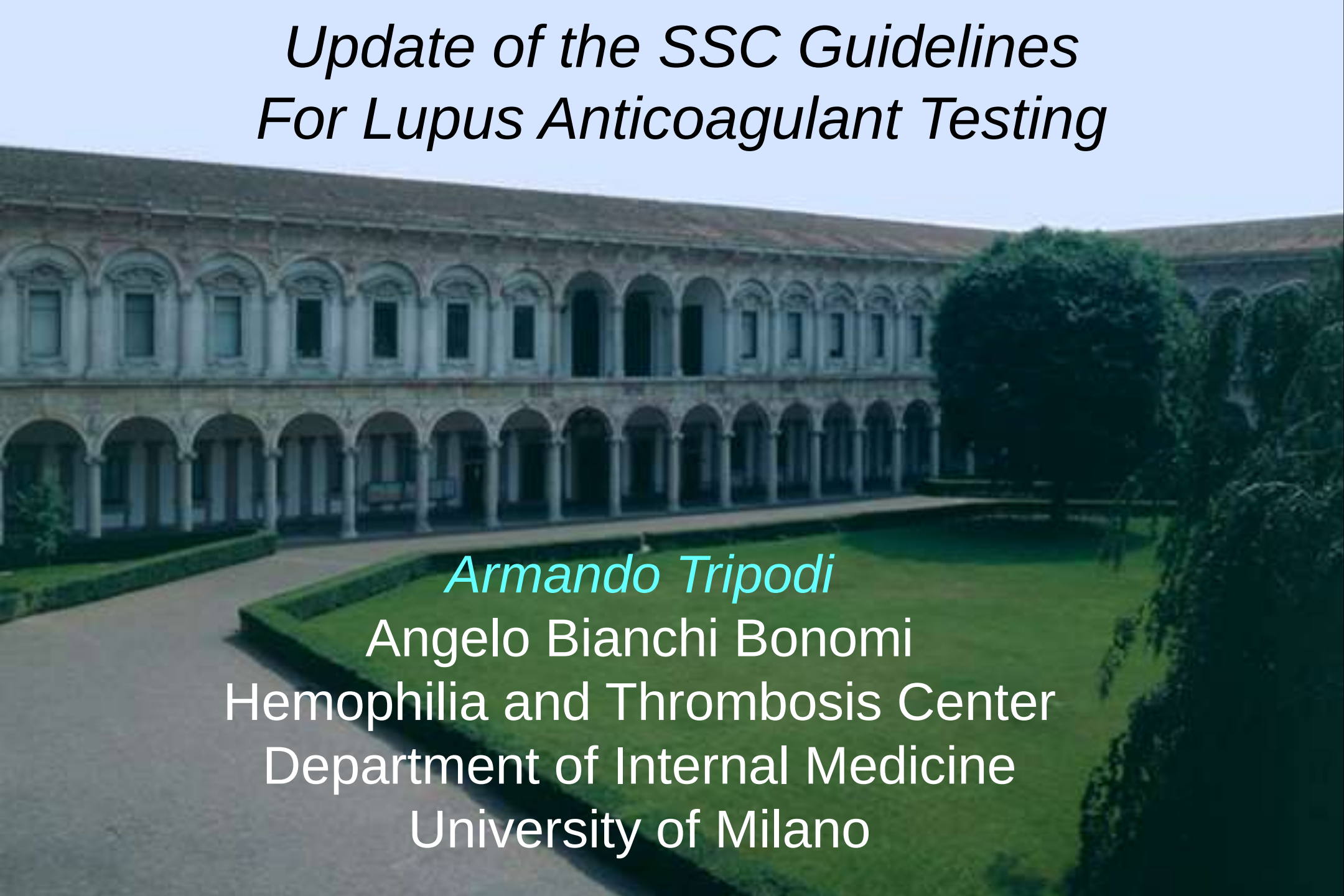
Update of the SSC Guidelines For Lupus Anticoagulant Testing



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Antiphospholipid Syndrome

The Syndrome is defined by one ***clinical*** and one **laboratory** criterion

Antiphospholipid Syndrome

- ***Laboratory criteria***
  - Repeated (2 times 12 weeks apart) LA and/or solid-phase antibody positive tests
- ***Clinical criteria***
  - Pregnancy complications, venous and/or arterial thrombosis

Antiphospholipid Antibodies

Definition

- ***Lupus Anticoagulant (LA)***

- Heterogenous category of Ig able to prolong PL-dependent coagulation tests

- ***Anti-cardiolipin, anti-β_2GPI***

- Heterogenous category of Ig able to bind protein-PL complexes immobilized on solid-phase surfaces

Antiphospholipid Syndrome
Laboratory Diagnosis

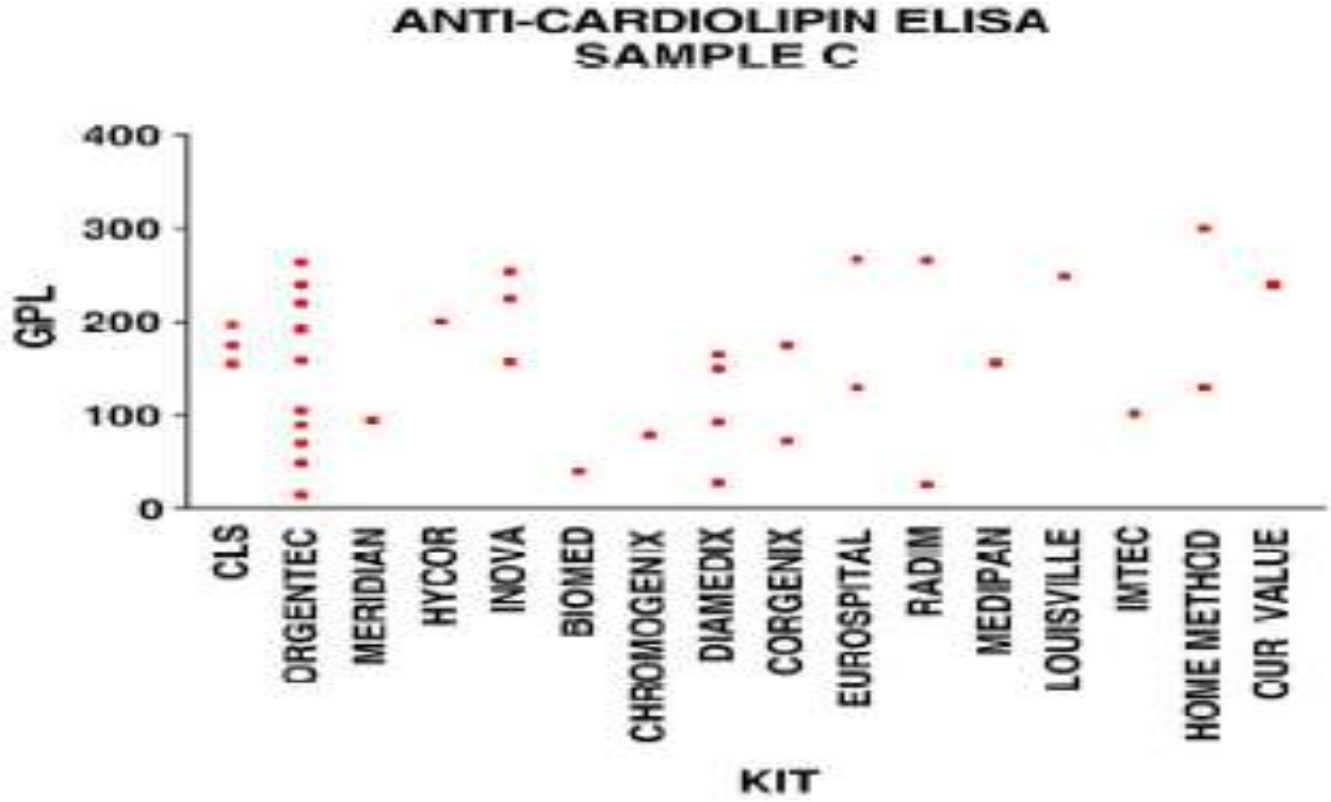
- LA ***and*** solid-phase aPL coexist in a limited proportion of patients with the syndrome

Diagnosis must be based on both LA and solid-phase aPL detection

Solid-phase Antiphospholipid Antibodies

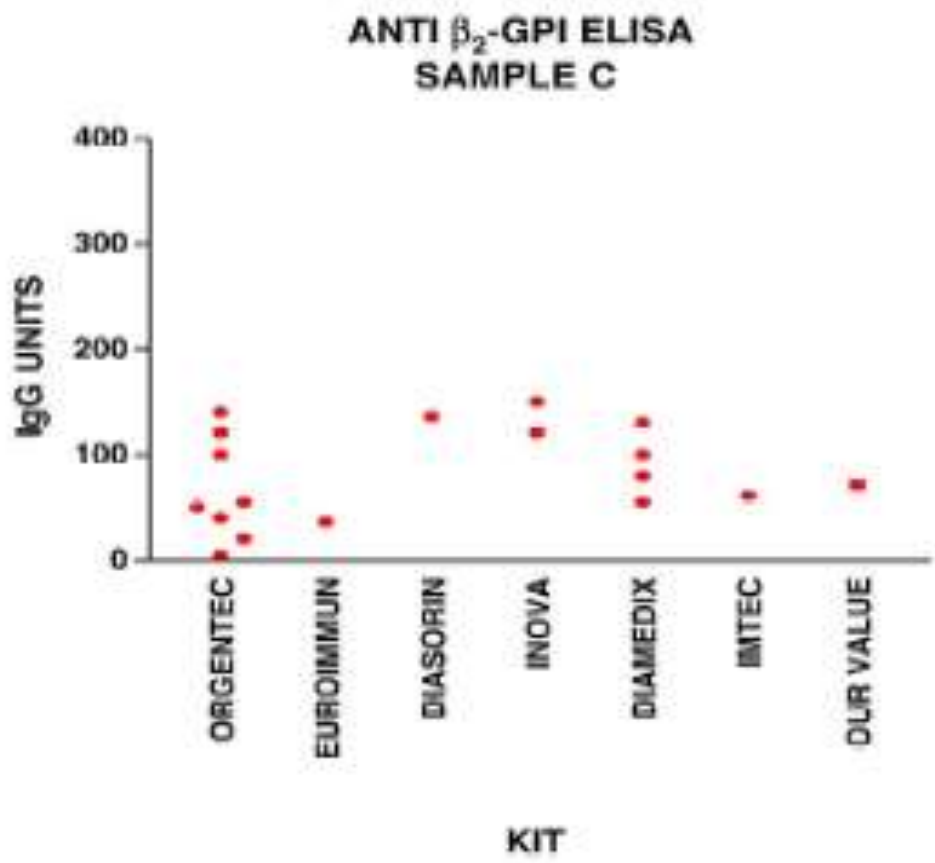
- ***Which Test(s)***
  - Anti-cardiolipin
  - Anti-β_2-GPI
- ***Which Isotype(s)***
  - IgG
  - IgM

Commercial Kits



Pengo V et al Thromb Res, 2006

Commercial Kits



Pengo V et al Thromb Res, 2006

Main Issues that Affect Assay Results
anti-Cardiolipin

- Type of surface on microplate
- Extent of cardiolipin oxidation
- Source of ß2-GPI
- Calibrators
- Cut off values

Main Issues that Affect Assay Results
aß2-GPI

- Type of surface on microplate
- Source and quality of ß2-GPI
- Calibrators
- Cut off values

Solid-phase aPL assay
Crucial issue to be resolved

To distinguish antibodies associated with the clinical feature of APS from those devoid of clinical relevance

de Laat et al, 2005
IgG antibodies to ß2-GPI domain I cause LA and are associated with thrombosis

Laboratory Detection of LA

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

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A. TRIPODI

Issues on LA Testing

- ***Who should be tested***
- Pre-analytical variables
- Which test(s)
- Diagnostic criteria
- When testing
- Results reporting

Indications to Search for the Antiphospholipid Syndrome

- Occurrence of (accidentally-found) prolongation of the APTT without known etiology
- Patients with venous and/or arterial thrombosis occurring at young age (<50 years)
- Patients with thrombosis at unusual sites, or associated with autoimmune diseases
- Women with pregnancy complications

Warning

Generalized searches on asymptomatic individuals or other categories of patients are highly discouraged as they increase the risk of false-positive results

Need to confirm the laboratory diagnosis

Once a patient has been identified as LA-, aCL- or aßGPI-positive, it is imperative that testing be repeated on a second occasion >12 weeks after initial testing

Issues on LA Testing

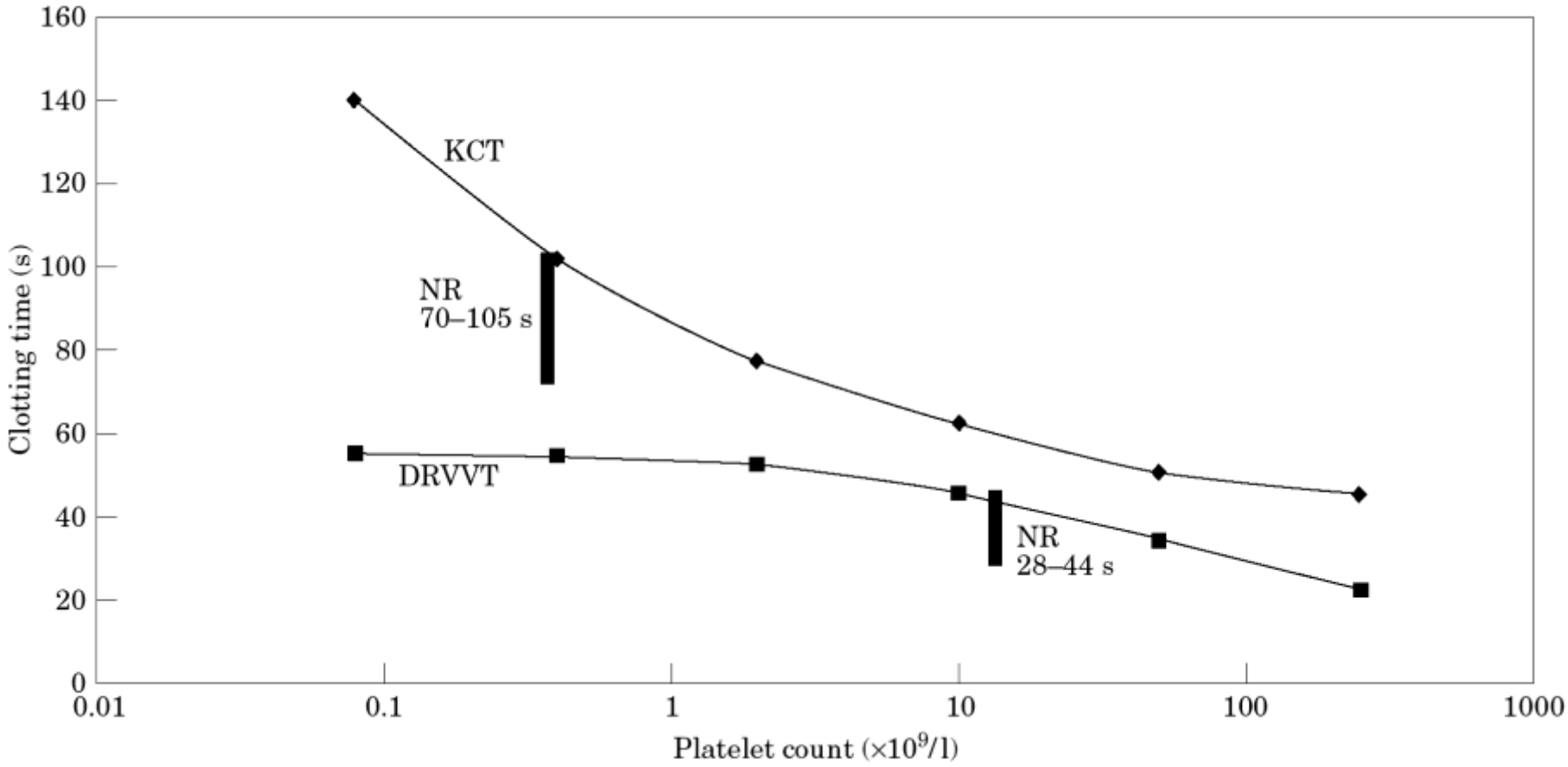
- Who should be tested
- ***Pre-analytical variables***
- Which test(s)
- Diagnostic Criteria
- When testing
- Results reporting

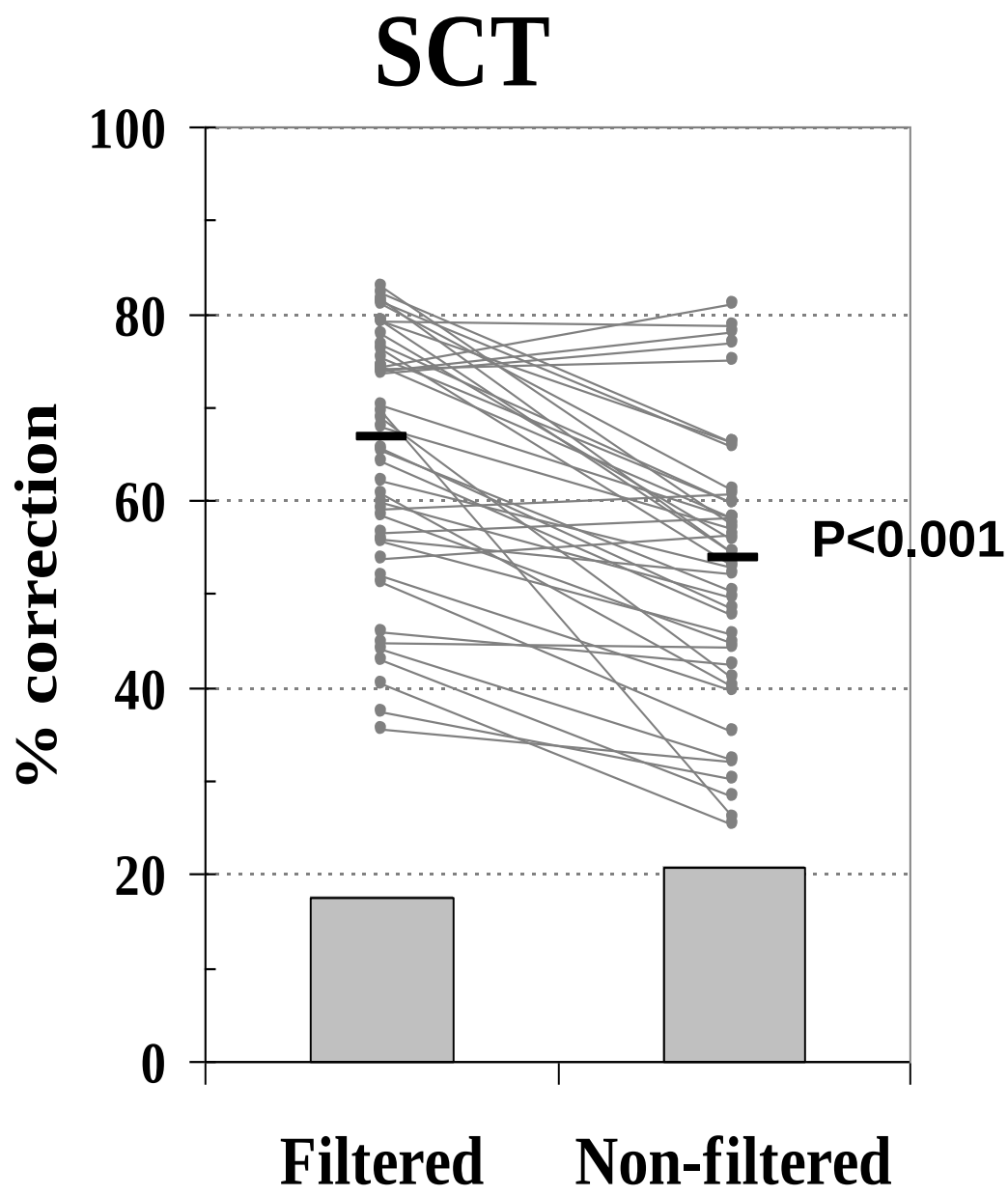
Pre-analytical Variables

- ***Problem (1)***
- Residual platelets affect PL-dependent tests especially after freezing-thawing

LA Diagnosis. Effect of Platelets

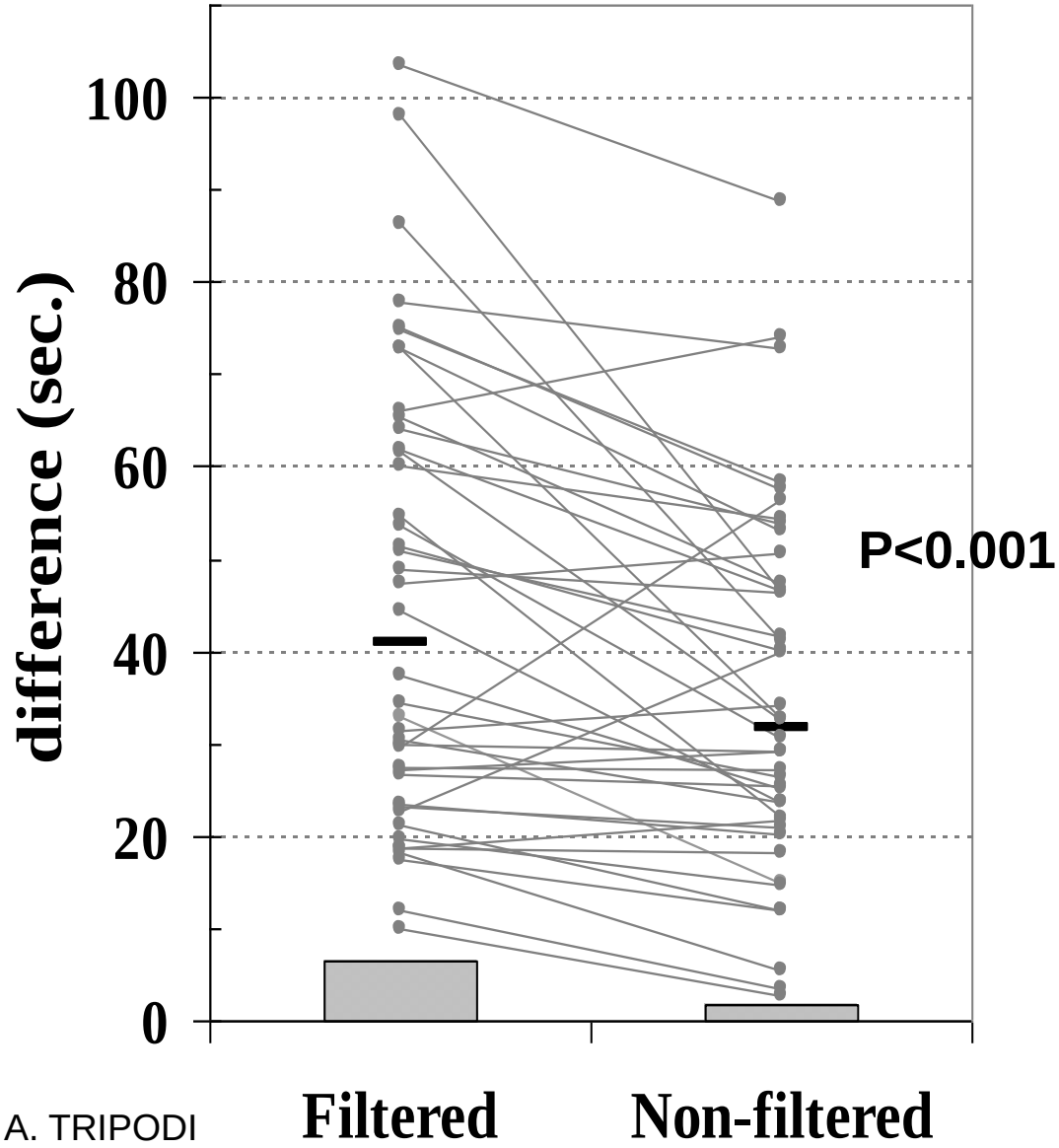
Exner, 2000





Chantarangkul et al.
Thromb Haemost 2002

STACLOT-LA



Chantarangkul et al.
Thromb Haemost 2002

Pre-analytical Variables

- ***Problem (1)***
- Residual platelets affect PL-dependent tests especially after freezing-thawing
- ***Recommendation***
- Double centrifugation

Pre-analytical Variables

- ***Problem (2)***
- Stability of coagulation factors
- ***Recommendation***
- Quickly frozen plasma is required if LA detection is postponed
- Frozen plasma must be thawed at 37°C

Issues on LA Testing

- Who should be tested
- Pre-analytical variables
- ***Which test(s)***
- Diagnostic Criteria
- When testing
- Results reporting

Which Test

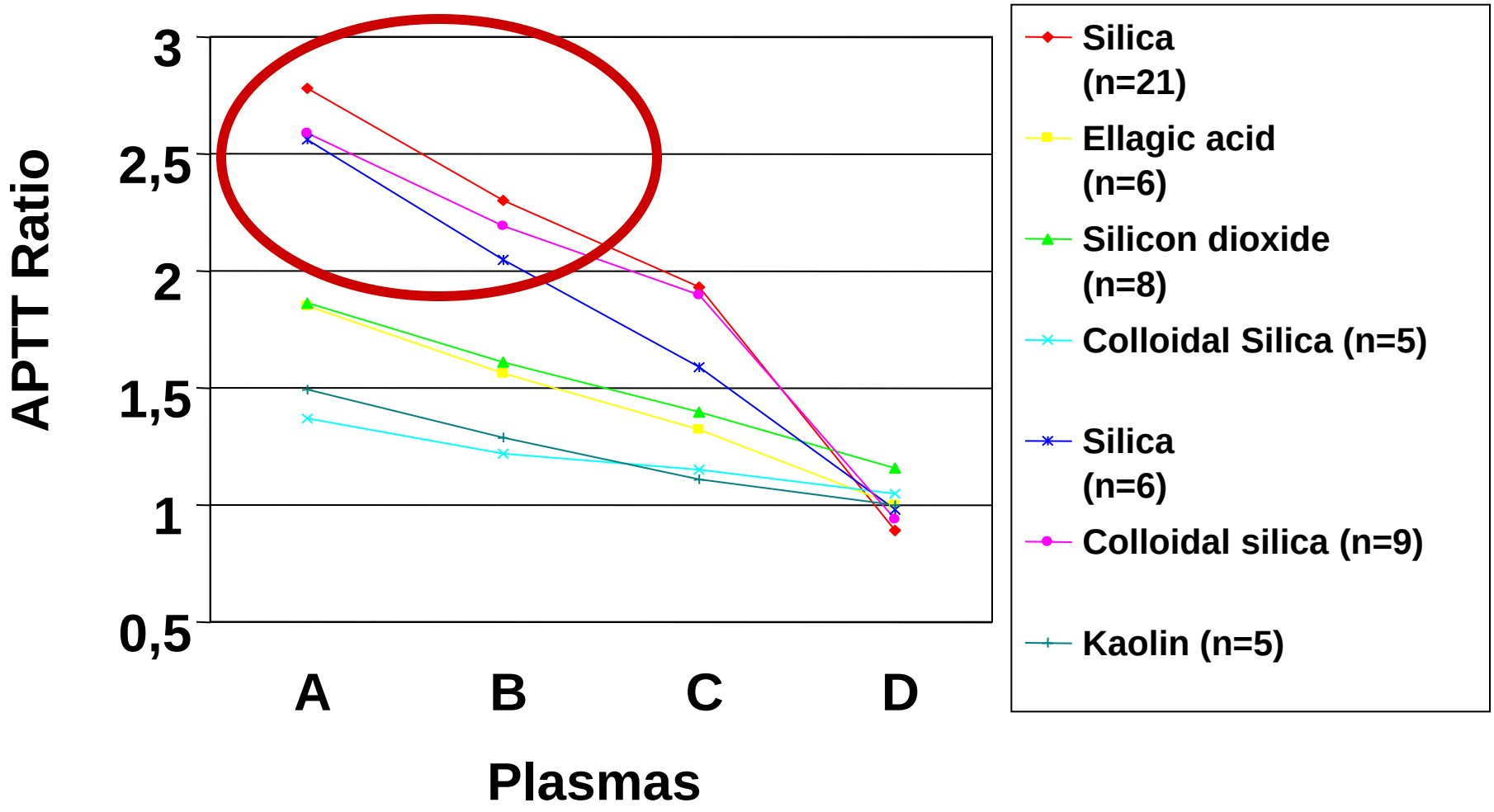
- ***Two tests based on different principles***

- dRVVT
- Sensitive aPTT-based test (low phospholipids and silica as activator)

LA should be considered as positive if at least one of the two tests gives a positive result

APTT Responsiveness to LA

Tripodi et al, Clin Chem 2003



Issues on LA Testing

- Who should be tested
- Pre-analytical variables
- Which test(s)
- ***Diagnostic Criteria***
- When testing
- Results reporting

Laboratory Diagnosis of LA

No specific test is available

Therefore….

Diagnosis must be based on

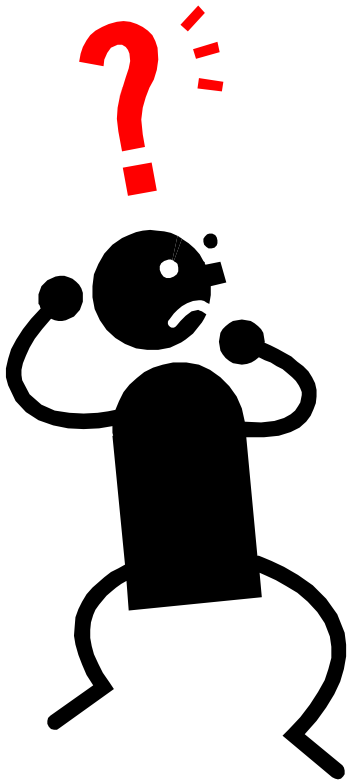
"DIAGNOSTIC CRITERIA"

Diagnostic Criteria for LA Detection

- ***Screening***
- Prolongation of one (or more) phospholipid-dependent clotting test
- ***Mixing***
- Evidence that the prolongation is due to the presence of an inhibitor
- ***Confirmation***
- Evidence that the inhibitor is directed against phospholipids

General Rules for Results Interpretation

- Determine local cut-off values and use them consistently for interpretation
- Do not use cut-off established elsewhere
- Use true positive or negative plasmas to validate local cut-off



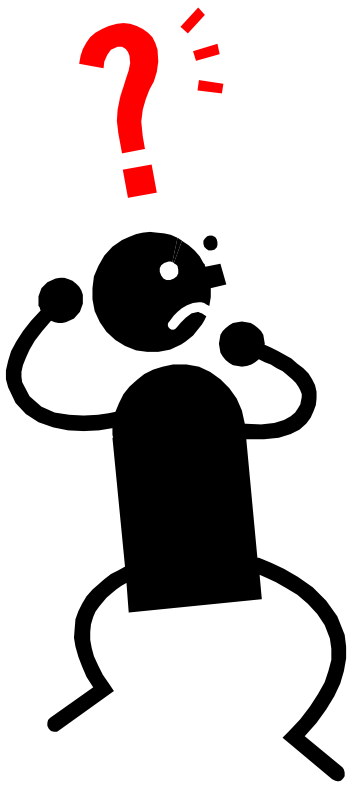
Screening

Screening
How to determine cut-off values

- Perform testing on plasmas from 40 healthy donors
- Take the cut-off as the value above the 99th percentile of the distribution

Screening
Interpretation

Results of screening tests are potentially suggestive of LA when their clotting times are longer than the local cut-off



Mixing

Mixing

- ***Problem***
- Poor quality of pooled normal plasma (PNP)
- ***Recommendations***
- PNP should be prepared to ensure no residual platelets and 100% activity for all clotting factors
- PNP must be stored frozen (-70°C) in small aliquots
- Commercial lyophilized or frozen PNPs can be used if they fulfil the above specifications

Mixing
How to determine cut-off values

- Perform testing on plasmas from 40 healthy donors, mixed with PNP at 1:1 proportion without preincubation
- Take the cut-off as the value above the 99th percentile of the distribution
- Alternatively, the cut-off may be the value of the ICA defined according to:

$$ICA = [(CT_{mix} - CT_{PNP}/CT_{patient})]x100$$

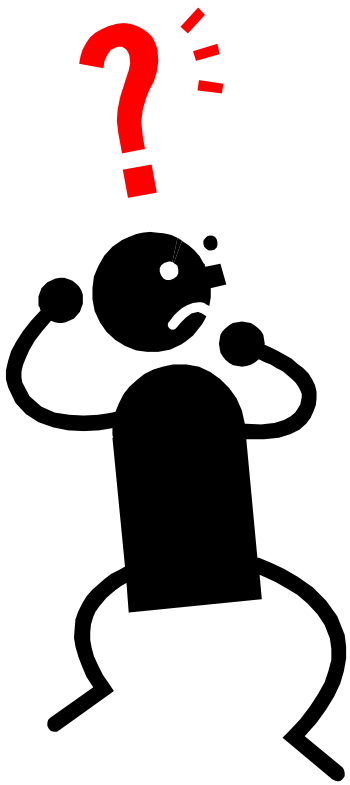
Mixing
Interpretation

- ***Results of mixing are suggestive of LA***

- If the clotting time is longer than the local cut-off

Or

- If the ICA is greater than the local cut-off



Confirmation

Confirmation

- ***Problem (1)***
- Type of phospholipids (PL)
- ***Recommendation***
- Confirmation must be performed by increasing the PL concentration of the abnormal screening test(s)
- Bilayer or Hexagonal PL should be used
- Freeze/thawed platelets are not recommended

Confirmation

How to determine cut-off values

- Perform testing on plasmas from 40 healthy donors at low (screen) and high (confirm) PL concentration
- Take the cut-off as the value corresponding to the mean of the individual % corrections calculated according to:

$$\% \text{ Corr.} = [(\text{screen} - \text{confirm})/\text{screen}] \times 100$$

Confirmation

Interpretation

Results are confirmatory of LA if the % correction is above the local cut-off value

Issues on LA Testing

- Who should be tested
- Pre-analytical variables
- Which test(s)
- Diagnostic Criteria
- ***When testing***
- Results reporting

When Testing

- ***Problem***

- Results interpretation is difficult because of acute thrombotic events and/or initiation of antithrombotic drugs (heparin & VKA)

- ***Recommendation***

- Blood should be collected before the start of any anticoagulant drug or after a sufficient period from its discontinuation

Effect of Heparin

- LA detection is not possible if the content of UFH in plasma exceeds the reagent neutralization capacity
  - ***Thrombin time helps***
- Although the experience is limited, LA detection is possible in LMWH-containing samples

Effect of Vitamin K Antagonists (VKA)

- It is recommended to postpone LA detection until after VKA discontinuation (1-2 weeks) or when INR is <1.5
  - ***Bridging VKA discontinuation with LMWH is a suitable alternative***
- Alternatively, if the INR is >1.5< 3.0, a 1:1 dilution (patient plasma:PNP) can be considered
- Other procedures are not recommended as they require critical evaluation

Effect of Other Drugs

- The effect of direct FIIa or FXa inhibitors is unknown
- Aspirin and clopidogrel do not interfere with LA detection

Issues on LA Testing

- Who should be tested
- Pre-analytical variables
- Which test(s)
- Diagnostic Criteria
- When testing
- ***Results reporting***

Results Reporting

- Results for screening, mixing and confirmation should be normalized against a PNP
- LA detection should be reported with analytical results and an interpretative comment (i.e., ***LA yes, or no***)
- Comments such as borderline or dubious LA are highly discouraged

Additional Recommendation

LA results should always be considered in the context of full aPL profile

- Triple positivity (LA + medium-high titer aCL & aβ_2GPI) identifies patients at high risk of thrombosis
- Less information is available on fetal loss
- Isolated LA positivity is more frequent in asymptomatic subjects