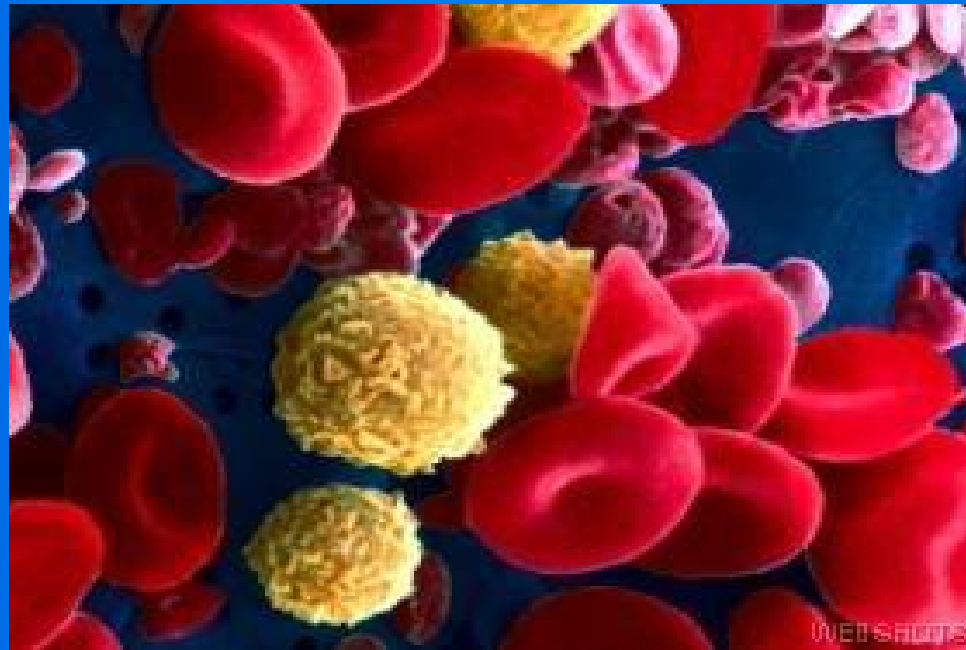


Lupus anticoagulant detection: Misconceptions and what the guidelines *don't* tell you



Dr Gary Moore

Lupus anticoagulants



Heterogeneous group of antibodies

Stronger association with thrombosis than ACA in adults

Occasionally present with bleeding

Detected by inference due to effects on phospholipid dependent coagulation assays

No gold standard assay

Variations in diagnostic performance of available assays

Available procedures for LA screening

- 1. Screening tests based on 'routine' coagulation screening tests**
 - Activated partial thromboplastin time (APTT)
 - Dilute APTT
 - Kaolin clotting time (KCT) and Exner curves
 - Silica clotting time (SCT)
 - Tissue thromboplastin inhibition (TTI)/dilute prothrombin time (DPT)
- 2. Screening tests using snake venom coagulation activators**
 - Dilute Russell's viper venom time (DRVVT)
 - Taipan snake venom time (TSVT)
 - Textarin time
- 3. Screening test using (human derived) serine protease as activator**
 - Activated seven lupus anticoagulant (ASLA) assay
- 4. Chromogenic assay**
 - Thrombin generation

Sensitivity & specificity of commercial reagents

No single test will detect all LA

Some LA react more potently in some tests than others

Some LA may only be detectable by one type of test

Sensitivity & specificity of commercial reagents

Personal experience of a DRVVT + DAPTT regime:

Positive in both DRVVT + DAPTT	40%
Positive in DRVVT alone	30%
Positive in DAPTT alone	30%

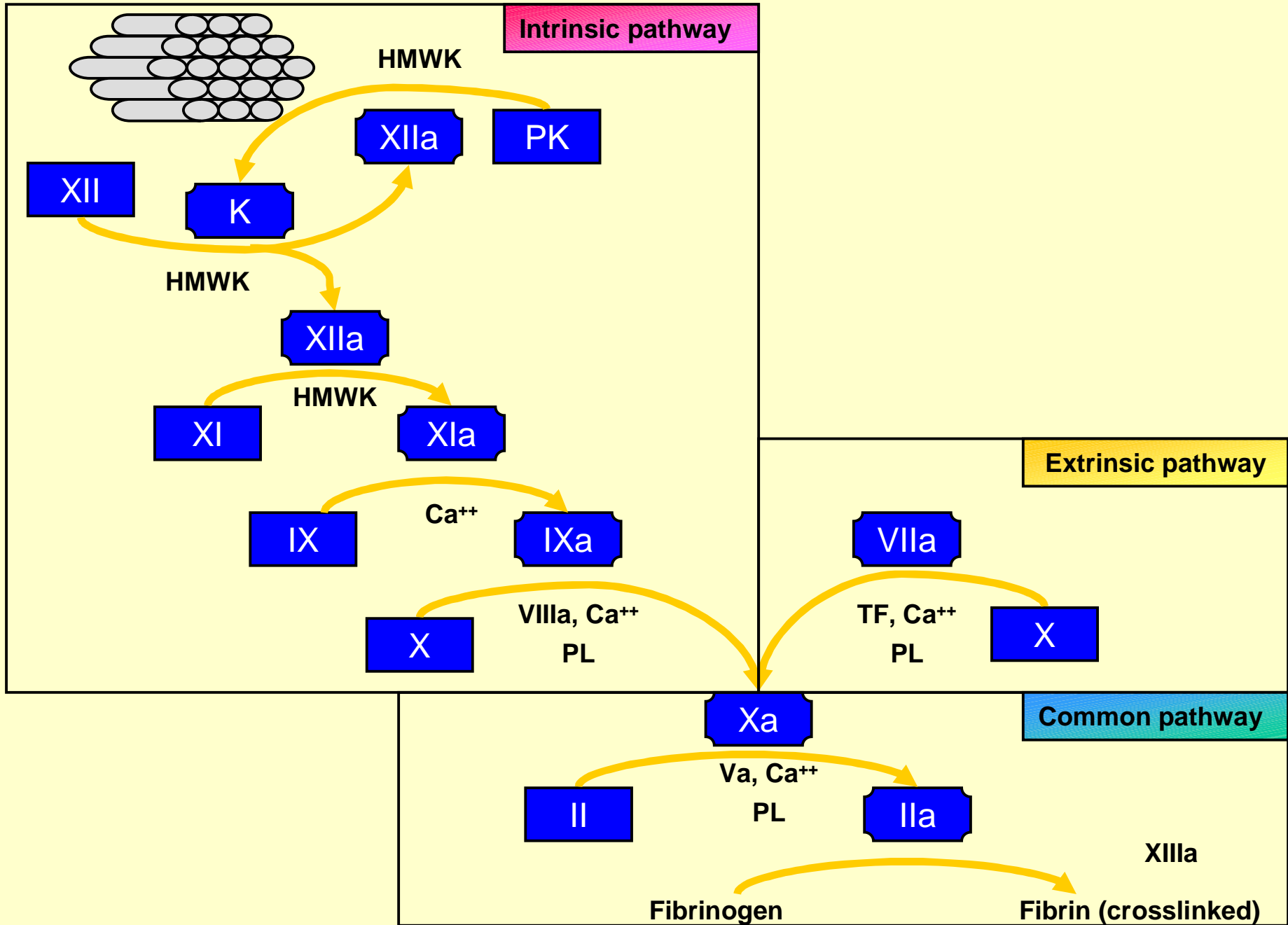
Sensitivity & specificity of commercial reagents

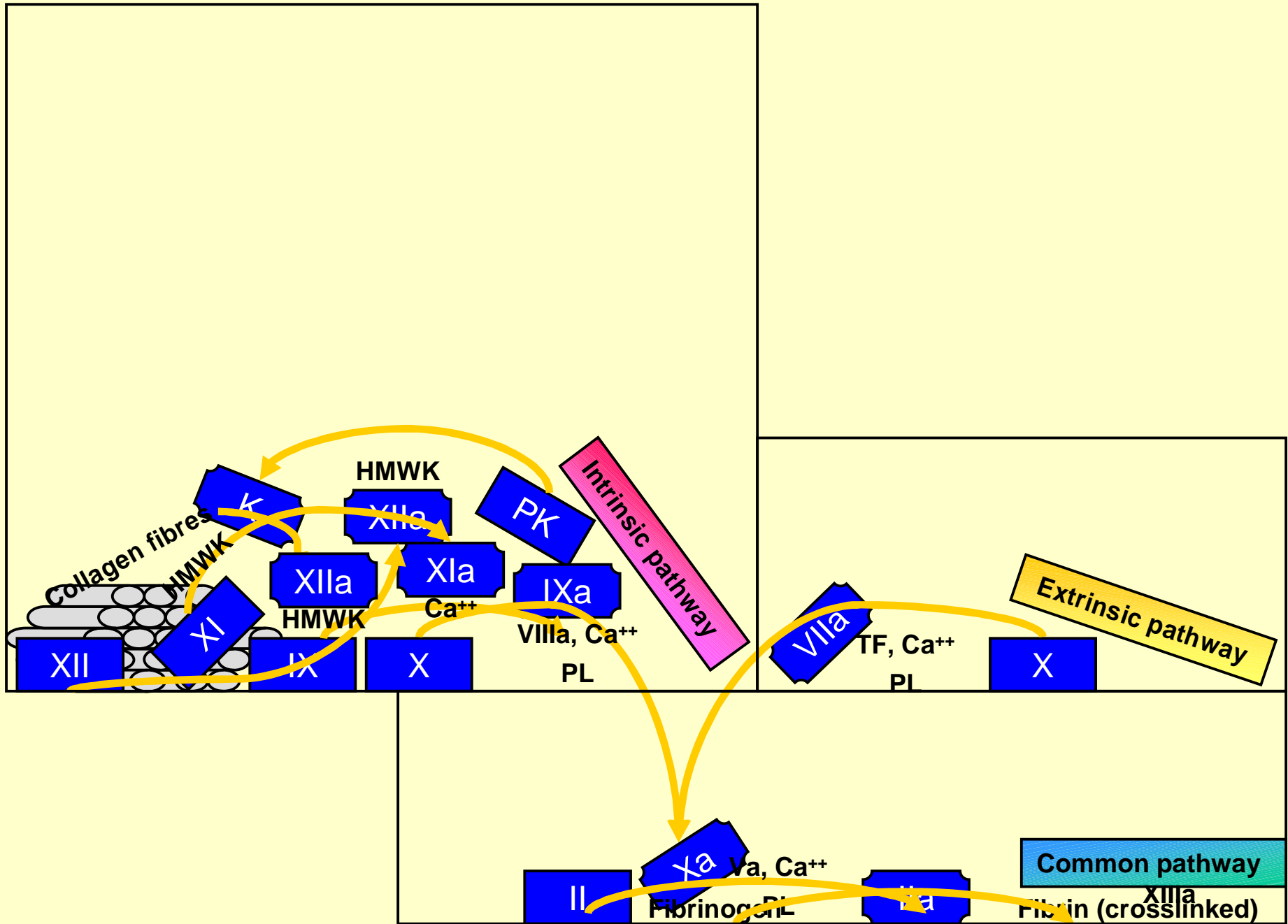
DRVVT + DAPTT is often quoted as having high detection rates

BUT:

is there more to it than that?

Collagen fibres





Sensitivity & specificity of commercial reagents

Although the three pathways of the classical cascade theory do not operate *in vivo*, assay design means they do *in vitro*

DAPTT is an intrinsic pathway based assay

DRVVT is a common pathway based assay

.....so something is missing

Extrinsic pathway based assays

Dilute prothrombin time (DPT)



Screening test uses a high dilution of thromboplastin

Confirmatory test uses lower dilution or neat thromboplastin

More sensitive with recombinant reagents

Identifies LA that are unreactive in DRVVT and DAPTT (& those that are....well, some)

Poor specificity relative to other LA assays

Wide variation in:

reagents

dilutions

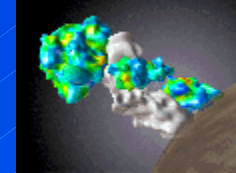
technique

result assessment

Standardised reagents becoming available

Extrinsic pathway based assays

Activated Seven Lupus Anticoagulant (ASLA) assay



Represents a new generation of LA assay

Activation of in vitro coagulation using a human derived coagulation factor

Coagulation activated by recombinant FVIIa (rFVIIa)

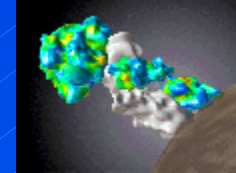
rFVIIa directly activates FX without tissue factor or thromboplastin

Dilute phospholipid for screen and PNP/high phospholipid reagent for confirmatory test

Results interpreted as for DRVVT

Extrinsic pathway based assays

Activated Seven Lupus Anticoagulant (ASLA) assay



Sensitive and specific extrinsic pathway based assay for LA

Sensitive to LA detectable in PT, APTT and snake venom based assays

Identifies LA undetectable in intrinsic and common pathway based assays

More specific than DPT, even when using recombinant thromboplastin

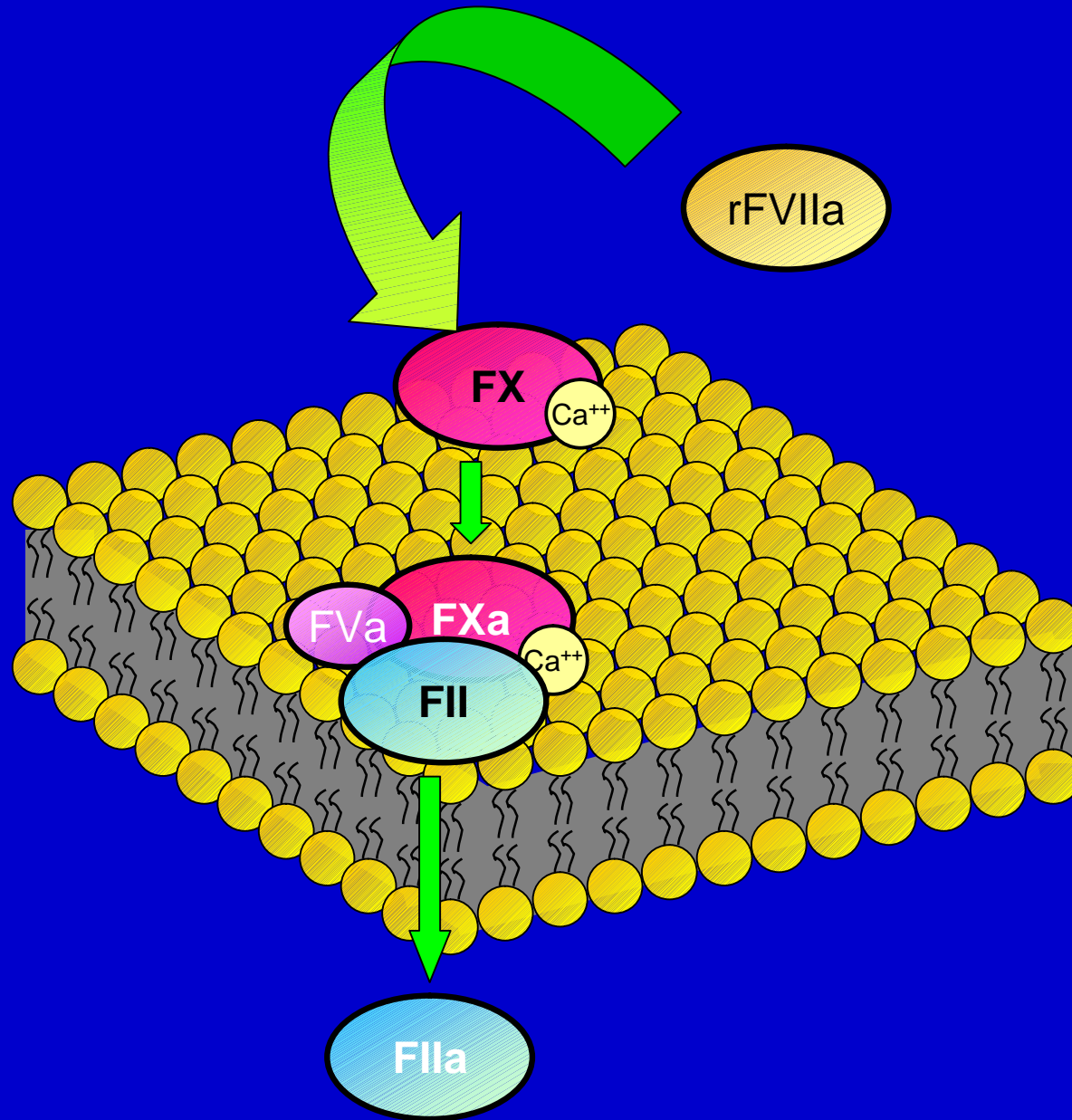
Affected by

factor deficiencies (II, V, X)

therapeutic anticoagulation

non-phospholipid dependent inhibitors

ASLA



Combinations of assays generating positive interpretations for LA in 26 patients with thrombotic disease previously classified as LA negative by a guideline compliant detection regime

	Assay or assay combinations								
	ASLA	DPT	ASLA DPT	ASLA DRVVT 1	ASLA DRVVT 2	ASLA DRVVT 1 DRVVT 2	ASLA DRVVT 2 DAPTT TSVT	DRVVT 3	DAPTT
Number of positive interpretations	10	3	5	2	1	1	1	1	2

So what does this tell us?

Primary detection regime was DRVVT + KCT (i.e. intrinsic and common pathway assays)

Total of 23 of 26 (88.5%) were positive in extrinsic pathway based assays

18 of 26 (69%) were **ONLY** positive in extrinsic pathway based assays

Epitope specificity

FVII

FVIIa

TF

HOLD ON A MINUTE

Combinations of assays generating positive interpretations for LA in 26 patients with thrombotic disease previously classified as LA negative by a guideline compliant detection regime

	Assay or assay combinations								
	ASLA	DPT	ASLA DPT	ASLA DRVVT 1	ASLA DRVVT 2	ASLA DRVVT 1 DRVVT 2	ASLA DRVVT 2 DAPTT TSVT	DRVVT 3	DAPTT
Number of positive interpretations	10	3	5	2	1	1	1	1	2

Dilute Russell's Viper Venom Time



DRVVT

Best thing since sliced bread.

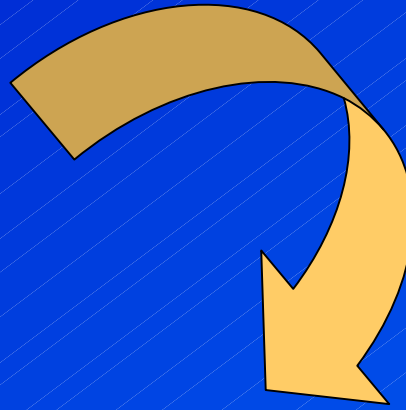


Photo: Sandilya Theuerkauf

Sensitivity & specificity of commercial reagents

Study used 5 sets of DRVVT reagents and also the Textarin/Ecarin ratio

Considerable variation in sensitivity & specificity between apparently identical reagents

Differences in sensitivity & specificity when same reagent is used on a different type of analyser

Thromb Haemost 1999; 81: 758-762

Sensitivity & specificity of commercial reagents

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Ameleung KC4A				
Manchester	76	96	96	78
Unicorn Diagnostics	83	81	83	81
Instrumentation Laboratory	90	42	63	79
American Diagnostica	72	100	100	76
Gradipore	90	54	68	82
Sysmex CA 6000				
Manchester	76	62	69	70
Unicorn Diagnostics	90	69	76	86
Instrumentation Laboratory	90	46	65	80
American Diagnostica	90	100	100	90
Gradipore	97	23	58	86
Textarin/Ecarin ratio	62	73	72	63

So why the discrepancies?

Phospholipid

source
degree of dilution
relative amounts of PS, PE, PC

Technique

clot detection method
combined or separate reagents

Antibodies

heterogeneity
epitope specificity

Venom

Native/purified venom
FX activator
FX + FV activator
Sub-species

**Pakistan Russell's Viper
(*Daboia russelli*)**



**Indian Russell's Viper
(*Daboia russelli russelli*)**



**Burmese/Siamese Russell's Viper
(*Daboia russelli siamensis*)**



**Sri Lankan Russell's Viper
(*Daboia russelli pulchella*)**



Taiwan Russell's Viper
(*Daboia russelli formosensis*)



**Javanese Russell's Viper
(*Daboia russelli sublimitis*)**



Indonesian Russell's Viper
(*Daboia russelli limitis*)



RVV heterogeneity

300 samples from a thrombotic population

48 of 300 (16%) positive for LA with primary reagent

All 300 samples analysed with 4 other RVV using identical phospholipid and technique

86 of 300 (28.6%) positive for LA

RVV reagent combination	Number of samples positive for LA in this combination
Sigma only	20
All 5 RVV reagents	15
American Diagnostica only	12
Diagen, Manchester, Diagnostica Stago, American Diagnostica	8
Sigma, American Diagnostica	5
Manchester only	4
Sigma, Diagen, American Diagnostica	3
Diagen, Manchester, Diagnostica Stago	3
Diagen, Diagnostica Stago	2
Diagen only	2
Diagen, Manchester, American Diagnostica	2
Other combinations where only one sample was positive	10

Which tests to use – and how many ?

Use of a single test to screen for LAs results in poor detection rates, is clinically dangerous and scientifically indefensible

DRVVT + DAPTT is good, but not good enough

DRVVT + DAPTT + ASLA or DPT more widely encompasses antibody heterogeneity/variation in epitope specificity

Which tests to use – and how many ?

β 2-GPI & prothrombin have been described as the main antigenic targets for APA with LA activity

FX / FVII / FVIIa / TF have also been implicated

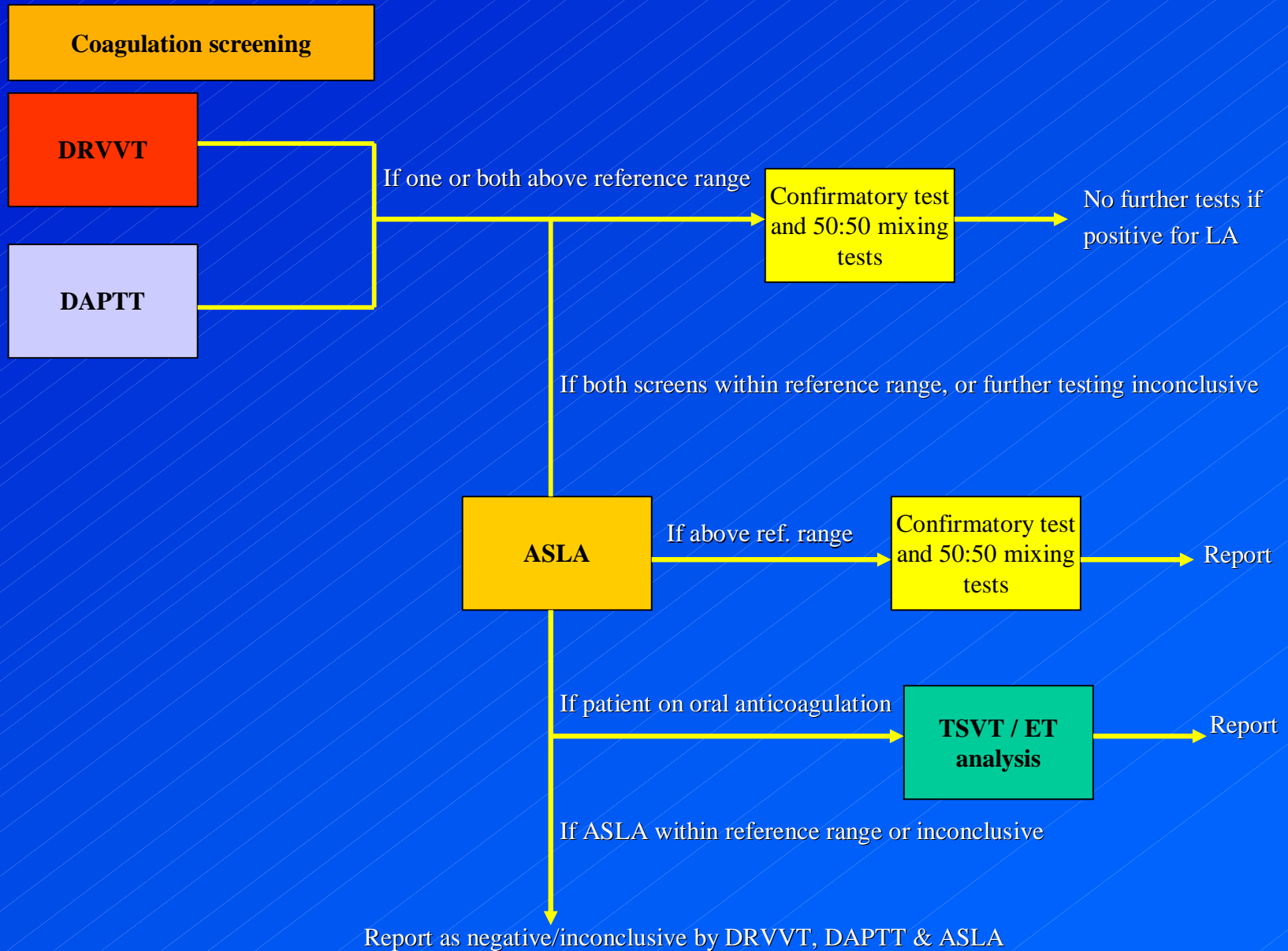
Antibodies to protein C, protein S, Annexin V not detected in clotting based assays

Likely outcome will be a recommendation to use 3 tests to detect LAs that affect different pathways

Intrinsic pathway APTT / DAPTT / KCT / SCT

Extrinsic pathway DPT / ASLA

Common pathway DRVVT / (TSVT / Textarin time)



Which tests to use – and how many ?

Even that will not detect all LA

DRVVT reagent and technique variation

APTT/DAPTT reagent and technique variation (be wary of your routine APTT reagent)

DPT reagent and technique variation

Availability of alternative reagents

Perform confirmatory test + mixing studies on assays that generate abnormal screening test

i.e. You cannot screen with APTT and then perform DRVVT + confirmatory tests to confirm that the abnormality in the APTT was due to a LA

Reference ranges

Kits		Manchester	Unicorn	American Diagnostica
Centre 1	Mean ratio	0.99	1.02	0.96
	± 2SD of mean	0.79 – 1.19	0.86 – 1.18	0.86 – 1.06
Centre 2	Mean ratio	0.96	1.01	0.97
	± 2SD of mean	0.86 – 1.06	0.87 – 1.15	0.87 – 1.07
Centre 3	Mean ratio	0.99	1.06	0.99
	± 2SD of mean	0.75 – 1.23	0.98 - 1.14	0.78 – 1.20
Centre 4	Mean ratio	1.07	1.03	1.02
	± 2SD of mean	0.95 – 1.19	0.91 – 1.15	0.90 – 1.14
All centres	Mean ratio	1.03	1.03	0.99
	± 2SD of mean	0.85 – 1.21	0.89 – 1.17	0.87 – 1.11

Reference ranges

Arbitrary DRVVT ratio of 1.1 as the cut-off value is inappropriate

Adoption of manufacturer's cut-off value is inappropriate

Reference ranges for all LA tests must be locally derived

Calculation used to assess phospholipid dependence affects interpretation

False negative screening test results

The ability of a given concentration of antibody to prolong a clotting time above the reference range may depend on the clotting time an individual plasma would have had without the influence of the LA



For example:

Baseline ratios:	DRVVT screen	0.90	(Ref. range 0.84 – 1.16)
	DRVVT confirm	0.91	(Ref. range 0.88 – 1.12)
Appearance of LA:	DRVVT screen	1.13	
	DRVVT confirm	0.91	
	% ratio correction	19.5	
	Normalised ratio	1.24	

False negative results

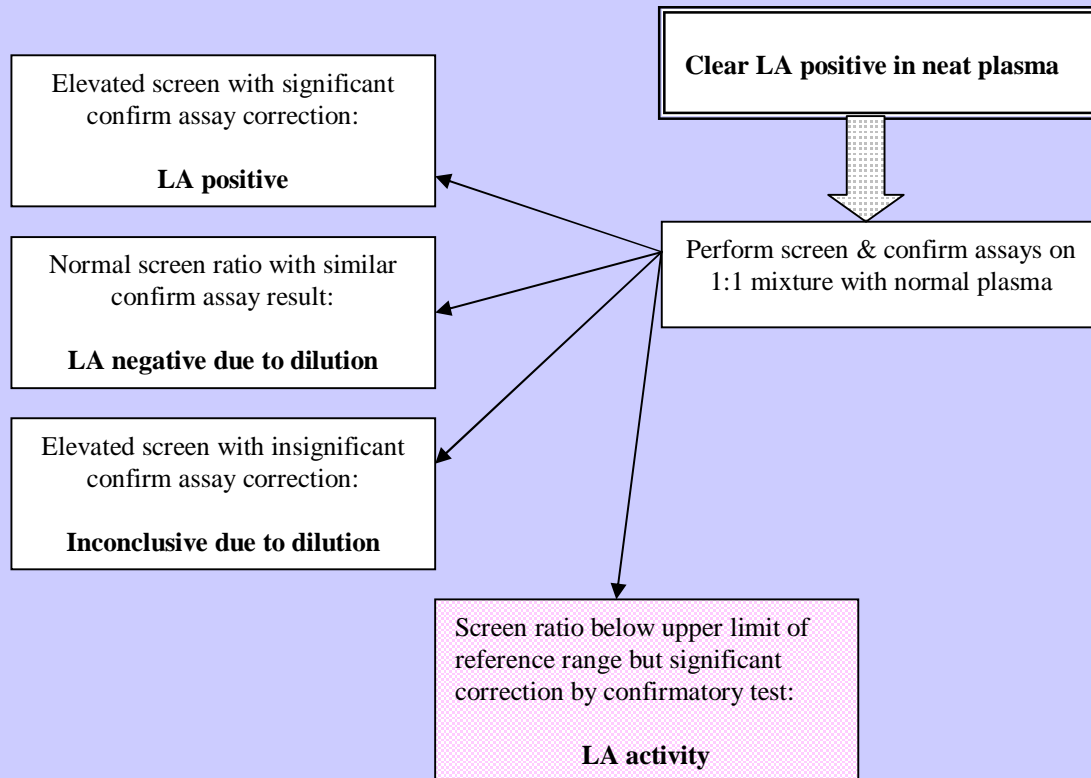
Test	Calculation	Sensitivity (%)
DAPTT (clotting time in seconds) as a screening test		53.5
Ratio of clotting times	$\frac{\text{DAPTT clotting time (s)}}{\text{Conc. PL APTT clotting time (s)}}$	68.1
DAPTT ratio as a screen	$\frac{\text{Test DAPTT clotting time (s)}}{\text{NP DAPTT clotting time (s)}}$	84.7
Normalised ratio of clotting times on 1:1 mixtures	$\frac{\text{Test screen (s) / Test confirm(s)}}{\text{NP screen (s) / NP confirm (s)}}$	95.1

Use of screening result as the only criterion for assessing abnormality does not take into account LA that do not prolong the clotting time above the reference range

All samples should be tested with screen and confirmatory reagents

Result should be evaluated in relation to each other and the results with normal plasma

False negative results

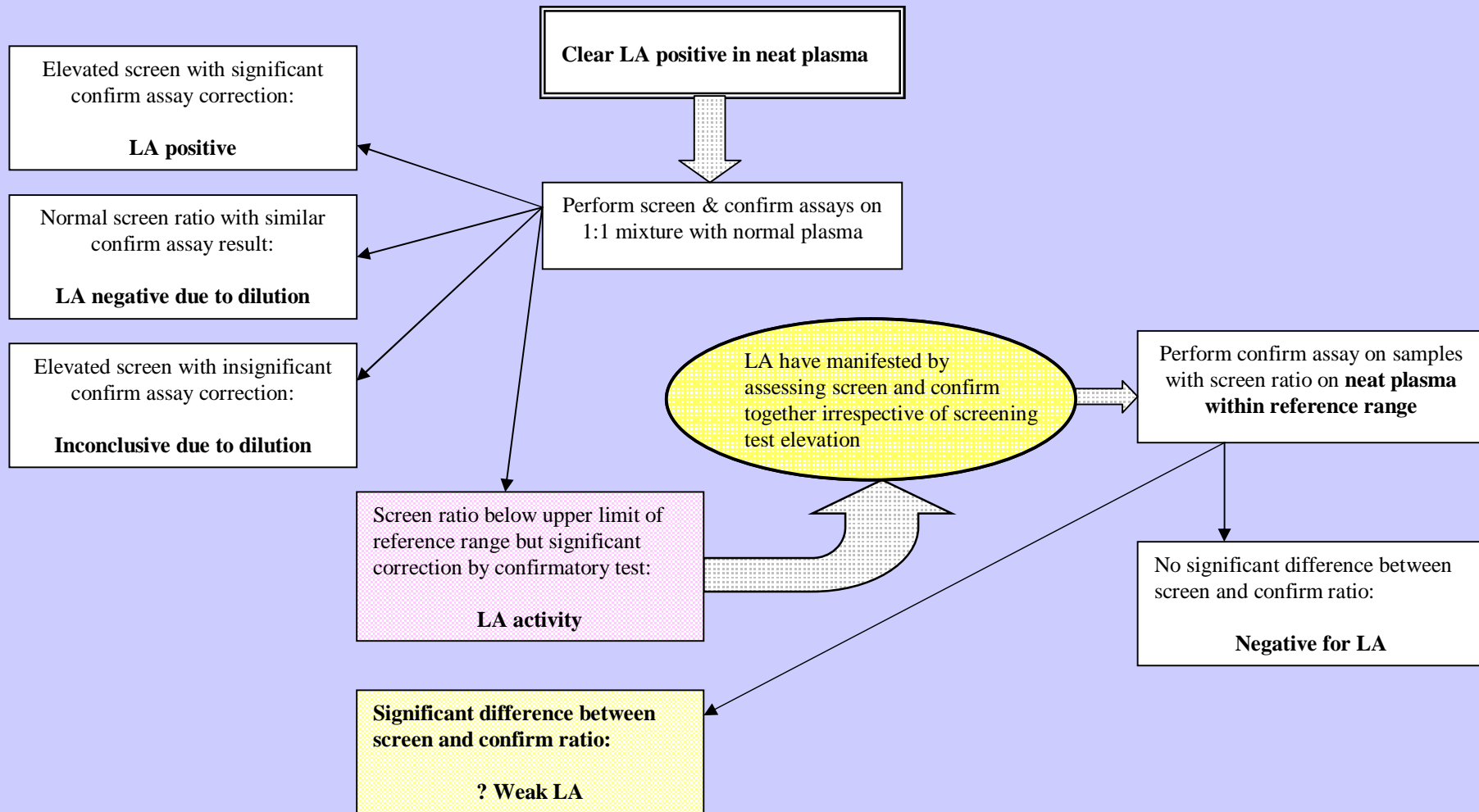


False negative results

Screen & confirm data on samples LA+ in neat plasma and with mixing test screens within reference ranges yet discordant confirm ratios

DAPTT	Screen ratio	Confirm ratio	1:1 Mixing study screen ratio	1:1 Mixing study confirm ratio	% correction of ratio (mix)	Normalised ratio (mix)
RR (ratio)	0.81 – 1.23	0.81 – 1.13	0.86 – 1.15	0.85 – 1.09		0.99 – 1.11
Result 1	1.43	1.09	1.11	0.93	16.2	1.19
Result 2	1.37	1.00	1.12	0.99	11.6	1.13
Result 3	1.38	1.09	1.12	0.96	14.3	1.17
Result 4	1.26	0.93	1.04	0.90	13.5	1.16
Result 5	1.26	1.08	1.12	0.98	12.5	1.14
Result 6	1.32	0.92	1.05	0.93	11.4	1.13
Result 7	1.29	1.02	1.08	0.96	11.1	1.13
Result 8	1.43	1.00	1.15	0.94	18.3	1.22
Result 9	1.40	0.97	1.11	0.93	16.2	1.19
Result 10	1.29	1.03	1.08	0.93	13.9	1.16
Result 11	1.51	0.94	1.09	0.93	14.7	1.17
Result 12	1.57	1.16	1.09	0.97	11.0	1.12
Result 13	1.39	0.93	1.13	0.88	22.1	1.28

False negative results



Diagrammatic representation of study design. Some LA are diluted in 1:1 mixing studies such that the screening test ratio is below the upper limit of the reference range, a sub-set of which retain screen and confirm discordance. If these genuine LA can be detected by assessing screen and confirm data in conjunction irrespective of screening test elevation above the reference range upper limit, could applying the same principle to results on neat plasma reveal weak LA that do not prolong a patient's baseline clotting time to above the reference range? LA: lupus anticoagulant

False negative results

	Percent correction of screen ratio by confirm ratio	Screen ratio range	Mean/median screen ratio	Confirm ratio range	Mean/median confirm ratio	Mean/median percent correction	Mean/median NR
DRVVT (n=166)	<10% (n=147)	0.79 – 1.17	0.97 / 0.96	0.84 – 1.34	0.98 / 0.97	-1.44 / -0.99	0.99 / 0.99
	>10% (n=19)	1.02 – 1.19	1.11 / 1.12	0.90 – 1.03	0.97 / 0.97	13.33 / 12.07	1.15 / 1.14
DAPTT (n=184)	<10% (n=121)	0.74 – 1.21	0.95 / 0.95	0.71 – 1.25	0.91 / 0.90	4.04 / 4.72	1.04 / 1.05
	>10% (n=63)	0.79 – 1.23	1.03 / 1.01	0.68 – 1.07	0.89 / 0.89	13.89 / 13.01	1.16 / 1.16
ASLA (n=117)	<10% (n=108)	0.81 – 1.14	1.01 / 1.01	0.82 – 1.43	1.02 / 1.01	-1.27 / 0.00	0.99 / 1.00
	>10% (n=9)	0.88 – 1.09	1.01 / 1.03	0.76 – 0.97	0.89 / 0.90	12.73 / 12.50	1.14 / 1.14

Screen and confirm data plus phospholipid dependence calculations for samples with screen ratios below the upper reference range limits by dilute Russell's viper venom time, dilute activated partial thromboplastin time or activated seven lupus anticoagulant assay

Diagnostic criteria for LA

ISTH guidelines

1. Prolongation of at least one phospholipid dependent test
 - 1a Assess screen and confirm discordance
 - 1b Minimum 3 tests, greater detail about supplementary tests
2. Evidence of inhibitory activity (mixing tests)
3. Evidence that inhibitory activity is dependent on phospholipid
4. Distinguish from other coagulopathies that may mask, mimic or co-exist

Can you always demonstrate inhibition ?

NO

Mixing with normal plasma results in unavoidable dilution of LA antibodies

Reduces sensitivity and can lead to false negative results

No consensus on how to interpret results

As transient LA disappear, mixing studies normalise before the abnormality in neat plasma

Negative mixing studies in LA +ve patients

Result interpretations for 600 plasmas +ve for LA by single assays/combinations of DRVVT, DAPTT, ASLA & TSVT

Assay	Total positive for LA in neat plasma	Number positive for LA in 1:1 mixing studies	Number negative for LA in 1:1 mixing studies	Number with elevated screen but insufficient correction by confirmatory test in 1:1 mixing studies
DRVVT	325	111	196	18
DAPTT	379	113	263	3
ASLA	69	19	38	12
TSVT	28	17	3	8
Total	801	260	500	41

Positive mixing studies defined as $\geq 10\%$ correction of elevated screen by confirmatory test

260 of 801 (32.5%) had positive mixing studies

Negative mixing studies in LA +ve patients

Reference ranges for DRVVT, DAPTT, ASLA & TSVT on Sysmex CA1500 analyser (n=40)

Assay	Screen (ratio) (Neat plasma)	Screen (ratio) (1:1 mix with NP)	Confirm (ratio) (Neat plasma)	Confirm (ratio) (1:1 mix with NP)
DRVVT	0.86 – 1.19	0.90 – 1.10	0.83 – 1.13	0.94 – 1.13
DAPTT	0.81 – 1.23	0.86 – 1.15	0.81 – 1.13	0.85 – 1.09
ASLA	0.82 – 1.14	0.89 – 1.09	0.86 – 1.10	0.91 – 1.07
TSVT	0.90 – 1.10	0.94 – 1.06	0.88 – 1.12	0.92 – 1.08

Lower upper reference range values for screening tests in 1:1 mixing tests

Further 33 positive mixing studies by DRVVT

51 by DAPTT

5 by ASLA

1 by TSVT

Total of 91 additional positive interpretations (11.2% of the total)

Left 451 of 801 (56.3%) still negative for LA

Negative mixing studies in LA +ve patients

Neat plasma screening test data for LA + samples

Assay	Neat plasma screen results for all 600 samples		Neat plasma screen results for samples with negative 1:1 mixing studies	
	Range of screening test ratios	Mean ratio	Range of screening test ratios	Mean ratio
DRVVT	1.20 – 5.51	1.47	1.20 – 1.87	1.30
DAPTT	1.24 – 4.68	1.55	1.24 – 2.43	1.37
ASLA	1.15 – 2.34	1.32	1.15 – 1.55	1.23
TSVT	1.13 – 2.25	1.54	1.13 – 1.20	1.16

Usually considered that it is weak LA that are diluted in mixing studies

Some strong LA in DRVVT & DAPTT & moderately strong in ASLA

? Contribution of epitope specificity

Do you report tests with LA in neat plasma but negative mixing studies as positive or negative ?

If you report as **POSITIVE**, you are contravening national and international guidelines

If you report as **NEGATIVE**, you are ignoring a clearly positive LA in neat plasma

Recognising the limitations of mixing studies, and providing that other causes of prolonged clotting times are excluded, report as **POSITIVE**

Many laboratory research studies on LA exclude mixing tests

Why bother with mixing studies if detecting LA in neat plasma only is sufficient to report a positive result ?

Exclusion of mixing studies contravenes national and international guidelines

LA are *in vitro* inhibitors, so demonstration of inhibitory activity increases the likelihood of obtaining the correct diagnosis

Although rare, the lupus co-factor effect can only be demonstrated in mixing studies

Mixing studies are useful when other coagulopathies co-exist with LA

Diagnostic criteria for LA

ISTH guidelines

1. Prolongation of at least one phospholipid dependent test
 - 1a Assess screen and confirm discordance
 - 1b Minimum 3 tests, greater detail about supplementary tests
2. Evidence of inhibitory activity (mixing tests)
 - 2a Useful to increase specificity but **cannot be** an essential diagnostic criterion
3. Evidence that inhibitory activity is dependent on phospholipid
4. Distinguish from other coagulopathies that may mask, mimic or co-exist

Available procedures for LA confirmation

1. **Mixing tests with normal plasma to demonstrate inhibition**

Usually 1:1 dilution

Can correct factor deficiencies and oral anticoagulant effect

Can dilute out LA to undetectable levels

1:4 (normal:test) may not dilute out oral anticoagulant effect

2. **Procedures for confirmation of phospholipid dependence**

Platelet neutralisation procedure (PNP)

Platelet derived vesicles

High phospholipid concentration

Pure phospholipids (i.e. inosithin, phosphatidyl serine)

Hexagonal phase phospholipids

LA insensitive reagents

Comparison of screening test result with a similar but LA insensitive test

Agarose gel diffusion

Demonstration of phospholipid dependence

Screening test



Confirmatory test



Coagulation factors

F

Phospholipid molecules

O

Lupus anticoagulant antibodies

Y

Demonstration of phospholipid dependence

Not all LA are neutralised/swamped/bypassed by high phospholipid confirmatory reagents

Study showed that 59.3% of elevated DRVVT ratios did not achieve significant correction (PT/APTT normal)

Concentration of PNP reagent doubled and venom concentration increased by 20%

Use of modified confirmatory test confirmed 82.2% as LA c.f. 40.7% with conventional test

Similar effect with higher concentration of rabbit brain phospholipid reagent

Concentration of rabbit brain PL does not need to be as high as platelet PL to achieve higher degree of correction

Conventional and modified PNP did not generate significant correction in non-LA coagulopathies

Demonstration of phospholipid dependence

Standard and modified PNP results on plasmas with elevated DRVVT in presence of normal PT/APTT

Sample	DRVVT (ratio) (RR 0.90 – 1.09)	% correction by PNP	% correction by modified PNP
1	1.15	2.6	10.4
2	1.19	4.2	10.9
3	1.29	5.4	19.4
4	1.10	5.5	11.9
5	1.36	5.9	27.9
6	1.19	6.7	16.0
7	1.14	7.0	16.7
8	1.32	7.6	18.2
9	1.92	8.3	22.9
10	1.17	9.4	23.1

Demonstration of phospholipid dependence

Percentage correction of ratio

Convert clotting times of screening and confirmatory tests to ratios:

$$\frac{\text{Clotting time of patient plasma (s)}}{\text{Clotting time of normal plasma (s)}}$$

Apply

$$\frac{\text{Screen ratio} - \text{Confirm ratio}}{\text{Screen ratio}} \times 100$$

>10% positive for LA

Normalised ratio

$$\frac{\text{Screening test ratio}}{\text{Confirmatory test ratio}}$$

> 1.2 positive for LA

% correction of clotting time

$$\text{Screen ratio} = \frac{\text{Test (s)} - \text{Control (s)}}{\text{Control (s)}}$$

$$\text{Confirm ratio} = \frac{\text{Test (s)} - \text{Control (s)}}{\text{Control (s)}}$$

Then apply:
$$\frac{(\text{Screen ratio} - \text{Confirm ratio}) \times 100}{\text{Screen ratio}}$$

> 65% is significant

Test - Confirm ratio

$$\frac{\text{Screening test (s)}}{\text{Confirmatory test (s)}}$$

Ratio >2SD above mean normal ratio indicates LA

Diagnostic criteria for LA

ISTH guidelines

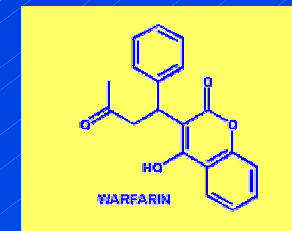
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 - 1a Assess screen and confirm discordance
 - 1b Minimum 3 tests, greater detail about supplementary tests
2. Evidence of inhibitory activity (mixing tests)
 - 2a Useful to increase specificity but **cannot be** an essential diagnostic criterion
3. Evidence that inhibitory activity is dependent on phospholipid
 - 3a **Crucial**; all confirmatory reagents are not what they seem
4. Distinguish from other coagulopathies that may mask, mimic or co-exist

Distinguish from other coagulopathies

- 1. Coagulation screen**
Use LA-insensitive APTT and approximately 75% will be normal but follow with LA-sensitive DAPTT + confirmatory tests in LA screening itself
Quality of normal plasma used in PT/APTT screening
- 2. Thrombin time + Reptilase time to exclude heparin**
Heparin neutralisers – beware false sense of security
PNP reagents
- 3. Warfarin – mixing studies or warfarin insensitive reagents**
- 4. Factor assays (multiple sample dilutions)**
Non-phospholipid dependent inhibitor assays
- 5. Other LA assays**



Detecting LA in orally anticoagulated patients



Mixing studies often inconclusive when using assays sensitive to the warfarin effect

INR	DRVVT	Confirm	1:1 Screen	1:1 Confirm	DAPTT	Confirm	1:1 Screen	1:1 Confirm
(2.0 – 4.5)	(0.86 – 1.19)	(0.83 – 1.13)	(0.90 – 1.10)	(0.94 – 1.13)	(0.81 – 1.23)	(0.81 – 1.13)	(0.86 – 1.15)	(0.85 – 1.09)
3.15	2.87	2.39	1.46	1.07	3.01	2.66	1.52	1.07

Neat plasma unreliable

Confirm in 1:1 mixture goes back into reference range

INR	DRVVT	Confirm	1:1 Screen	1:1 Confirm	DAPTT	Confirm	1:1 Screen	1:1 Confirm
3.15	2.87	2.39	1.46	1.23	3.01	2.66	1.52	1.28

Confirm in 1:1 mixture does not go back into reference range – suggestive of LA

INR	DRVVT	Confirm	1:1 Screen	1:1 Confirm	DAPTT	Confirm	1:1 Screen	1:1 Confirm
3.15	2.87	2.39	1.07	1.00	3.01	2.66	1.11	1.05

Neat plasma unreliable, mixing studies negative BUT a weak LA cannot be excluded

Textarin Time



Textarin venom fraction directly activates prothrombin (FII)

Dependent on FV, phospholipid and calcium ions

Most conventional assays require mixing tests for warfarinised patients

Textarin time insensitive to the effects of warfarin

Dilution of phospholipid makes Textarin time sensitive to LA

Originally described with Ecarin time as confirmatory test

Can also be used with high phospholipid confirmatory test

Australian Eastern Brown Snake (*Pseudonaja textilis*)



Ecarin time (ET)



Ecarin venom fraction directly activates prothrombin (FII)

Independent of other coagulation factors, phospholipid and calcium ions

ET insensitive to the effects of warfarin

Originally described as confirmatory test for Textarin time

Saw-scaled or Carpet Viper (*Echis carinatus*)





Textarin/Ecarin ratio



Textarin and Ecarin venom fractions directly activate FII but differ in phospholipid requirements

Ecarin time is totally insensitive to LAs

Textarin (PL & Ca⁺⁺) and Ecarin reagents are titrated to generate similar clotting times with normal plasma

Results are expressed as the Textarin/Ecarin ratio:
$$\frac{\text{Textarin time (s)}}{\text{Ecarin time (s)}}$$

If a LA is present, Textarin time should be elevated and Ecarin time unaffected - giving raised TE ratio

Similar clotting times with factor deficiencies, oral anticoagulation, heparin therapy, specific inhibitors

Upper limit of normal: TE ratio of 1.3

False positives with FV deficiency or FV inhibitors

Taipan snake venom time (TSVT)



Taipan venom fraction directly activates prothrombin (FII)

Venom fraction contains FXa-like sub-unit and FVa-like co-factor unit

Dependent on phospholipid and calcium ions

TSVT insensitive to the effects of warfarin

Works in the presence of des carboxyprothrombin to generate thrombin and meizothrombin

Dilution of phospholipid makes TSVT sensitive to LA

Originally described with PNP as confirmatory test

Interpreted as for DRVVT and confirmatory tests

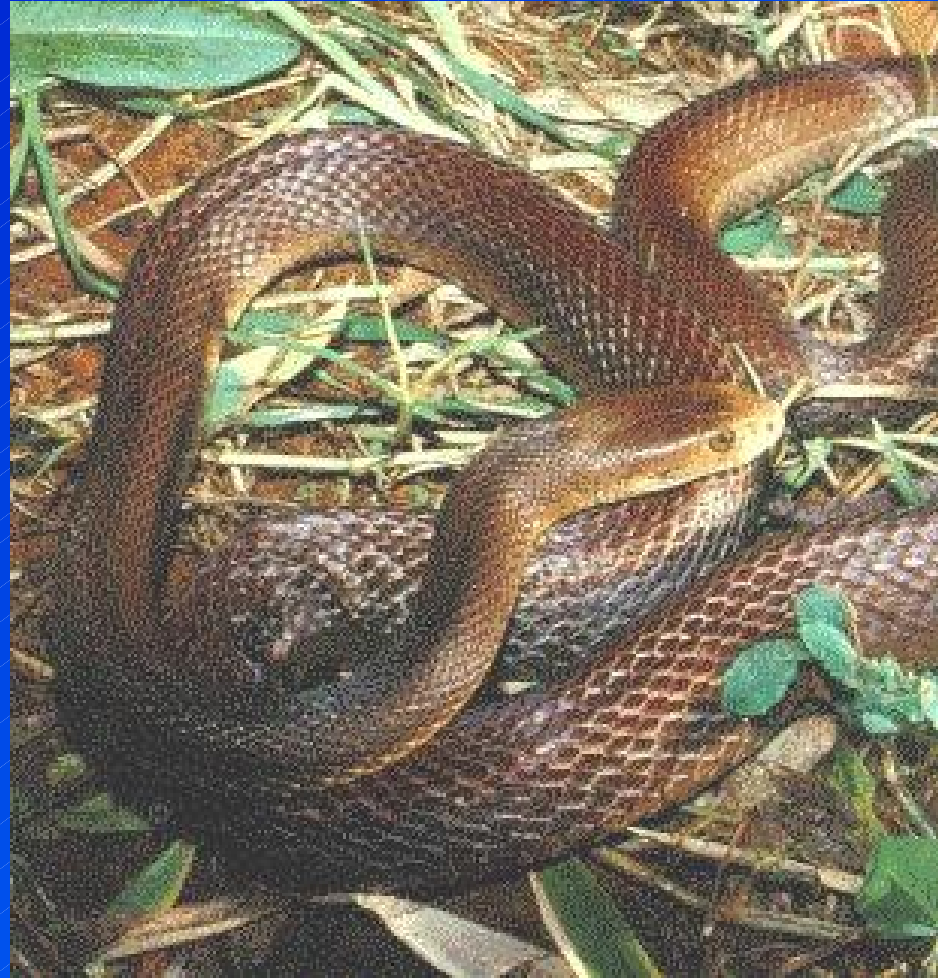
Use of Ecarin time as confirmatory test increases sensitivity to LA in warfarinised patients

TSVT/ET for warfarinised patients

Analysis of warfarinised known LA by TSVT + PNP and ET

Sample	TSVT ratio (RR: 0.91 – 1.07)	% correction by PNP	% correction by Ecarin time ratio
1	1.02		
2	1.05		
3	0.95		
4	1.07		
5	1.25	16.8	24.8
6	1.29	14.0	23.3
7	1.13	7.1	14.2
8	1.17	-1.3	17.1
9	1.44	9.7	35.8
10	1.20	-0.8	14.2
11	1.27	10.2	26.8
12	1.13	0.0	11.5
13	1.45	6.9	26.9
14	1.56	12.2	33.3
15	1.21	5.0	18.1
16	1.30	-6.2	20.8

Coastal Taipan (*Oxyuranus scutellatus*)



Diagnostic criteria for LA

ISTH guidelines

1. Prolongation of at least one phospholipid dependent test

1a Assess screen and confirm discordance

1b Minimum 3 tests, greater detail about supplementary tests

2. Evidence of inhibitory activity (mixing tests)

2a Useful to increase specificity but **cannot be** an essential diagnostic criterion

3. Evidence that inhibitory activity is dependent on phospholipid

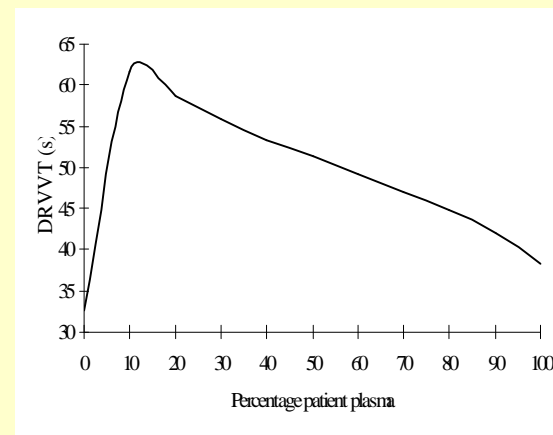
3a **Crucial**; all confirmatory reagents are not what they seem

4. Distinguish from other coagulopathies that may mask, mimic or co-exist

4a **Crucial**; be sure everything is excluded but sometimes you can't see the LA for the trees

Interesting patient

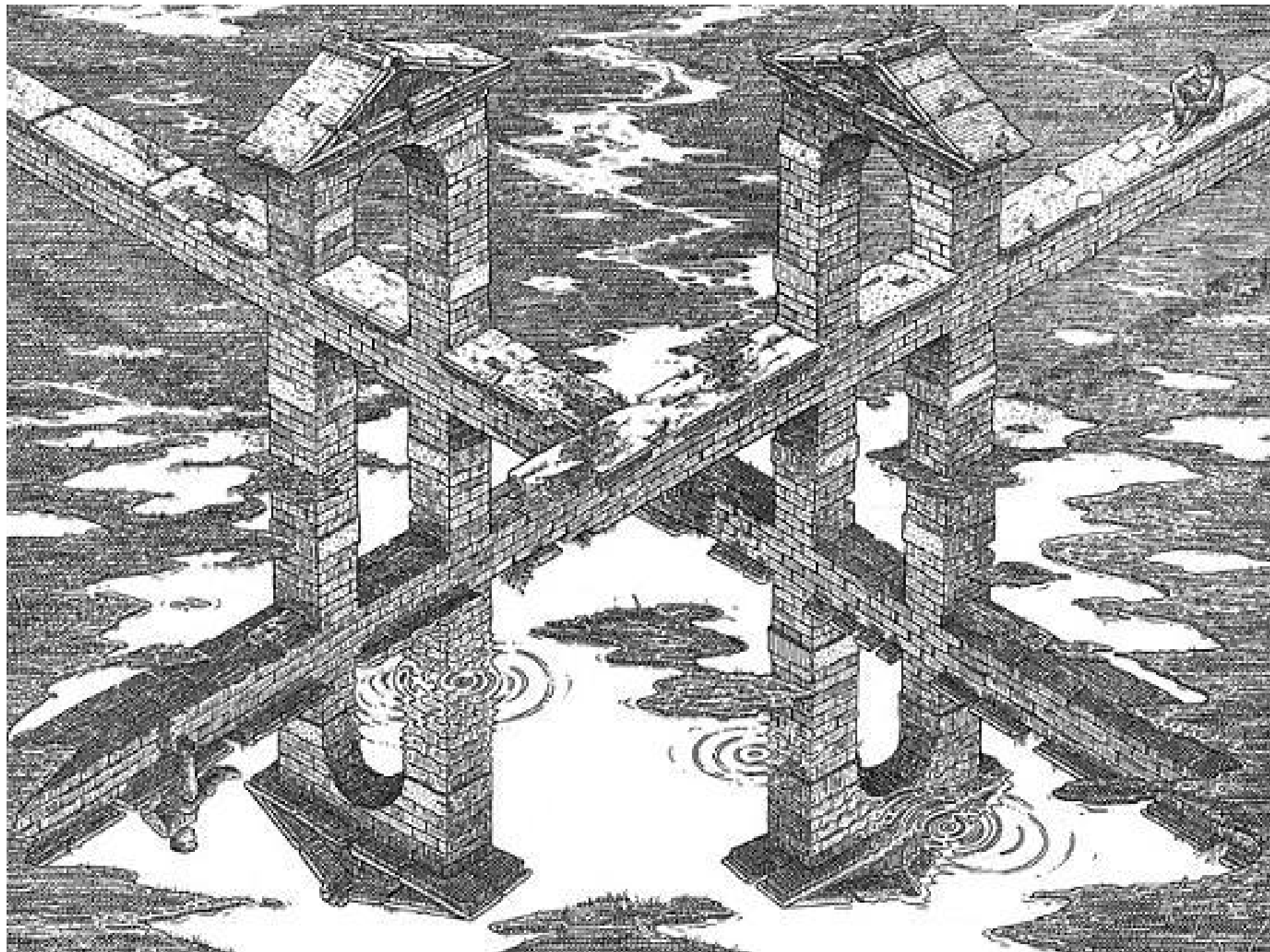
Assay	Result	Units	Reference range
Prothrombin time (INR) with Innovin®	2.75	Ratio	0.90-1.10
50:50 mixture INR with Innovin®	3.08	Ratio	0.90-1.10
Prothrombin time (INR) with brain thromboplastin	1.10	Ratio	0.80-1.10
50:50 mixture INR with brain thromboplastin	1.00	Ratio	0.80-1.10
APTT with Actin FS®	0.78	Ratio	0.85-1.16
50:50 mixture APTT with Actin FS®	0.80	Ratio	0.85-1.16
APTT with Platelin®	0.89	Ratio	0.82-1.18
50:50 mixture APTT with Platelin®	0.88	Ratio	0.82-1.18
Clauss fibrinogen	6.40	g/L	1.67-5.43



DRVVT based Exner curve demonstrating the lupus anticoagulant co-factor effect. The patient is deficient in a plasma co-factor essential for the lupus anticoagulant to exert its *in vitro* anticoagulant effect. Mixing with normal plasma supplies the co-factor, the effect of which is reduced as the amount of co-factor deficient patient plasma is increased.

Assay	Screen (ratio)	Reference range	PNP (ratio)	Reference range	Modified PNP (ratio)	Screen EVM (ratio)	PNP EVM (ratio)	Ecarin time (ratio)	Reference range	Ecarin EVM (ratio)
DRVVT	1.23	0.90-1.09	1.25	0.90-1.09	0.85	1.77	1.31	-	-	-
ASLA	1.32	0.88-1.12	1.77	0.90-1.11	1.11	2.00	1.40	-	-	-
TSVT	1.11	0.91-1.07	1.91	0.90-1.06	-	1.33	1.30	0.89	0.92-1.08	0.90







International guidelines (1995)	British guidelines (2000)	Review in light of recent evidence ?
Attention to pre-analytical variables	Attention to pre-analytical variables Quality of normal control plasma	No , mainly platelet reduction
Coagulation screen to exclude factor deficiency, anticoagulants, inhibitors. Factor assays with at least 2 dilutions	Coagulation screen to exclude factor deficiency, anticoagulants, inhibitors	No , crucial but could suggest use of LA-insensitive APTT
2 or more tests of different principles At least one should be based on low PL concentration	Use sensitive APTT If prolonged; mixing tests &/or PNP 2 nd test, preferably KCT or DRVVT Supplementary tests – DPT, other venoms	Yes 3 tests, one from each ‘pathway’ All must have confirmatory test Greater emphasis on supplementary tests Greater emphasis on 2 nd line reagents for same test KCT is not suitable as 2 nd test to APTT
Prolongation of PL dependent test	Prolongation of PL dependent test	Yes Assess screen and confirm on all patients
Inhibitory activity using mixing tests Can be incorporated into initial screen	Inhibitory activity using mixing tests Loss of sensitivity due to dilution effect	Yes Improves specificity but cannot be an essential criterion Do not use in initial screen
Confirmatory test Based on method giving abnormal screen	Confirmatory test	Yes Crucial but make aware of HNR LA TSVT/ET for HNR LA
Little about warfarin	DRVVT 1:1 mixing studies (with confirm) TSVT/Textarin time + confirm & mixtures	Yes More prescriptive about what is available
Neutraliser &/or mix to counteract heparin False positives with PNP	Rarely necessary in heparinised patients Some reagents contain heparin neutraliser	Yes , nothing new but better detail required



Thank you





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