #2) | APTT Screen 1:1 Mix Test (s) | APTT Screen 1:1 Mix Test N Ratio | dRVVT Screen (s) | dRVVT Screen N Ratio | dRVVT Confirm (s) | dRVVT Confirm N Ratio | dRVVT Screen to Confirm N Ratio | dRVVT Screen to Confirm N Ratio % Correction | dRVVT Screen 1:1 Mix (s) | dRVVT Screen 1:1 Mix N Ratio | dRVVT Confirm 1:1 Mix (s) | dRVVT Confirm 1:1 Mix N Ratio |
| | Reference Interval | 10–12 | 30–40 | 9–11 | 31–39 | <1.20 | 29–37 | 30–38 | <4 | 31–37 | <1.15 | 33–43 | <1.18 | 32–41 | <1.15 | <1.15 | <10% | 33–40 | <1.10 | 33–39 | <1.10 |
| 1 | LA Not Detected | 11 | 32 | 10 | 35 | 1.00 | ND | ND | ND | ND | ND | 37 | 0.98 | 35 | 0.96 | 1.02 | 2.0 | ND | ND | ND | ND |
| 2 | LA Present | 11 | 50 | 10 | 53 | 1.51 | 48 | 32 | 16 | 46 | 1.35 | 56 | 1.47 | 33 | 0.90 | 1.63 | 38.8 | 46 | 1.26 | ND | ND |
| 3 | LA Present but Assay Dependent | 11 | 37 | 9 | 35 | 1.00 | ND | ND | ND | ND | ND | 53 | 1.39 | 34 | 0.93 | 1.49 | 33.1 | 48 | 1.31 | ND | ND |
| 4 | LA Present With Dilution Effect in 1:1 Mixing Test | 10 | 40 | 11 | 41 | 1.17 | 38 | 33 | 5 | 36 | 1.06 | 46 | 1.20 | 35 | 0.96 | 1.25 | 20.0 | 39 | 1.07 | ND | ND |
| 5 | Indeterminate Results | 10 | 39 | 10 | 41 | 1.18 | 37 | 35 | 2 | 35 | 1.03 | 46 | 1.21 | 41 | 1.12 | 1.08 | 7.4 | 40 | 1.09 | ND | ND |
| 6 | UFH (High Levels) | 11 | 180 | 150 | 171 | 4.88 | 160 | 120 | 40 | 85 | 2.50 | 80 | 2.10 | 78 | 2.14 | 0.98 | <1.0 | 60 | 1.64 | ND | ND |
| 7 | LA Present but With UFH (Lower Levels) | 11 | 60 | 75 | 60 | 1.71 | 65 | 60 | 5 | 43 | 1.26 | 46 | 1.20 | 34 | 0.93 | 1.29 | 22.5 | 43 | 1.18 | ND | ND |
| 8 | VKA | 33 (INR=3.0) | 42 | 10 | 41 | 1.17 | 43 | 41 | 2 | 35 | 1.03 | 59 | 1.55 | 55 | 1.51 | 1.03 | 2.6 | 40 | 1.09 | 37 | 1.03 |
| 9 | LA Present Despite VKA | 33 (INR=3.0) | 42 | 10 | 47 | 1.34 | 40 | 36 | 4 | 41 | 1.20 | 61 | 1.60 | 50 | 1.37 | 1.17 | 14.3 | 44 | 1.21 | 34 | 0.94 |
| 10 | DTI | 47 | 139 | >180 | 146 | 4.17 | 138 | 135 | 3 | 99 | 2.91 | 74 | 1.95 | 71 | 1.94 | 1.00 | 0.5 | 55 | 1.51 | 69 | 1.92 |
| 11 | FVIII Inhibitor | 12 | 130 | 9 | 105 | 3.00 | 98 | 95 | 3 | 70 | 2.06 | 34 | 0.89 | ND | ND | ND | ND | ND | ND | ND | ND |
| | Legend | Mean of reference interval in seconds: APTT=35; APTT Screen 1:1 Mix Test=34 | | | | | | | | | | | | | | | | | | | |
| | | Mean of reference interval in seconds: dRVVT Screen=38; dRVVT Confirm=36.5; dRVVT Screen 1:1 Mix Test=36.5; dRVVT Confirm 1:1 Mix Test=36 | | | | | | | | | | | | | | | | | | | |
| | | * Other paired test systems: intrinsic pathway assays (SCT) or extrinsic pathway (dPT). | | | | | | | | | | | | | | | | | | | |
| | | Normalized ratios for screen assays are discussed in Section 10; Normalized ratios for independent systems and normalized ratios and % corrections for paired test systems are discussed in Section 11; Normalized ratios for screen and confirm mix test are discussed in Section 12; Appendix G2 provides suggestions for interpretive comments and rationale for those interpretive comments based upon the data presented above. | | | | | | | | | | | | | | | |
| | | Abbreviations: APTT, activated partial thromboplastin time; DTI, direct thrombin inhibitor(s); dPT, dilute prothrombin time; dRVVT, dilute Russell's viper venom time; FVIII, factor VIII; N, normalized; ND, not done; PL, phospholipid(s); PNP, platelet neutralization procedure; PT-INR; prothrombin time-international normalized ratio; RI, reference interval(s); s, seconds; SCT, silica clotting time; TT, thrombin time; UFH, unfractionated heparin(s); VKA, vitamin K antagonist(s). | | | | | | | | | | | | | | | | | | | |

# Summary

| Area of recommendation | 2009 | 2012 | 2014 |
|---|---|---|---|
| Sample preparation | Double centrifugation | | |
| Assays to use | dRVVT & APTT | dRVVT<br>plus APTT or others | dRVVT & APTT<br>+/- others |
| Testing order | Screen – Mix - Confirm | | Screen – Confirm - Mix |
| Ratio derivation | NPP denominator | | RI mean denominator |
| Reference interval/cut-offs | 99$^{th}$ percentile | 97.5$^{th}$ percentile<br>(if Gaussian) | 97.5$^{th}$ percentile<br>(if Gaussian) |
| Phospholipid-dependence calculations | % correction of screen by confirm<br><br>LA ratio (screen/confirm) | | |
| Mixing test | Perform on 1:1 mixture with NPP<br><br>Interpret with ICA<br>or<br>mixing test-specific cut-off | Perform on 1:1 mixture with NPP | Perform on 1:1 mixture with NPP<br><br>Interpret with ICA<br>or<br>mixing test-specific cut-off |
| Testing patients on VKAs | Undiluted plasma if INR <1.5<br><br>Mix with NPP if INR >1.5 <3.0 | Screen & confirm on 1:1 mix with NPP<br><br>TSVT + ET or PNP | Screen & confirm on 1:1 mix with NPP<br><br>TSVT + ET or PNP |
| Testing patients on UFH | Interpret with caution | Can detect LA in some cases where heparin neutraliser is effective | |
| Interpretive reporting | Recommended | | |

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