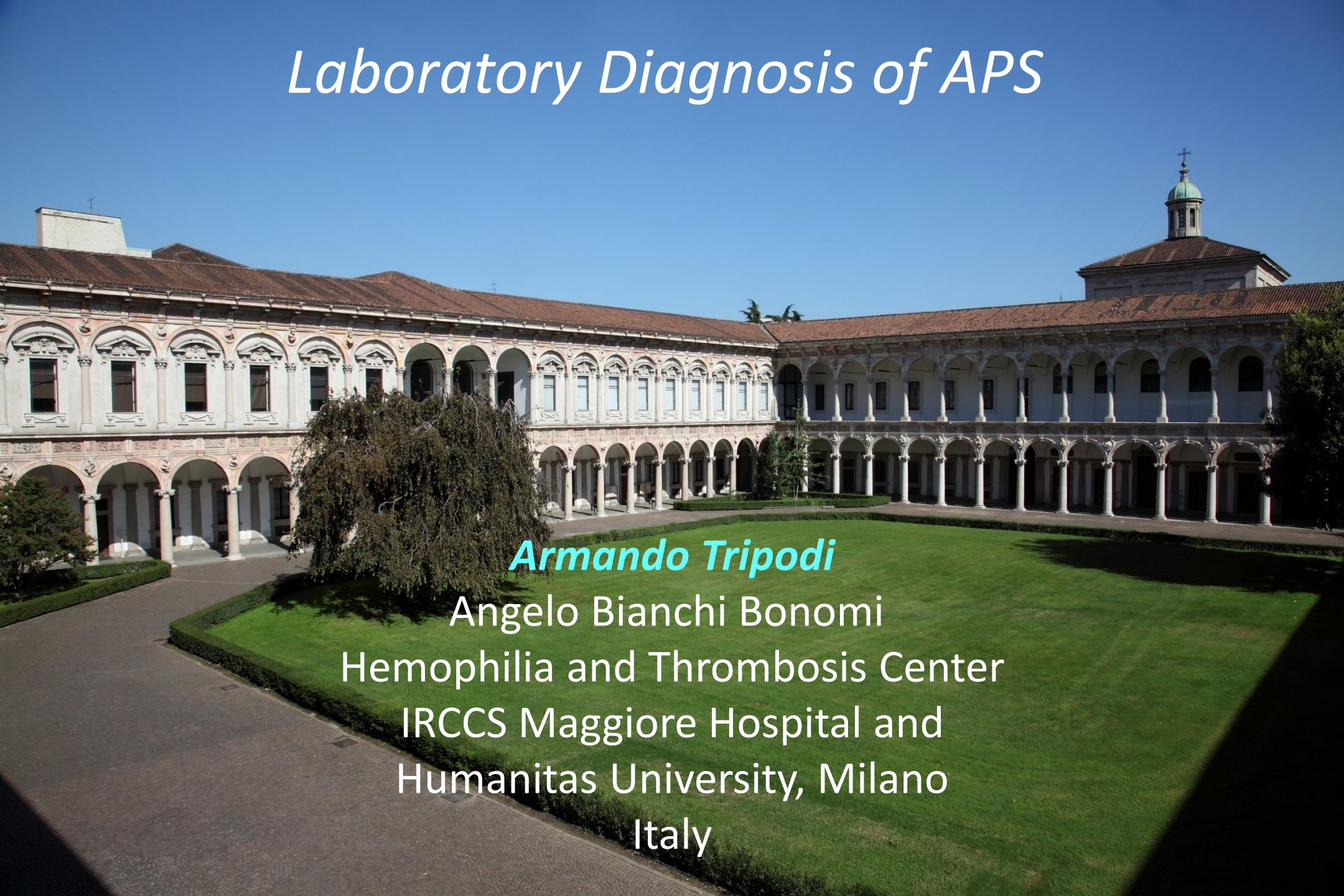
Laboratory Diagnosis of APS



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Journal of Thrombosis and Haemostasis, **16**: 809–813 DOI: 10.1111/jth.13976

RECOMMENDATIONS AND GUIDELINES

Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH

K. M. J. DEVREESE,* T. L. ORTEL,† V. PENGO‡ and B. DE LAAT§¶FOR THE SUBCOMMITTEE ON LUPUS ANTICOAGULANT/ANTIPHOSPHOLIPID ANTIBODIES

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Antiphospholipid Syndrome

Condition defined by clinical and lab criteria

- *Laboratory criteria*

- Lupus anticoagulant and/or solid-phase antiphospholipid antibody positive tests (confirmed on 2 occasions 12 weeks apart)

- *Clinical criteria*

- Pregnancy complications, venous and/or arterial thrombosis

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Solid-phase Antiphospholipid Antibodies

- *Which Test(s)*
  - Anti-cardiolipin
  - Anti-β_2GPI
  - Anti-PS/PT
- *Which Isotype(s)*
  - IgG
  - IgM

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There are many commercial assays available for aCL or a-ß$_2$GPI

But they are not yet standardized across laboratories

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Assays for aCL and a-ß$_2$GPI

- Many commercial ELISA-based assays
  - Poorly standardized. Gross degree of variation across labs
- Chemiluminescence-based assays
  - Closed systems (easy handling of reagents and samples)

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Laboratory Detection of LA

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OFFICIAL COMMUNICATION OF THE SSC

Update of the guidelines for lupus anticoagulant detection

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Issues on LA Detection

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

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Indications to search for APS

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

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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 is positive

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Issues on LA Detection

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- ***Diagnostic Criteria***
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Diagnostic Criteria for LA Detection

- ***Screening***
- Prolongation of 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

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Screening
How to determine cut-off values

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

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Mixing

How to determine cut-off values

- Perform testing on plasmas from healthy donors, mixed with PNP at 1:1 proportion
- 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:

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

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Confirmation

How to determine cut-off values

- Perform testing on plasmas from healthy donors at low (screen) and high (confirm) phospholipid concentrations
- Take the cut-off as the value above the 99th percentile of the distribution of the individual % corrections calculated according to:

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

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Journal of Thrombosis and Haemostasis, **15**: 1180–1190 DOI: 10.1111/jth.13678

ORIGINAL ARTICLE

Variability of cut-off values for the detection of lupus anticoagulants: results of an international multicenter multiplatform study

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Essentials

- Cut-off values for LA detection were calculated in 11 labs each testing plasma from 120 donors with 3 commercial platforms
- Major variations were observed even within the same platform
- Cut-off values determined in any given lab are not necessarily interchangeable

Issues on LA Detection

- Who should be tested
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- ***When testing***
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When Testing

- ***Problem***
- Results interpretation is difficult during acute thrombosis and/or during antithrombotic drugs
- ***Recommendation***
- Blood should be collected before starting anticoagulation or after a sufficient period from its discontinuation

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Effect of Anticoagulation on LA Testing

- Heparin mimics LA
  - *Many LA tests do contain heparin neutralizers*
- LMWH may mimic LA
  - *Depending on the brand of LMWH used*
  - *Especially at peak*
- VKA give rise to false-positive (or negative) LA
- DOAC give rise to false-positive LA

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Approaches to Overcome Anticoagulation

- Dilution (1:1) of patient plasma into pooled normal plasma (PNP)
- Integrated assays (screen and confirm)
- Tests (reportedly) less affected by anticoagulants
- Antidotes or neutralizers to quench *in vitro* the activity of anticoagulants
- Discontinuation of anticoagulation

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Dilution (1:1) of patient plasma into PNP

- *Rationale*
  - Deficiency of coagulation will be corrected by the PNP
- Limitations
  - Applicable only to VKA
  - Good quality PNP
  - Dilution reduces (by 50%) the LA potency
  - Correction by PNP is dependent on the APTT or dRVVT used for testing
  - No conclusive evidence on the value of the procedure
  - False-negative or false-positive LA should be expected

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Schematic representation of Integrated LA Test

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Integrated LA Tests

- Earlier reports suggested that screen and confirm integrated tests in the presence of VKA or UFH are proportionally prolonged
- Hence, they are reliable for the majority of patients even in the presence of UFH or VKA
- Later reports showed that screen and confirm in the presence of DOAC are not proportionally prolonged
- Screen tends to be more prolonged than confirm
- Consequently, the ratio screen/confirm tends to be higher than expected and may lead to false-positive LA

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Approaches to Overcome Anticoagulation

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Tests (reportedly) less affected by anticoagulants

- Snake venoms (Taipan & Ecarin) might be useful to detect LA during anticoagulation, as they are able to activate FII
- Taipan is a PL- and calcium-dependent activator, whilst Ecarin is not
- If used in combination they may help detecting LA during anticoagulation
- There is information from literature on their diagnostic efficacy on patients on VKA, but not conclusive evidence

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Approaches to Overcome Anticoagulation

- Dilution (1:1) of patient plasma into pooled normal plasma (PNP)
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Antidotes/Neutralizers to Quench Anticoagulants

- Idarucizumab added in vitro to neutralize dabigatran
- Andexanet alfa added in vitro to neutralize anti-FXa drugs
- DOAC-Stop®, or DOAC-Remove®

– *Activated charcoal added in vitro to adsorb DOAC*

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Thromb Res 2019; 180:10-19

Neutralising rivaroxaban induced interference in laboratory testing for lupus anticoagulant (LA): A comparative study using DOAC Stop and andexanet alfa

Emmanuel J. Favaloro[a,b,*], Grace Gilmore[c], Sandya Arunachalam[d], Soma Mohammed[a], Ross Baker[c]

Essentials

Rivaroxaban caused clotting time prolongation for most LA tests and generated falsely elevated dRVVT screen/confirm ratio results that mimicked the presence of LA

Rivaroxaban plasma treated with DOAC-Stop showed correction of the clotting time Prolongation and the screen/confirm ratio for most LA tests

Participants in the study correctly identified the rivaroxaban plasma treated with DOAC-Stop as LA-negative

Andexanet-alfa corrected the prolonged clotting time induced by rivaroxaban, but displayed over-correction of the screen/confirm ratio

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Approaches to Overcome Anticoagulation

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- Integrated assays (screen and confirm)
- Tests (reportedly) less affected by anticoagulants
- Antidotes or neutralizers to quench *in vitro* the activity of anticoagulants
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Discontinuation of Anticoagulation

- Oral anticoagulation may be temporarily stopped and switched to LMWH
- LMWH would protect from thrombosis, making LA detection possible
- This strategy may be considered in individual patients after full consideration of pros and cons

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Issues on LA Detection

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

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Results Reporting

LA detection should be reported with analytical results and an interpretative comment

(i.e., ***LA yes, or no***)

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Issues on LA Detection

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

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Clinical interpretation of results

- *Interpretation should consider the results of all the three tests*
- The syndrome is defined if at least one of the tests (LA, aCL or aß$_2$GPI) is positive
- Positivity for all the three tests *(triple positivity)* identify patients at very high risk

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LA Detection
Main unresolved issues

- Standardization of existing procedures
- *Application of SSC guidelines*
- Urgent need for LA specific tests
- U*nderstanding of pathogenic mechanisms may help*
- Tests able to distinguish LA patients who develop clinical events from those who do not
- *dRVVT better than APTT-based tests ?*
- *aβ2-GPI domain I*
- Quantification of LA potency
- *Establishment of "international standards" ?*

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Journal of Thrombosis and Haemostasis, **7**: 1767–1773 DOI: 10.1111/j.1538-7836.2009.03588.x

ORIGINAL ARTICLE

The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study

B. DE LAAT,* V. PENGO,† I. PABINGER,‡ J. MUSIAL,§ A. E. VOSKUYL,¶ I. E. M. BULTINK,¶ A. RUFFATTI,** B. ROZMAN,†† T. KVEDER,†† P. DE MOERLOOSE,‡‡ F. BOEHLEN,§§ J. RAND,¶¶ Z. ULCOVA-GALLOVA,*** K. MERTENS* and P. G. DE GROOT†††

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Table 2 Association between aPL and thrombosis

	Odds ratio (95% confidence interval)
Anti-domain I IgG	**3.5 (2.3–5.4)***
Non-domain I Anti-beta2GPI IgG	0.4 (0.3–0.6)
Anti-beta2GPI IgM	0.9 (0.6–1.3)
LAC	**1.8 (1.1–3.1)***
aCL	1.1 (0.6–2.1)

To estimate whether there is a significant increase in association of anti-domain I IgG antibodies with thrombosis an odds ratio was calculated within the total population of 511 patients. *One is not included in 95% confidence interval. Bold: Significant association of assay with clinical symptom.

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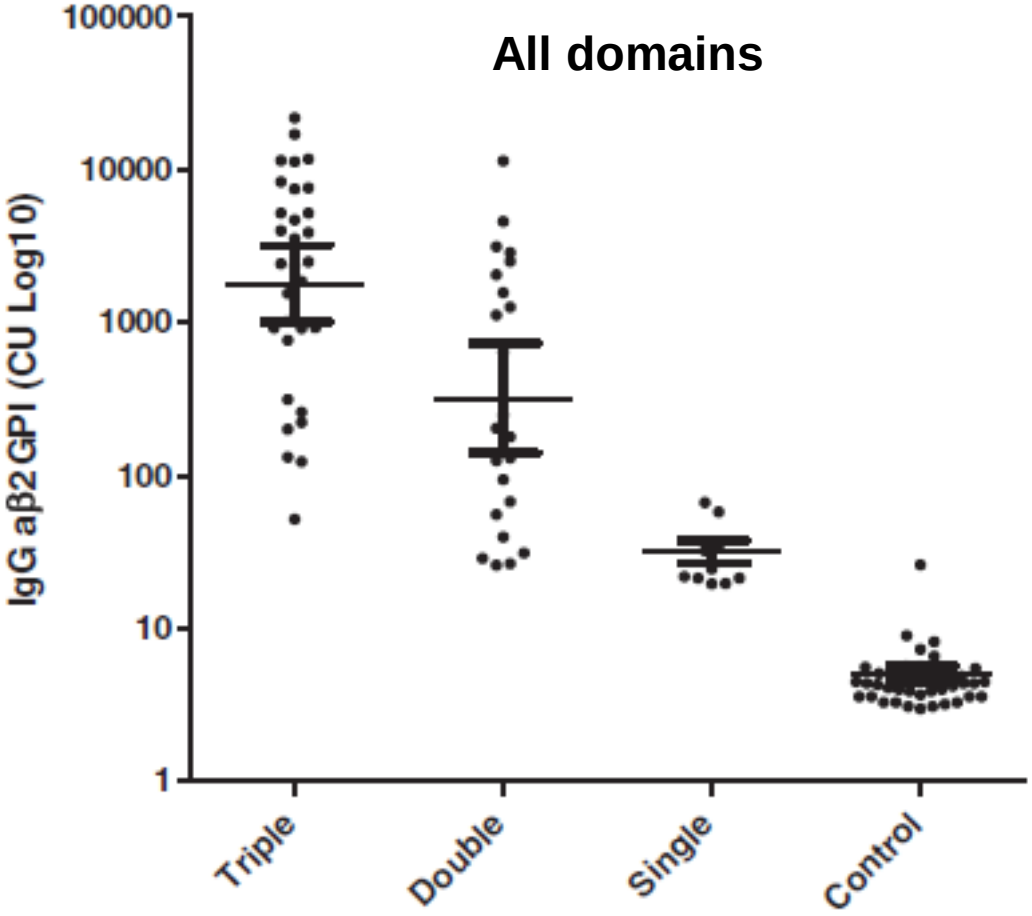
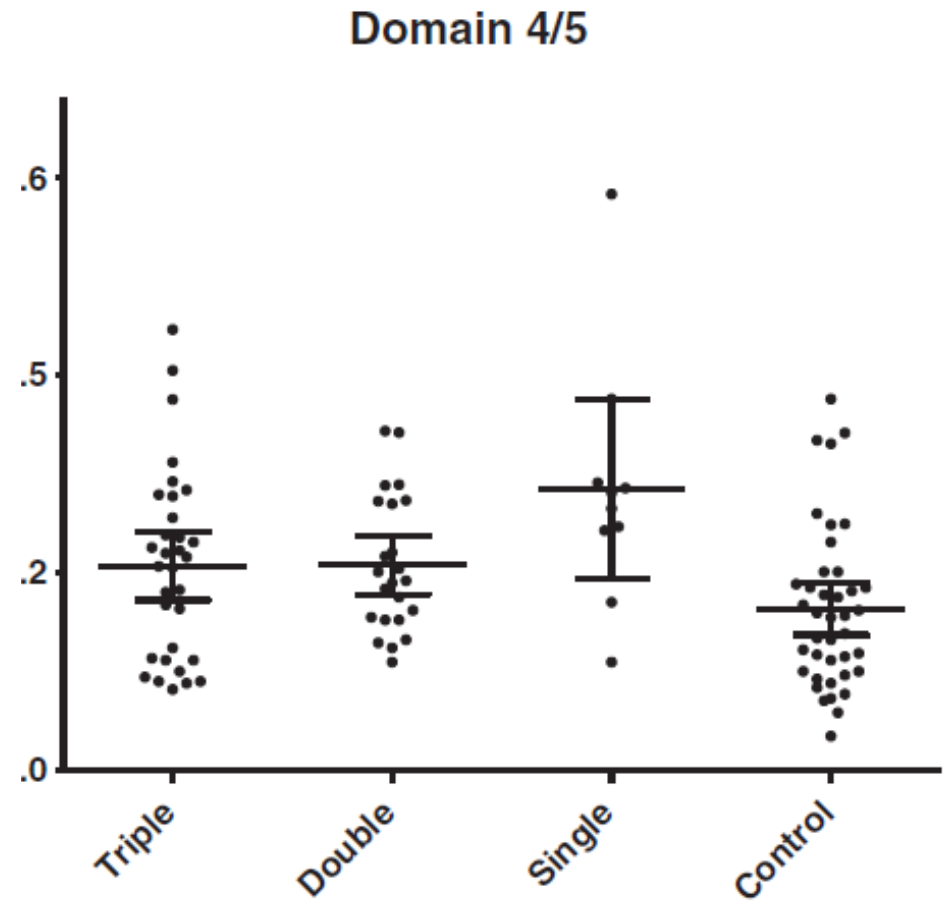
Thromb Res 2915; 136: 161-63

Antibodies to Domain 4/5 (Dm4/5) of β2-Glycoprotein 1 (β2GP1) in different antiphospholipid (aPL) antibody profiles

V. Pengo [a,b,*], A. Ruffatti [a,b], M. Tonello [a,b], A. Hoxha [a], E. Bison [a], G. Denas [a], S. Padayattil Jose [a], G. Zoppellaro [a], A. Bracco [a], A. Banzato [a]





Summary & Conclusions

- Accuracy of lab diagnosis is essential as APS patients are candidates for long term anticoagulation
- Diagnosis should be established far from acute events and off therapy
- APS requires one the following
  - *Positive APTT-based or dRVV tests*
  - *aCL (aß2GPI-dependent) IgG or IgM above normal limits*
  - *aß2GPI, IgG or IgM above normal limits*
- Triple positivity identify patients at high risk

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