APSFORUMMAASTRICHT

Focus on new tests and techniques in laboratory diagnosis of APS

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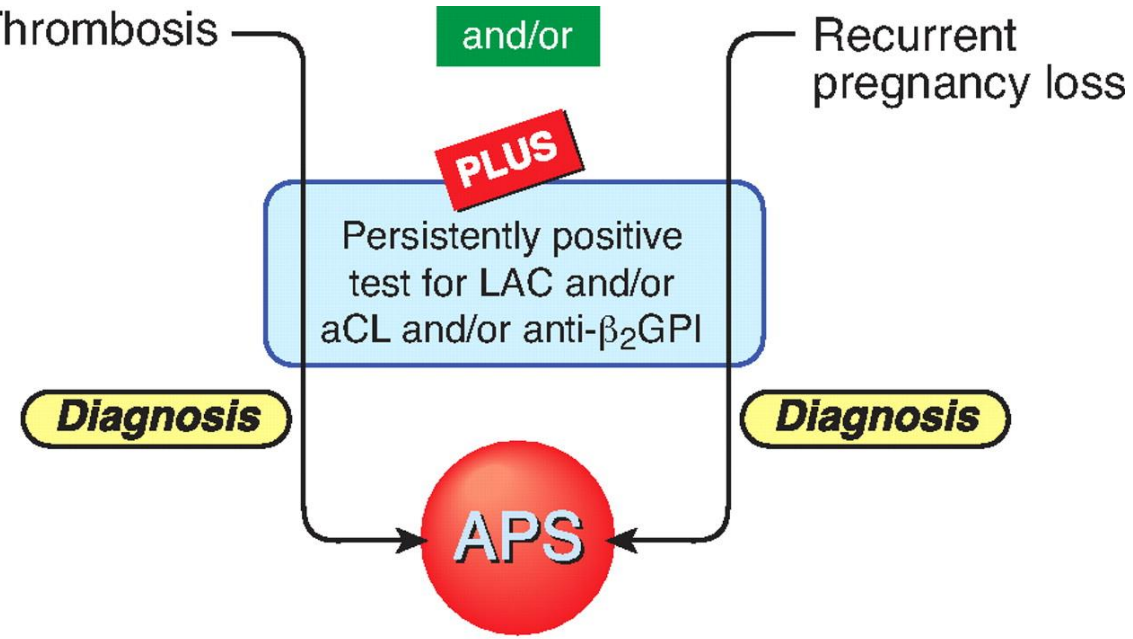
Antiphospholipid syndrome (APS)

- autoimmune disease
- antiphospholipid antibodies (aPL)

- thrombosis
- pregancy morbidity



GIANNAKOPOULOS ET AL. BLOOD 2009;113:985-994; MIYAKIS ET AL, J THROMB HAEMOST 2006; 4: 295-306

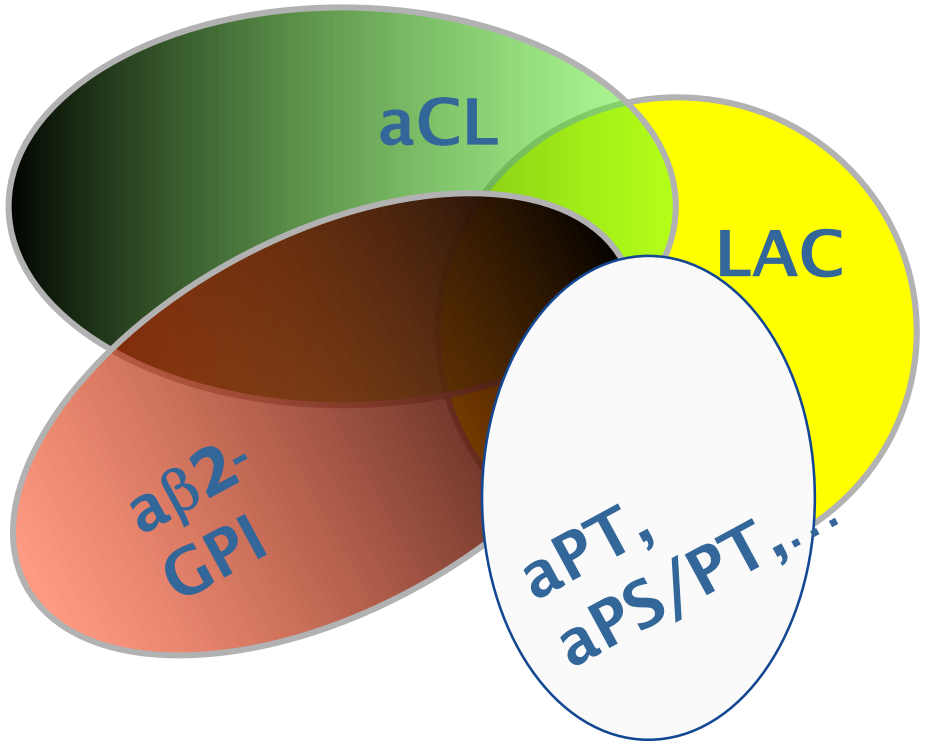
Antiphospholipid antibodies (aPL)

Lupus anticoagulants (LAC)

Anticardiolipin antibodies (aCL)

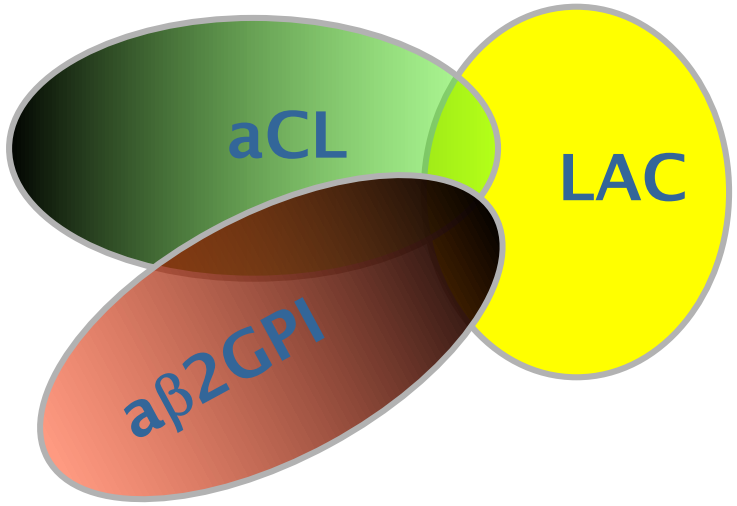
β2-glycoprotein I antibodies (aß2GPI)

Other Antiprothrombin, …



Antiphospholipid antibodies (aPL)

- Lupus anticoagulants (LAC)
- Anticardiolipin antibodies (aCL)
- Beta-2-glycoprotein I antibodies (aß2GPI)



International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS)) ***MIYAKIS ET AL, J THROMB HAEMOST 2006; 4: 295-306***

Testing for Antiphospholipid antibodies with Solid Phase Assays: Recommendations from the Subcommittee on Lupus Anticoagulant/Phospholipid-Dependent Antibodies.

DEVREESE ET AL, J THROMB HAEMOST 2014; **5:792-795**

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

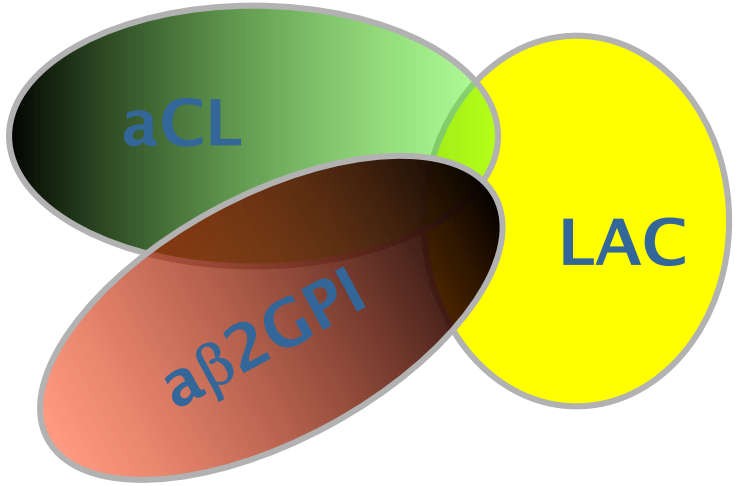
DEVREESE ET AL, J THROMB HAEMOST 2018; 16: 809–13

Antiphospholipid antibodies (aPL)

Lupus anticoagulants (LAC)

Coagulation assays

Functional antibodies: “all” aPL: ß2GPI antibodies, prothrombin antibodies, other?



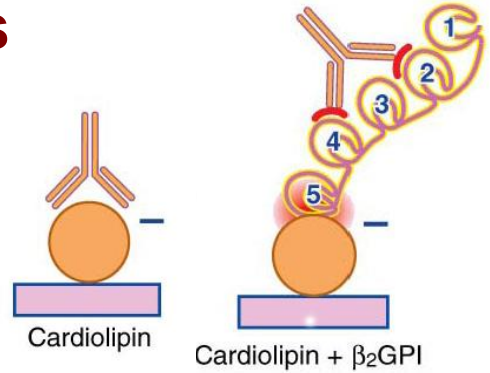
Anticardiolipin antibodies (aCL)

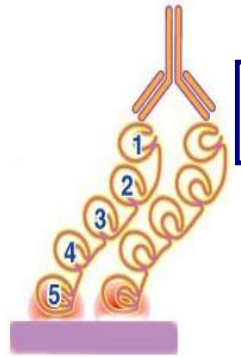
Beta-2-glycoprotein I antibodies(aß2GPI)

Solid phase assays

One group of antibodies

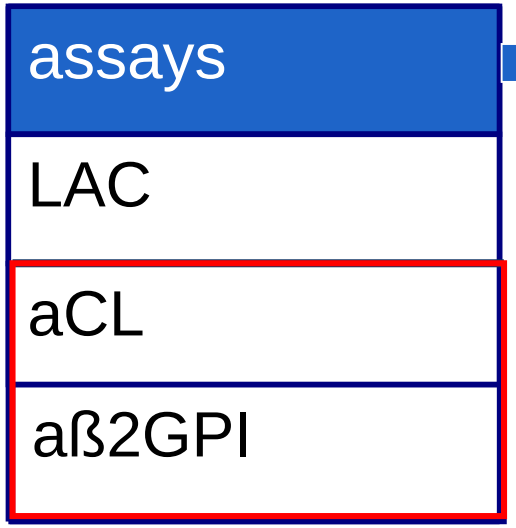
aCL





aβ2GPI

Laboratory diagnosis of APS



Lack of **standardization**
-different sensitivity and specificity
-inter-assay and -laboratory variation
-overdiagnosis/misdiagnosis

Need reliable tests!

Variability due to pre-, post- and analytical conditions:

- Specific issues related to the assays
  - methodological problems due to the heterogeneity of aPL
  - variation in local working conditions
  - differences in calibration
- Difficulties in interpretation of results
- Calculation of cut-off values

Thrombotic risk in APS

- Serological and clinical factors
  - Type and level of aPL
  - Coexistence of predisposing thrombotic risk factors
  - Association with underlying autoimmune diseases (SLE)
- the laboratory parameters in risk stratification for clinical complications in APS

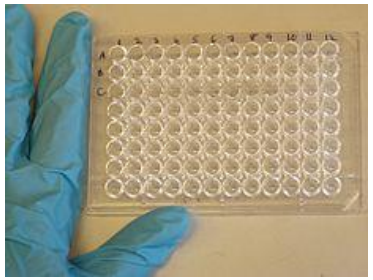
DEVREESE. THROMB RES 2012;130 SUPPL 1:S37-40

aCL and aβ2GPI: methods

- Measured by standardized ELISA

International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS)

MIYAKIS ET AL, J THROMB HAEMOST 2006; 4: 295-306



- Guidelines: focused on ELISA

Guidelines on the investigation and management of antiphospholipid syndrome

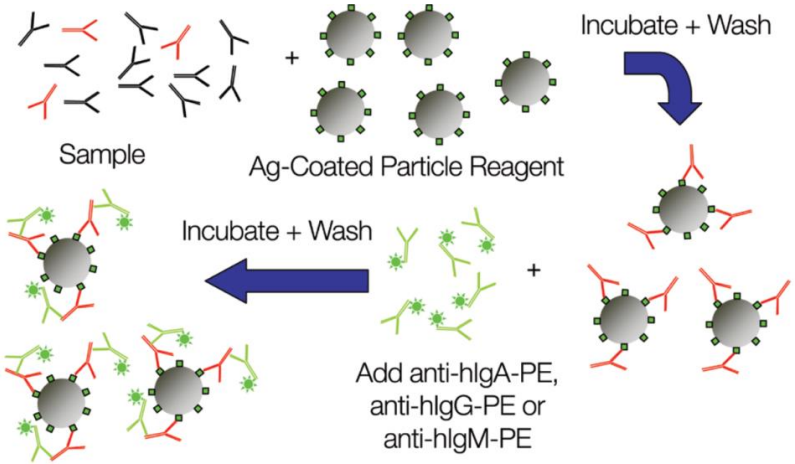
KEELING ET AL, BR J HEMATOL 2012

- New technologies and automated platforms: alternative solid phases and detection systems

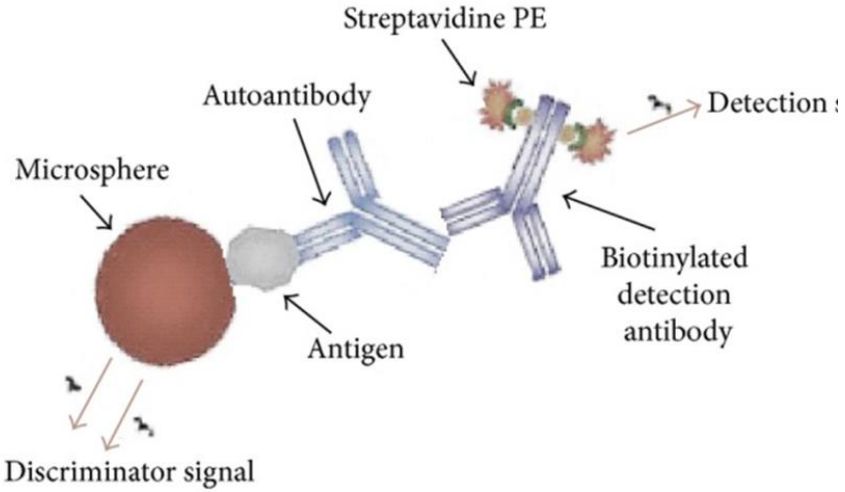
Testing for Antiphospholipid antibodies with Solid Phase Assays: guidance from the SSC of the ISTH

DEVREESE ET AL, J THROMB HAEMOST 2014; **5:792-795**

aCL and aβ2GPI: methods



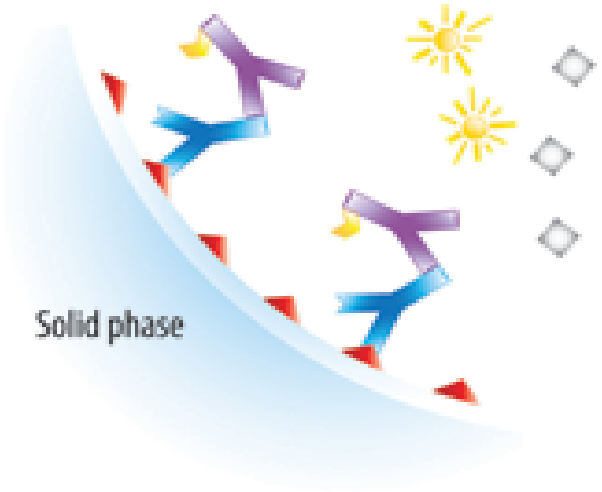
- Multiplex format
  - Microbead-based
    - Bioplex 2200 (BioRad): multiplex flow immunoassay technology, fluorochrome
    - Luminex (Theradiag.)



  - Multi-line Immunodot assay

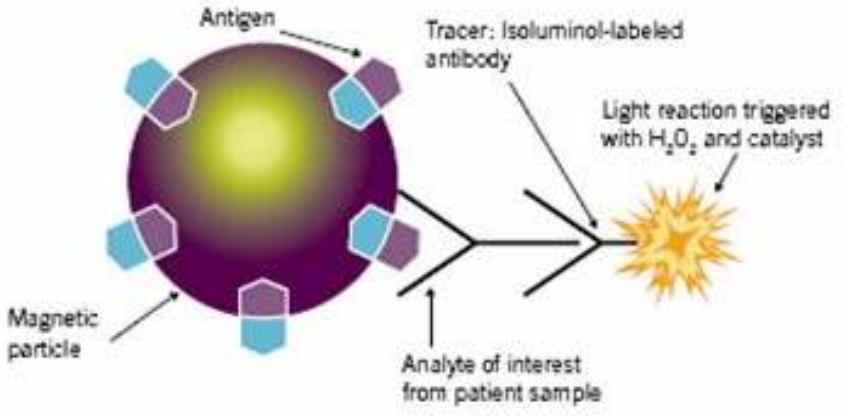
Sénat et al, J Immunol Res 2015, 983094 ; Roggenbuck et al, Autoimmunity Reviews, 11, 2012, 821–826

aCL and aβ2GPI: methods



►Coated polystyrene cups

- EliA (Phadia-ThermoFischer): fluorescense enzyme immunoassay



►Magnetic beads, chemiluminescence

- Acustar, BioFlash (Werfen; IL, Inova)
- Zenit RA (Menarini)

aCL and aβ2GPI: methods

- Automated systems

  - Easy to perform
  - More strict protocols
  - Harmonized working conditions

    - Good reproducibility
    - Less inter-lab and intra-lab variation for one system

aCL and aβ2GPI: precision

Between-run imprecison

- ELISA: < 20% (15%)
- Automated systems: < 10%

DEVREESE ET AL, ISTH-SSC RECOMMENDATIONS, J THROMB HAEMOST 2014; 12: 792-5

	aCL IgG			aCL IgM			aβ2GPI IgG			aβ2GPI IgM		
	L	cutoff	H	L	cutoff	H	L	cutoff	H	L	cutoff	H
AcuStar												
mean	10,3	19,8	103	7,0	20,6	84		20,9	401	4,4	18,1	57
n	82	17	82	82	17	82		83	83	82	16	82
CV (%)	**7,46**	**4,31**	**6,04**	**6,27**	**2,53**	**4,6**		**8,41**	**7,3**	**5,7**	**6,07**	**9,30**
BioPlex												
mean	1,8	22,8	61,7	neg	24,3	51	neg	26,1	46	neg	20,8	45
n	9	17	44		17	43		17	44		17	44
CV (%)	**3,27**	**4,23**	**7,00**		**2,42**	**4**		**6,69**	**8,3**		**4,36**	**7,5**
EliA ImmunoCAP												
mean			48,7			47			58,0			50
n			46			46			46			46
CV (%)			**7,05**			**7,6**			**6,2**			**10**

Between-run imprecision
over 1 year

aCL and aβ2GPI: precision

Between-run imprecison

- ELISA: < 20% (15%)
- Automated systems: < 10%

DEVREESE ET AL, ISTH-SSC RECOMMENDATIONS, J THROMB HAEMOST 2014; 12: 792-5

aCL and aβ2GPI: precision

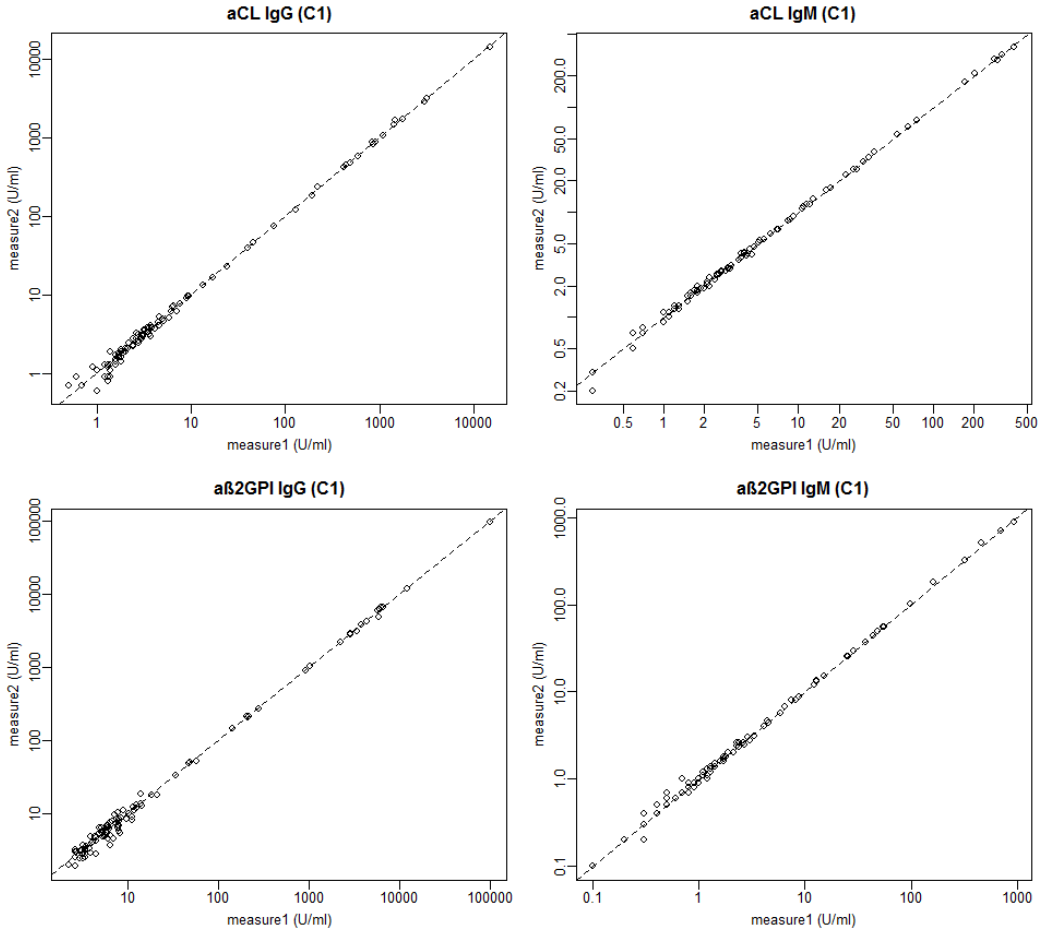
Intra-lab variation

N=100 samples analysed in duplicate with AcuStar

(APS, AID, DC, NC)

-very good repeatability

-no samples differed in classification (pos/neg) on first/second measurement

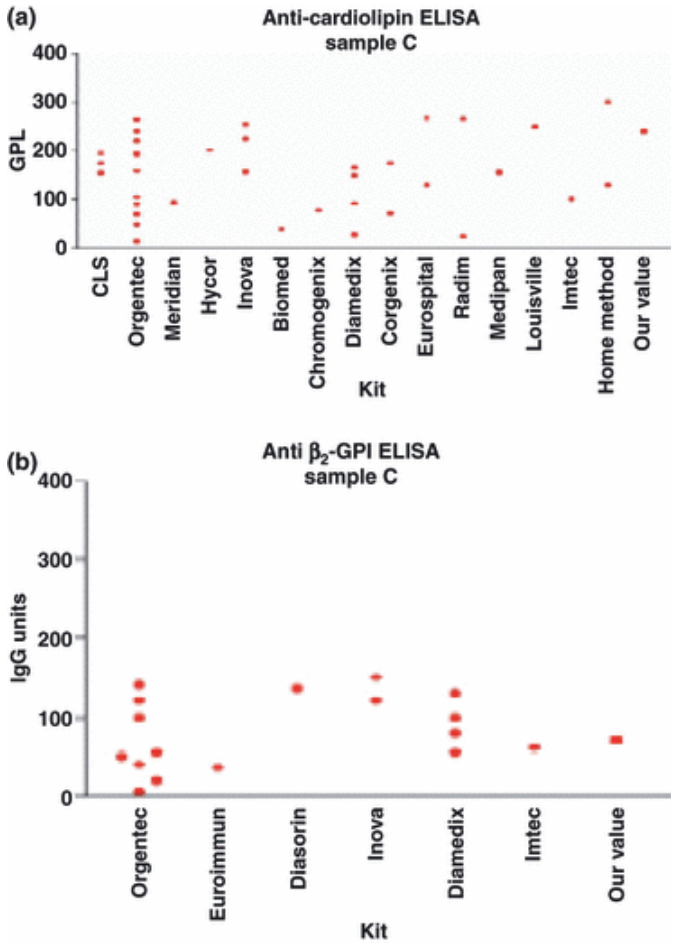


DEVREESE ET AL, A MULTICENTER STUDY TO ASSESS THE REPRODUCIBILITY OF ANTIPHOSPHOLIPID ANTIBODY RESULTS PRODUCED BY AN AUTOMATED SYSTEM. J THROMB HAEMOST. 2017 JAN;15(1):91-95

aCL and aβ2GPI: inter-lab variation

Comparison of ELISA

Commercial ELISAs: same samples, same method, tested in different labs

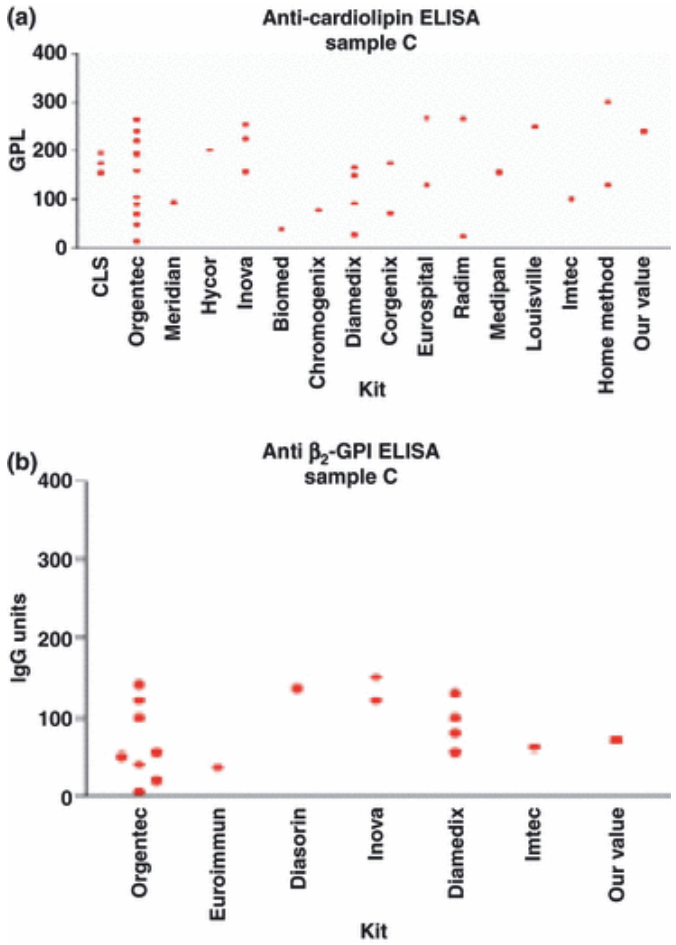


PENGO ET AL, THROMB RES. 2007;120:127-33

aCL and aβ2GPI: inter-lab variation

Comparison of ELISA

Commercial ELISAs: same samples, same method, tested in different labs



PENGO ET AL, THROMB RES. 2007;120:127-33

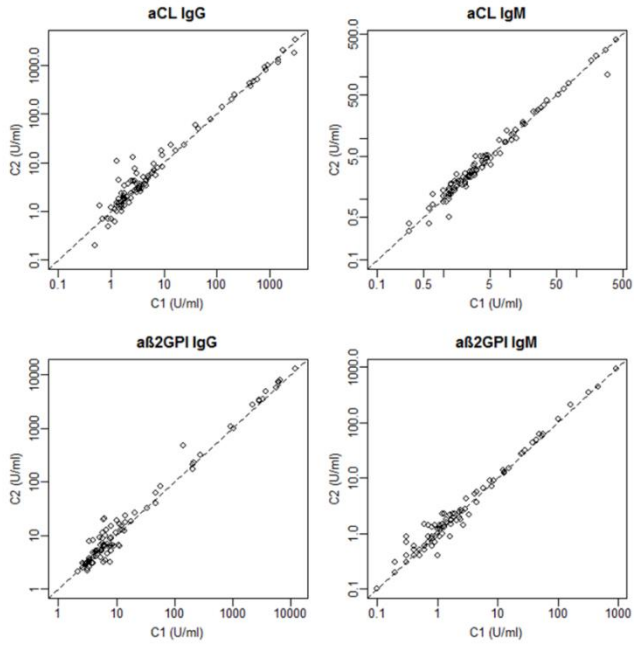
Comparison automated system

N=99 samples analysed in three centers with AcuStar

Comparison Center 1 and Center 2:

-good correlation for titers

-4 samples differed in classification (pos/neg) with values around the cut-off



DEVREESE ET AL,. J THROMB HAEMOST. 2017 JAN;15(1):91-95

aCL and aβ2GPI: inter-lab variation

ECAT 2016-1 (clearly positive aCL and aβ2GPI IgG)

IgG	n	assigned value	CV (%)	range
Total Group				-
GPL	117	135.5	35.2	17.6 - 823.0
Aeskulisa Diagnotic GmbH	5	175.0		152.0 - 238.0
INOVA Quanta Flash	5	102.0		82.0 - 108.0
INOVA Quanta Lite	14	106.5	10.6	87.1 - 164.0
Orgentec (Elisa)	19	93.7	24.5	34.0 - 120.0
Thermo Scientific EliA	51	167.8	12.0	114.0 - 208.0
U/mL	11	102.9	15.4	64.0 - 1816.0
Euroimmun	6	104.6		64.0 - 123.0
CU/mL	33	931.5	8.7	805.0 - 5594.5
I.L. Acustar Automate	25	943.5	9.5	805.0 - 5594.5

ELISA CV% 10.6-24,5 Automates CV% 9.5-12.0

IgG	n	assigned value	CV (%)	range
Ratio	22	10.4	109.0	0.1 - 60.0
				-
U	87	123.2	45.8	1.8 - 1400.0
Aeskulisa Diagnotic GmbH	6	88.3		1.8 - 427.0
INOVA Quanta Flash	5	61.0		49.0 - 84.0
INOVA Quanta Lite	9	67.6		52.6 - 93.0
Orgentec (Elisa)	16	83.5	19.2	61.8 - 102.0
Thermo Scientific EliA	37	156.2	10.3	112.0 - 203.0
µg/mL	3	76.3		4.7 - 144.0
U/mL	14	160.4	32.4	20.0 - 5424.6
Euroimmun	5	192.1		160.0 - 194.0
CU/mL	26	5942.4	13.5	930.6 - 7799.7
I.L. Acustar Automate	23	6053.7	12.5	930.6 - 7799.7

ELISA CV% 19.2 Automates CV% 10.3-12.5

ECAT 2018-1 (clearly positive aCL IgG)

IgG	n	assigned value	CV (%)	range
Total Group				-
GPL	134	209.7	74.5	3.1 - 601.3
Aeskulisa Diagnotic GmbH	8	197.0		45.0 - 273.0
INOVA Quanta Lite	23	61.9	23.9	36.1 - 87.0
Orgentec (Alegria)	5	118.9		87.9 - 276.8
Orgentec (Elisa)	19	92.4	25.6	59.0 - 237.5
Reaads	6	99.5		87.8 - 131.7
Thermo Scientific EliA	60	348.8	13.6	3.1 - 456.0
U/mL	17	95.6	47.3	33.0 - 2115.0
Euroimmun	12	77.0	22.6	33.0 - 106.0
CU/mL	42	943.7	11.6	759.5 - 1208.4
I.L. Acustar / INOVA Quanta Flash	42	943.7	11.6	759.5 - 1208.4

ELISA CV% 23,9-25,6 Automates CV% 11,6-13,6

- Inter-lab variation is lower for automated systems compared to ELISA

aCL and aβ2GPI: inter-assay variation

Comparison chemiluminescence assays

Qualitative AcuStar – Zenit (n=314)				
	aCL IgG	aβ2GPI IgG	aCL IgM	aβ2GPI IgM
Agreement (%)	93,90	96,2	95,50	95,90
Kappa	0,481	0,555	0,510	0,559

314 plasma samples were including:
- 30 APS patients
-146 thrombotic patients

Persijn et al, Evaluation of a new set of automated chemiluminescence assays for anticardiolipin and anti-beta2-glycoprotein I antibodies in the laboratory diagnosis of the antiphospholipid syndrome, Throm Res 2011, 565-569

aCL and aβ2GPI: inter-assay variation

- Lack of uniformity in calibration
  - Harris standards (polyclonal IgG and IgM)
  - Secundary standards (heterogenous)
  - Monoclonal antibodies HCAL, EY2C9
  - ”internal standard”

- Differences in kits
  - differences in production of the kits:
    type of microtiter plate, PL, blocking agents, source of β2GPI, coating…

	Zenit RA		HemosIL Acustar		BioPlex 2200	
	aCL IgG/IgM	aβ2GPI IgG/IgM	aCL IgG/IgM	aβ2GPI IgG/IgM	aCL IgG/IgM	aβ2GPI IgG/IgM
Calibration	Harris reference sera	internal reference standard not further specified by manufacturer	internal standard correlated with reference chimeric antibody HCAL and EY2C9		internal standard not further specified by manufacturer	

aCL and aβ2GPI: inter-assay variation

New technologies: performance

study	patients	assay	sensitivity (%)				specificity (%)			
Some examples			aCL		aβ2GPI		aCL		aβ2GPI	
			IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Montarulli 2016	77/190 APS	EliA Phadia	55,3	35,5	55,8	29,9	75,5	79,1	82,3	70,8
		CliA Zenit	54,5	27,3	59,7	29,9	78,8	76,1	80,5	76,1
		CliA BioFlash	51,9	23,4	55,8	18,2	77,9	79,5	67,3	77,7
		ELISA	55,8	54,5	44,2	31,2	76,1	55,8	83,2	70,8
Vandevelde 2016 (poster)	66/354 APS	CliA Acustar	19,7	25,8	24,2	10,6	99	92,4	92,4	97,6
		BioPlex 2200	19,7	12,1	18,2	13,6	96,6	97,2	97,2	96,9
Devreese 2013 (poster)	81/239 APS	CliA Acustar	69,1	39,5	75,3	33,3	96,8	94,3	92,4	96,2
		BioPlex 2200	72,8	35,8	72,8	38,3	98,7	96,2	98,1	96,2
Tozzoli 2014	50/131 APS	BioPlex 2200	86,0	38,0	88,0	42,0	92,6	95,1	95,1	97,6
Van Hoecke 2012	26/134 APS	CliA Zenit	56,3	75	57,1	60	100	98,3	100	98,3
		CliA Acustar	50	50	71,4	40	100	98,3	99,1	100
Ghillani 2012	79/190 APS	CliA Zenit	54,4	24,1	53	20,8	97,3	95,5	91	95,1
		ELISA	68,3	20,2	40	15,2	95,5	97,3	91	98,1
Persijn 2011	30/314 APS	CliA Zenit	30	16,7	26,7	6,7				
		ELISA TBS	46,7	6,7	46,7	13,3				
Devreese 2011	34/164 APS	CliA Acustar	25,7	11,4	52,9	11,4	100	96,1	87,4	99,2
		ELISA	48,6	11,4	42,9	11,4	96,1	96,6	95,3	96,9
Villalta 2009	62/185 APS	EliA Phadia	69,4	65,4	64,5	53,7	81,9	86,7	98,8	92,8
de Moerloose 2010	85/218 APS	CliA Acustar	55,4	33,7	66,3	30,4	95,6	94,3	89,5	95,2
		ELISA	46,7	31,5	28,3	29,3	96,5	94,3	98,7	92,6

aCL and aβ2GPI: inter-assay variation

New technologies: performance

study	patients	assay	sensitivity (%)				specificity (%)			
Some examples			aCL		aβ2GPI		aCL		aβ2GPI	
			IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Montarulli 2016	77/190 APS	EliA Phadia	55,3	35,5	55,8	29,9	75,5	79,1	82,3	70,8
		CliA Zenit	54,5	27,3	59,7	29,9	78,8	76,1	80,5	76,1
		CliA BioFlash	51,9	23,4	55,8	18,2	77,9	79,5	67,3	77,7
		ELISA	55,8	54,5	44,2	31,2	76,1	55,8	83,2	70,8
Vandevelde 2016 (poster)	66/354 APS	CliA Acustar	19,7	25,8	24,2	10,6	99	92,4	92,4	97,6
Villalta 2009	62/185 APS	EliA Phadia	69,4	65,4	64,5	53,7	81,9	86,7	98,6	92,6
de Moerloose 2010	85/218 APS	CliA Acustar	55,4	33,7	66,3	30,4	95,6	94,3	89,5	95,2
		ELISA	46,7	31,5	28,3	29,3	96,5	94,3	98,7	92,6

assay	sensitivity (%)				specificity (%)			
	aCL		aβ2GPI		aCL		aβ2GPI	
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
EliA Phadia	55-69	35-65	56-64	30-54	76-82	79-87	82-99	71-93
CliA Zenit	30-56	24-75	27-60	7-60	79-100	76-98	81-100	76-98
CliA BioFlash/AcuStar	26-55	11-50	53-71	11-40	78-100	80-98	67-99	78-100
Multiplex BioPlex 2200	20-86	12-38	18-88	14-42	93-99	95-97	95-98	96-98

- Performance of assays varies depending on the study design

aCL and aβ2GPI: assay agreement

Multicenter solid phase assay study

Agreement between commercial assays for aPL detection

- 1168 samples from eight European centers, including APS, AID, diseased controls, healthy controls.
- aCL and aβ2GPI IgG/IgM, determined by a single technician, 4 platforms

Participants	Center
K. Devreese	Ghent University Hospital
D. Wahl/ S. Zuily	Centre hospitalier universitaire de Nancy
A. Tripodi	Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milano
G. Moore	Diagnostic Haemost & Thromb Laboratory, St Thomas' Hospital, London
J. Musial	Jagiellonian University Medical College, Krakow, Poland
P. Fontana	University Hospital Geneva, Switzerland
J. Remijn	Gelre hospitals, Apeldoorn, the Netherlands
J.C. Gris	Centre Hospitalier Universitaire, Nîmes, France

Manufacturer	
AcuStar HemosIL	Werfen/IL
BioPlex 2200	BioRad
EliA	Phadia/ThermoFisher
Quanta Lite ELISA	Werfen/INOVA

aCL and aβ2GPI: agreement

Agreement between methods

Multicenter solid phase assay study; total population n= 1168 APS thrombosis n=259, Non-APS thrombosis n=204, AID+HC n=390, APS obstetric n= 122, non-APS obstetric n= 33, normal pregnancy n= 100, unspecified APS n=60

- good-very good agreement between methods for aCL IgG, aβ2GPI IgG and aβ2GPI IgM positivity
- moderate agreement for aCL IgM positivity

UNPUBLISHED DATA, COURTESY TO WALID CHAYOUA

aCL and aβ2GPI: agreement

Clinical performance: agreement

- aPL positive samples not in agreement across the platforms showed lower median aPL titers than positives in agreement
- the majority of discrepant patients showed clinical manifestations of APS

UNPUBLISHED DATA, COURTESY TO WALID CHAYOUA

aCL and aβ2GPI: agreement

Multicenter solid phase assay study
APS thrombosis n=259, Non-APS thrombosis n=204
+ AID+HC: n=390

Clinical performance: sensitivity and specificity

- Sensitivity and specificity differs between platforms

UNPUBLISHED DATA, COURTESY TO WALID CHAYOUA

aCL and aβ2GPI: agreement

Clinical performance: antibody profiles

Agreement of triple positivity detection

Multicenter solid phase assay study

APS thrombosis n=258, Non-APS thrombosis n=204, AID+HC n=389

Agreement	
>0.90	Almost perfect
0.80-0.90	Strong
0.60-0.79	Moderate

	BioPlex® 2200	ImmunoCap® EliA	ACL AcuStar™	Quanta Lite ELISA®
BioPlex® 2200	1	0.84	0.95	0.79
ImmunoCap® EliA	0.84	1	0.86	0.85
ACL AcuStar®	0.95	0.86	1	0.85
Quanta Lite ELISA®	0.79	0.85	0.85	1

CHAYOUA ET AL, J THROMB HAEMOST. 2018; DOI - 10.1111/JTH.14261

aCL and aβ2GPI: agreement

Clinical performance: antibody profiles

Agreement of triple positivity detection

Multicenter solid phase assay study
APS thrombosis n=258, Non-APS thrombosis n=204, AID+HC n=389

Agreement	
>0.90	Almost perfect
0.80-0.90	Strong
0.60-0.79	Moderate

	BioPlex® 2200	ImmunoCap® EliA	ACL AcuStar™	Quanta Lite ELISA®
BioPlex® 2200	1	0.84	0.95	0.79
ImmunoCap® EliA	0.84	1	0.86	0.85
ACL AcuStar®	0.95	0.86	1	0.85
Quanta Lite ELISA®	0.79	0.85	0.85	1

Number of triple positives according to the platform

	Lupus anticoagulant positive	Triple positives			
		BioPlex® 2200	ImmunoCap® EliA	ACL AcuStar®	QUANTA Lite ELISA®
APS Thrombosis (n=258)	202	118	101	111	89
Non-APS Thrombosis (n=204)	0	0	0	0	0
AID controls (n=196)	56	22	18	19	14
Controls (n=193)	16	6	6	4	3
Total patient population (n=851)	274	146	125	134	106

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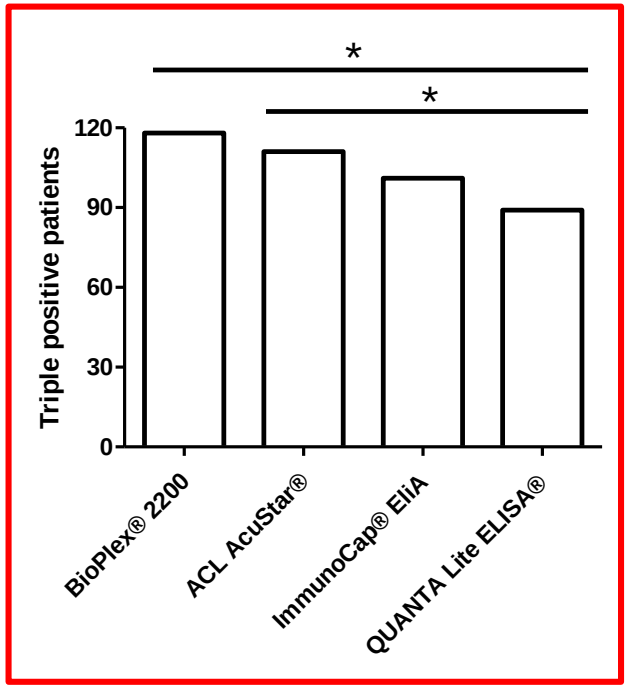


Number of triple positives differs according to the platform

	Lupus anticoagulant positive	Triple positives			
		BioPlex® 2200	ImmunoCap® EliA	ACL AcuStar®	QUANTA Lite ELISA®
APS Thrombosis (n=258)	202	118	101	111	89
Non-APS Thrombosis (n=204)	0	0	0	0	0
AID controls (n=196)	56	22	18	19	14
Controls (n=193)	16	6	6	4	3
Total patient population (n=851)	274	146	125	134	106

- triple positivity detection differs between platforms

CHAYOUA ET AL, J THROMB HAEMOST. 2018; DOI - 10.1111/JTH.14261

aCL and aβ2GPI: clinical performance

Multicenter solid phase assay study
APS thrombosis n=259, Non-APS thrombosis n=204
+ AID+HC: n=390

Odds ratio for thrombosis

- Association of aCL/aβ2GPI IgG and IgM positivity and thrombosis is variable per method detection

UNPUBLISHED DATA, COURTESY TO WALID CHAYOUA

aCL and aβ2GPI: clinical performance

Clinical performance: antibody profiles

Multicenter solid phase assay study
APS thrombosis n=259, Non-APS thrombosis n=204
+ AID+HC: n=390

- Clinical association was globally concordant between solid phase test systems considering antibody profiles
- Considering all aPL, OR differ, measuring the four aPL with one test system

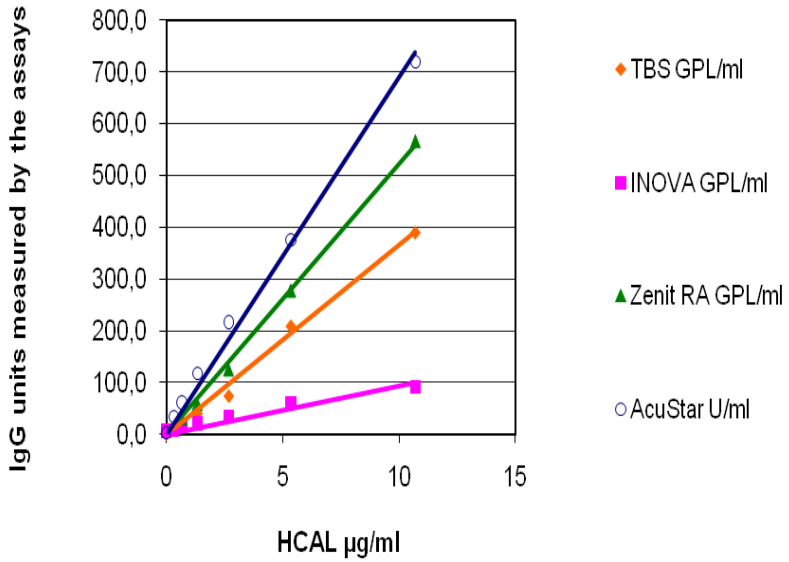
CHAYOUA ET AL, J THROMB HAEMOST. 2018; DOI - 10.1111/JTH.14261; UNPUBLISHED DATA

aCL and aβ2GPI: agreement

Titer comparison between methods

IgG Sapporo standard (HCAL) 10.7 µg/ml
-ELISA (Inova, The Binding Site)
-automated systems (Zenit, AcuStar)
both CLIA

aCL IgG



VAN HOECKE ET AL, INT J LAB HEMATOL, 2012, **34; 410-416**

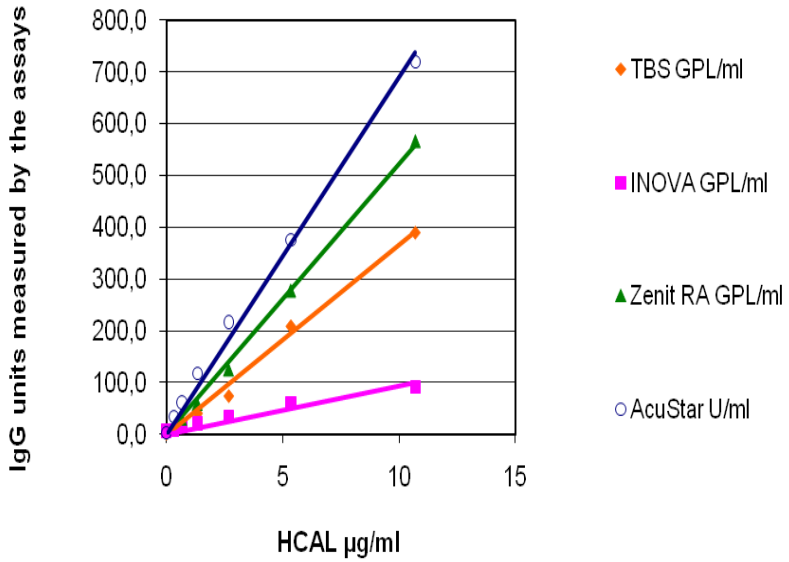
aCL and aβ2GPI: agreement

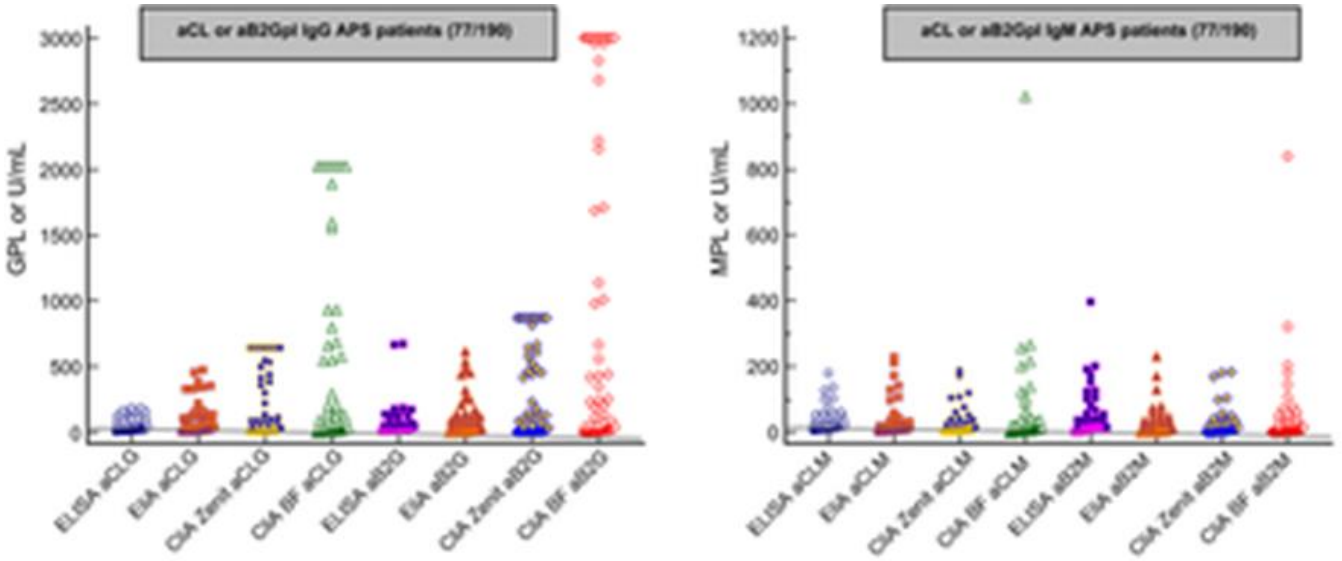
Titer comparison between methods

IgG Sapporo standard (HCAL) 10.7 µg/ml
-ELISA (Inova, The Binding Site)
-automated systems (Zenit, AcuStar)
both CLIA

Comparison of different immunoassays in APS and non-APS patients
-ELISA (Inova)
-automated systems (Phadia, Zenit, BioFlash)

aCL IgG





VAN HOECKE ET AL, INT J LAB HEMATOL, 2012, 34; 410-416

MONTARULI ET AL, INT J LAB HEMATOL 2016, 38 , 172-182

aCL and aβ2GPI: agreement

Titer correlation between methods

Multicenter solid phase assay study; total population n= 1168 APS thrombosis n=259, Non-APS thrombosis n=204, AID+HC n=390, APS obstetric n= 122, non-APS obstetric n= 33, normal pregnancy n= 100, unspecified APS n=60

- aCL/aβ2GPI IgG and aβ2GPI IgM titer showed a good-very good correlation
- Apart from Bioplex-Acustar, there is only a moderate aCL IgM titer correlation between the platforms

UNPUBLISHED DATA, COURTESY TO WALID CHAYOUA

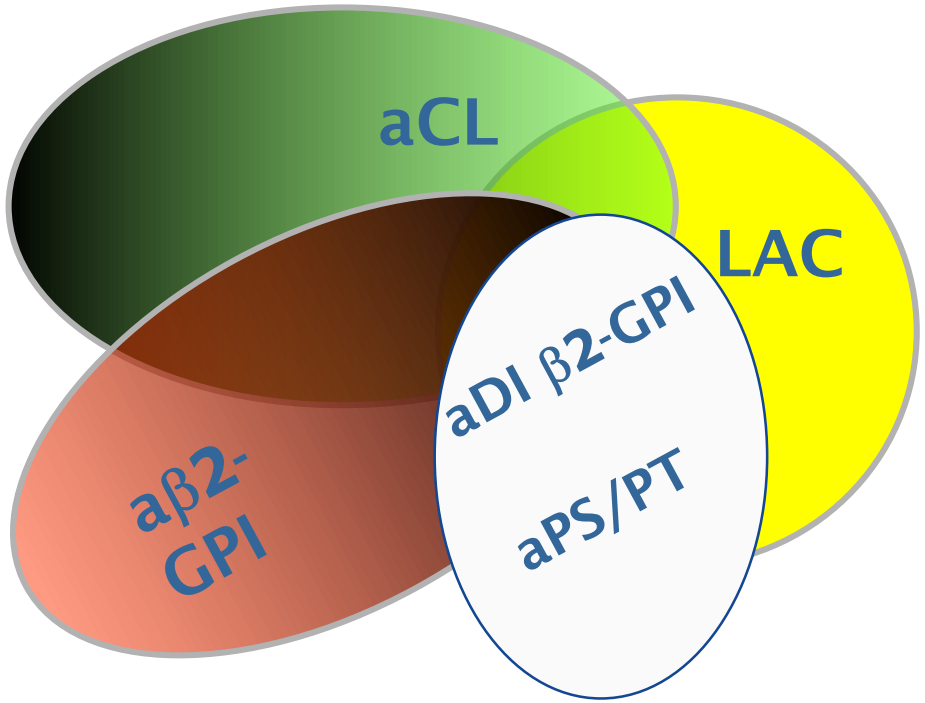
Antiphospholipid antibodies (aPL)

Lupus anticoagulants (LAC)

Anticardiolipin antibodies (aCL)

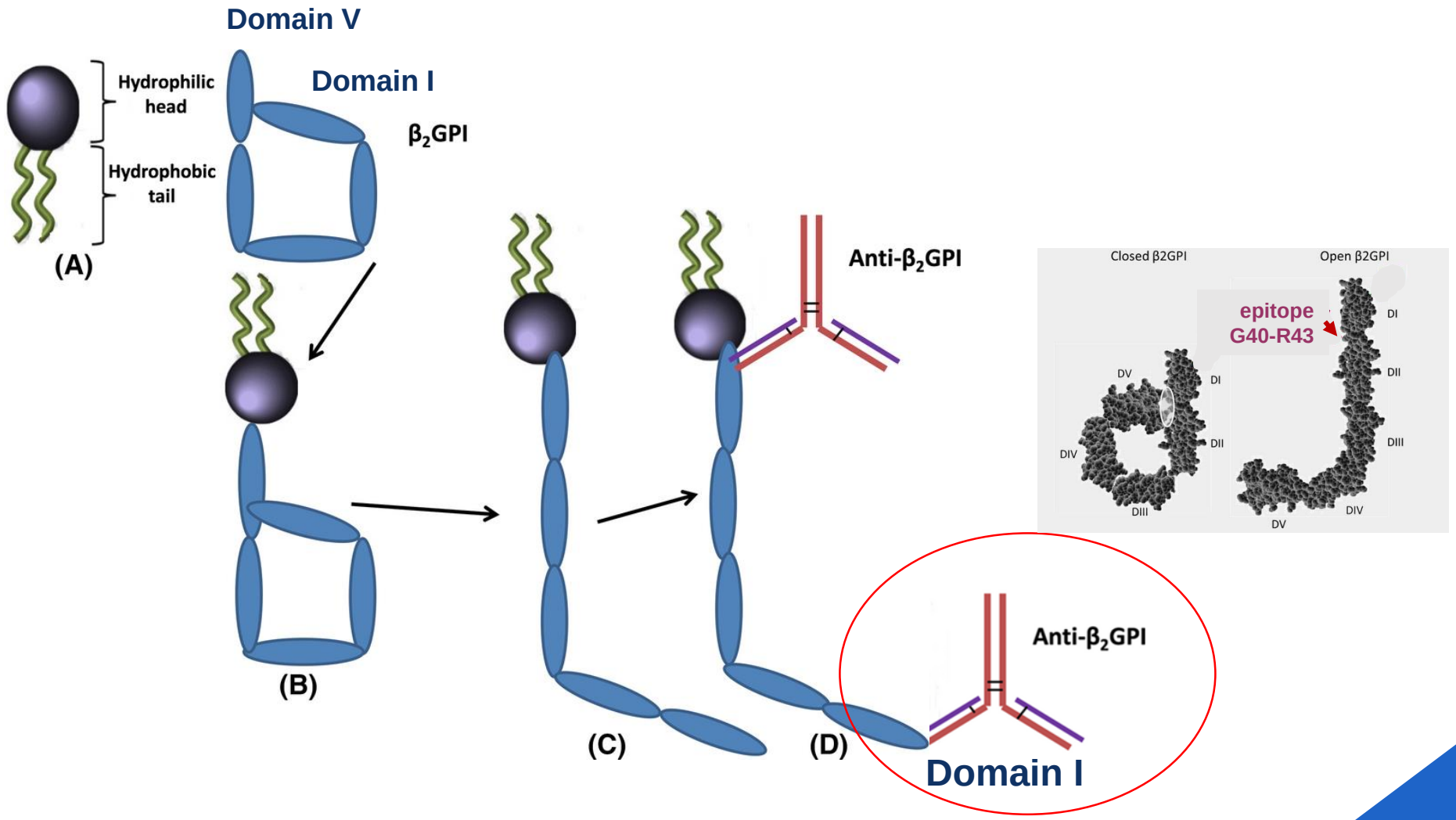
β2-glycoprotein I antibodies (aß2GPI)

Other aPL



"Non-criteria aPL"

Non-criteria aPL: subgroup of aß2GPI



ARACHCHILLAGE ET AL. BR J OF HAEMATOL 2017; 178: 181-195; VAN OS ET AL. J THROMB HAEMOST 2011; 9: 2447-2456; I. DIENAVA-VERDOOLD ET AL, J THROMB HAEMOST 2011; 9: 738-47; PELKMANS ET AL PLOS ONE. 2013;8:E71402

Non-criteria aPL: anti-domain I β2GPI

- First in-house ELISA
  - increased association with thrombosis
    - OR 18.9 *(DE LAAT ET AL, BLOOD 2005; 105:1540-5)*
    - OR 3.5 *(DE LAAT ET AL, J THROMB HAEMOST. 2009;7:1767-73)*

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)

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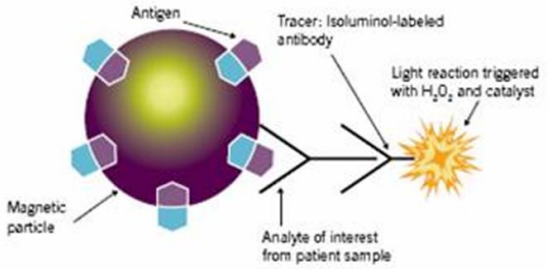
aCL	1.1 (0.6–2.1)

- Two other types of in-house ELISA
  - A direct aDI ELISA *(COUSINS ET AL. ANN RHEUM DIS 2015; 74: 317-319; PERICLEOUS ET AL. PLOS ONE 2016; 11: E0156407)*
  - A competitive inhibition ELISA *(POZZI ET AL, PROTEIN SCI 2010; 19:1065-1078; BANZATO ET AL. THROMB RES 2011; 128:583-586)*

- Commercial ELISA (INOVA) *(ANDREOLI ET AL, ANN RHEUM DIS 2011; 70: 380-383; ANDREOLI ETVAL, ARTHRITIS RHEUMATOL 2015; 67: 2196-2204; AKHTER ET AL. J RHEUMATOL 2013; 40: 282-286)*

- Chemiluminescense assay QUANTA Flash® assay (BioFlash/ Acustar, Werfen)
  - Since 2014
  - 17 published studies



YIN ET AL, AUTOIMMUNITY REVIEWS, 2018, IN PRESS

Non-criteria aPL: anti-domain I β2GPI

- significant correlation with thrombosis
- lower sensitivity compared to β2GPI IgG, higher specificity

	aβ2GPI (20 CU)	aβ2GPI (164 CU)	β2GPI-aD1 (20 CU)	β2GPI-aD1 (190 CU)
Sensitivity %			34.9	
Specificity %			99.5	
OR	2.3	4.1	4.0	8.7

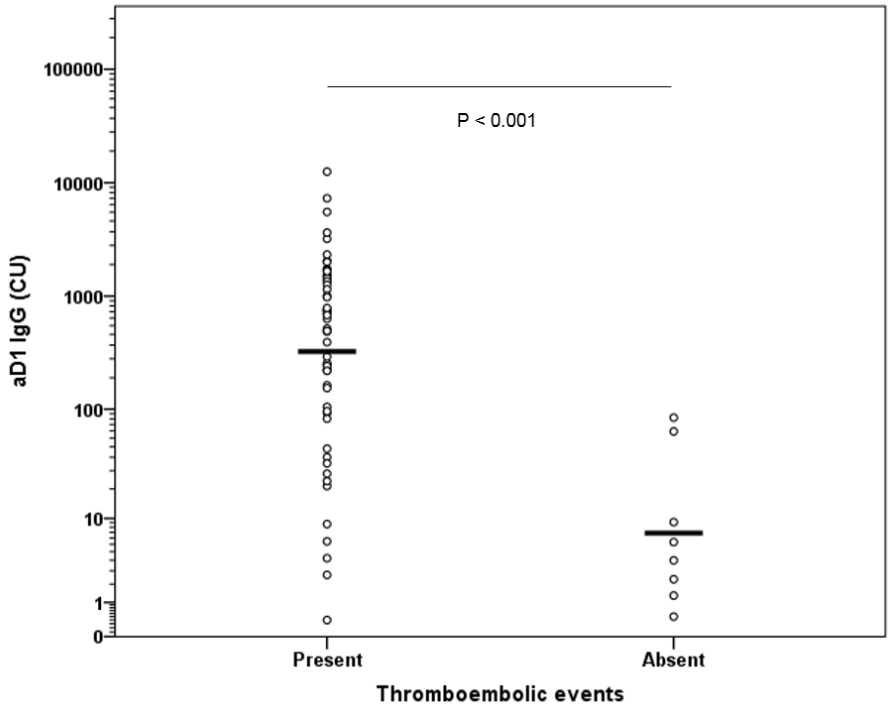
MAHLER ET AL, LUPUS 2016, 25:911-916

	aβ2GPI (20 CU)	aβ2GPI (60 CU)	β2GPI-aD1 (20 CU)	β2GPI-aD1 (44 CU)
sensitivity		56.4	53.5	48.5
specificity		99.1	97.8	99.1
OR		139	52.2	101

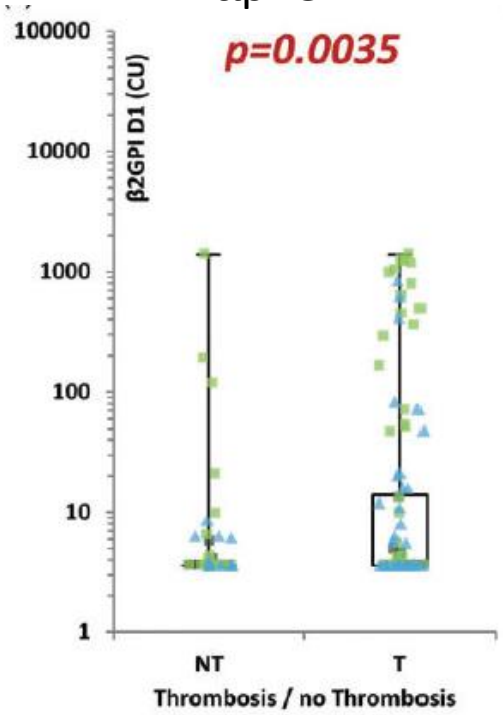
DE CRAEMER ET AL, J THROMB HAEMOST 2016, 14:1779-87

Non-criteria aPL: anti-domain I β2GPI

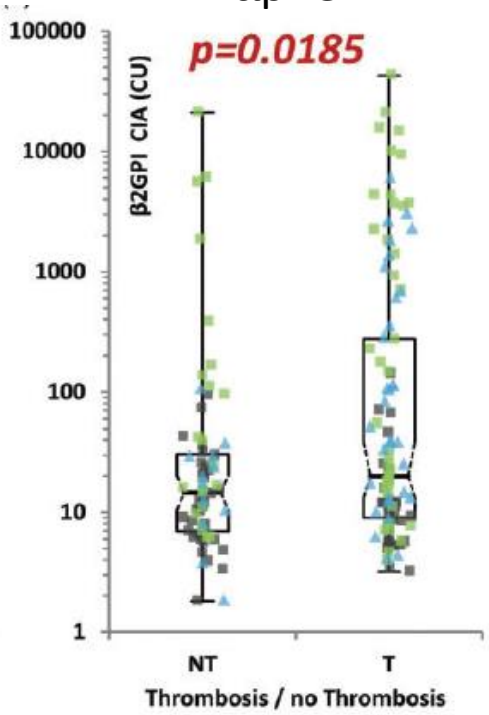
- higher titers of aDI in thrombotic patients



aβ2GPI-DI



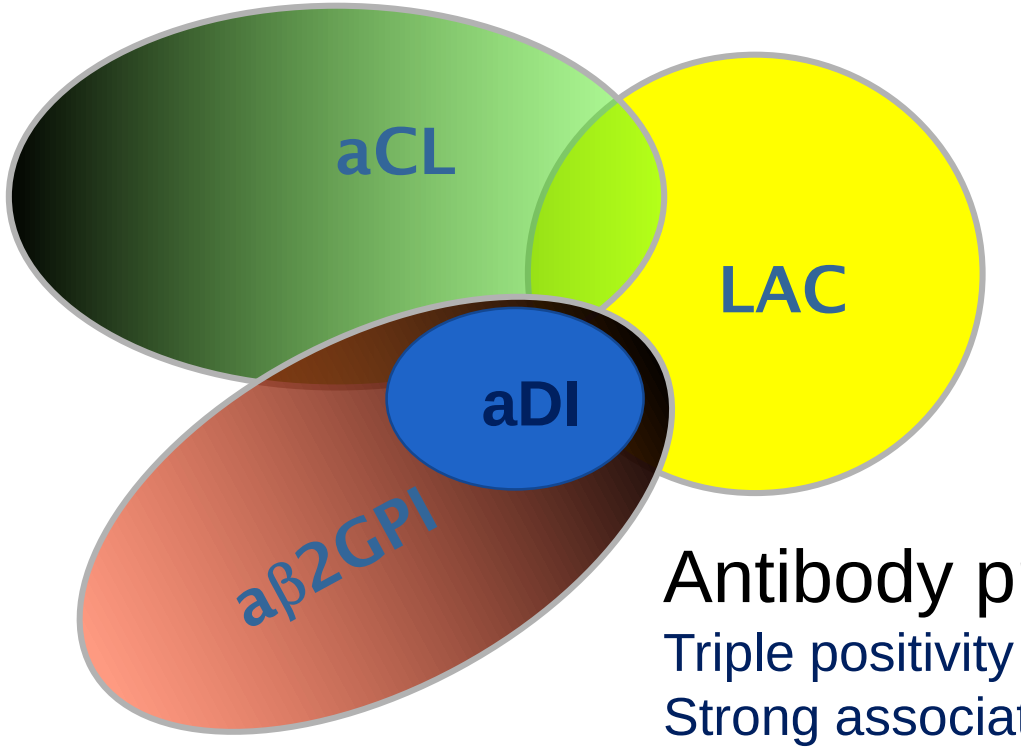
aβ2GPI



DE CRAEMER ET AL, J THROMB HAEMOST 2016, 14:1779-87

MAHLER ET AL, LUPUS 2016, 25:911-916

Non-criteria aPL: anti-domain I β2GPI



Antibody profiles:

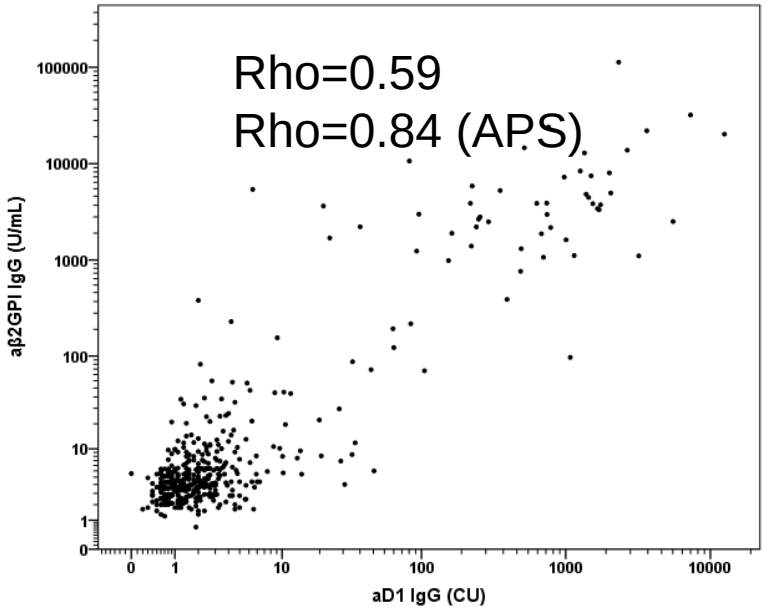
Triple positivity = patients at risk

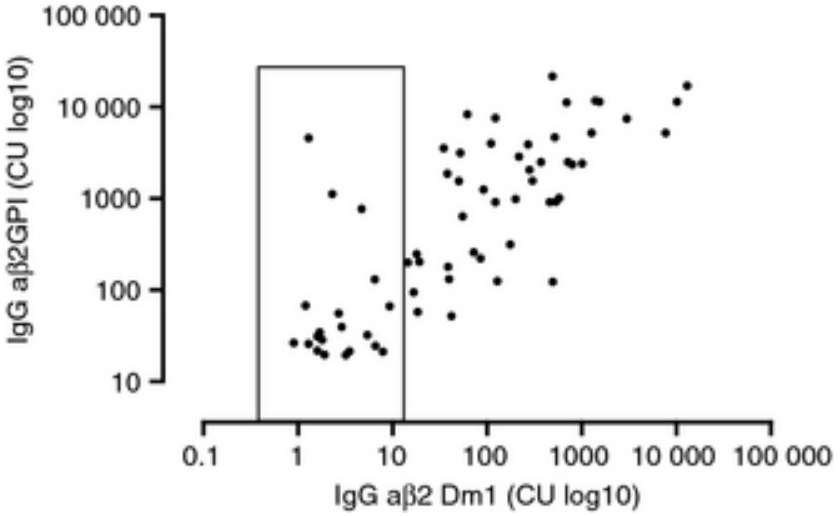
Strong association with anti-domain I β2GPI (aDI)

Meneghel et al. Clin Chim Acta. 2015;446:201-5;
Mondejar et al. Clin Chim Acta. 2014;431:174-8;
Pengo et al.. J Thromb Haemost. 2015;13:782-7;
Mahler M et al. Lupus 2016; 25:911-916;
De Craemer ET AL. J Thromb Haemost 2016, 14:1779-87

Non-criteria aPL: anti-domain I β2GPI

- good correlation for anti-domain I and aβ2GPI for commercial assay QUANTA Flash® assay





qualitative agreement aDI /aβ2GPI	
de Laat, 2009	*positive agreement 55%*
Pengo, 2015	positive agreement 69%
Mondejar, 2014	overall agreement 91%
Meneghel, 2015	overall agreement 91%
Devreese, 2016	positive agreement 92%

DE CRAEMER ET AL, J THROMB HAEMOST 2016, 14:1779-87

PENGO ET AL, J THROMB HAEMOST 2015, 14:1779-87

Non-criteria aPL: anti-domain I β2GPI

- Added value of the commercial aDI assay compared to criteria aPL: inconsistency in results
  - 4 Yes: *Lee et al, Clin Chem Lab Med 2017; 55: 882-889; Pericleous et al, PLoS One 2016; 11: e0156407; Nakamura et al, Arthritis Care & Research 2017: 1-46; Nojima et al, Thromb Res 2017; 153: 83-84*
  - 3 No: *Iwaniec et al, Thromb Res 2017; 153: 90-94; De Craemer et al, J Thromb Haemost 2016; 14: 1779-1787; Marchetti et al, J Thromb Haemost 2016; 14: 675-684*

Covariates		AUC predicted probability
LAC + aCL IgG/IgM	+ aβ$_2$GPI IgG	0.77
LAC + aCL IgG/IgM	+ aD1 IgG	0,76
LAC + aCL IgG/IgM + aβ$_2$GPI IgG	+ aD1 IgG	0,77

De Craemer et al, J Thromb Haemost 2016, 14:1779-87

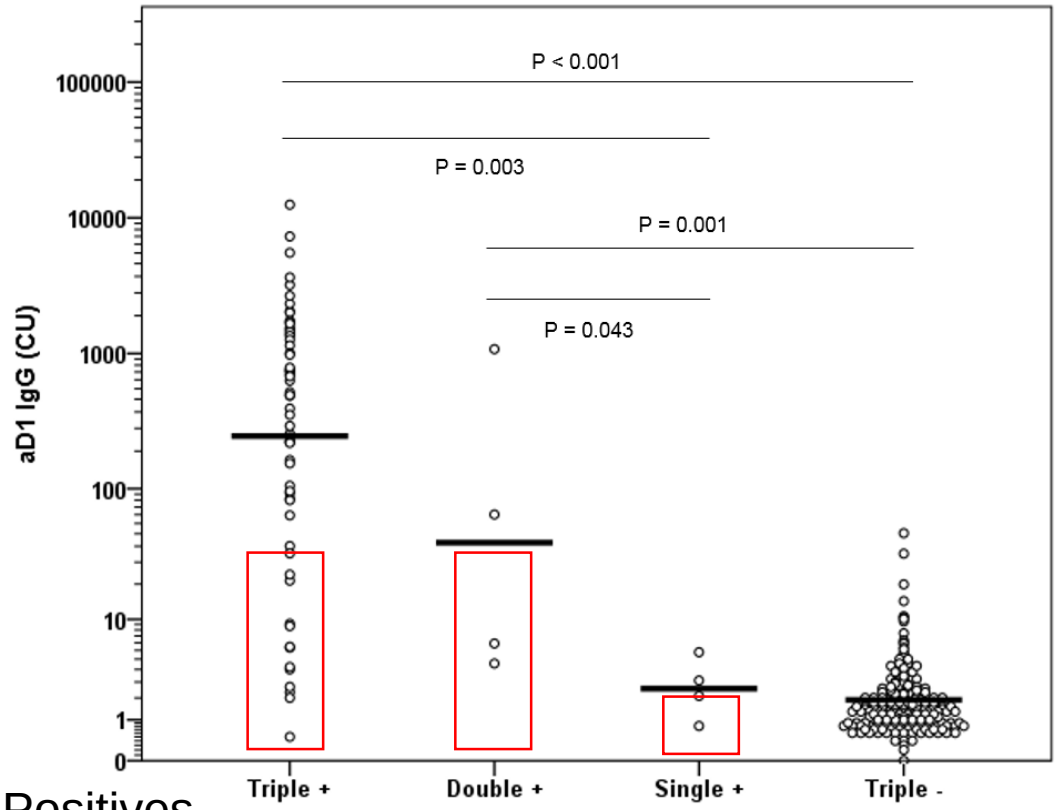
		AUC
LAC		0.872
LAC	+ aD1	0.755
aβ2GPI IgG		0.770
aβ2GPI IgG	+ aD1	0.728
Triple pos		0.829
Triple pos	+aD1	0.755

Iwaniec et al, Throm Res 2017, 153: 90-94

Poster Dongmei Yin et al

Non-criteria aPL: anti-domain I β2GPI

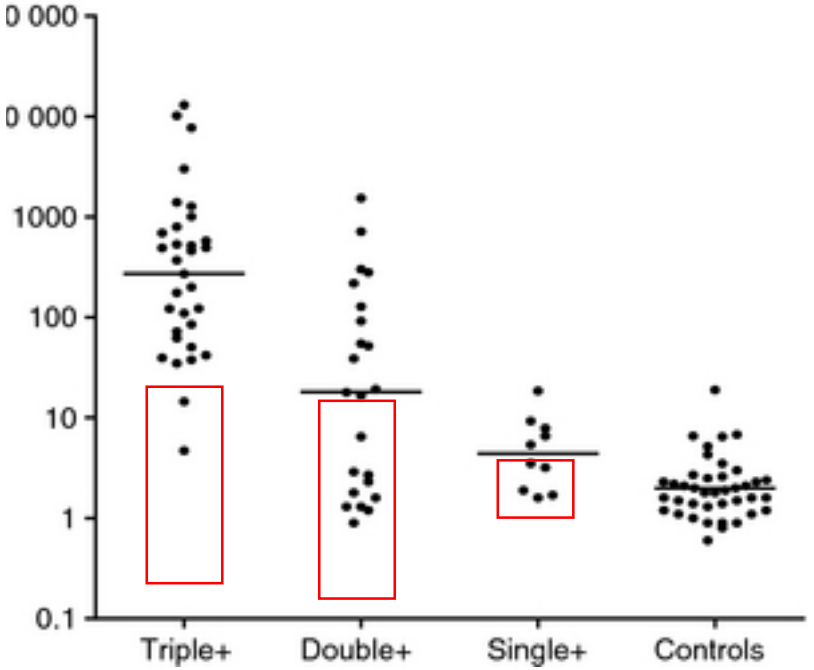
292/ 426 patient samples (APS, AID, DC, HC), positive for IgG aβ2GP1



Positives /group 52/62 2/4 0/4 2/222

DE CRAEMER ET AL, J THROMB HAEMOST 2016, 14:1779-87

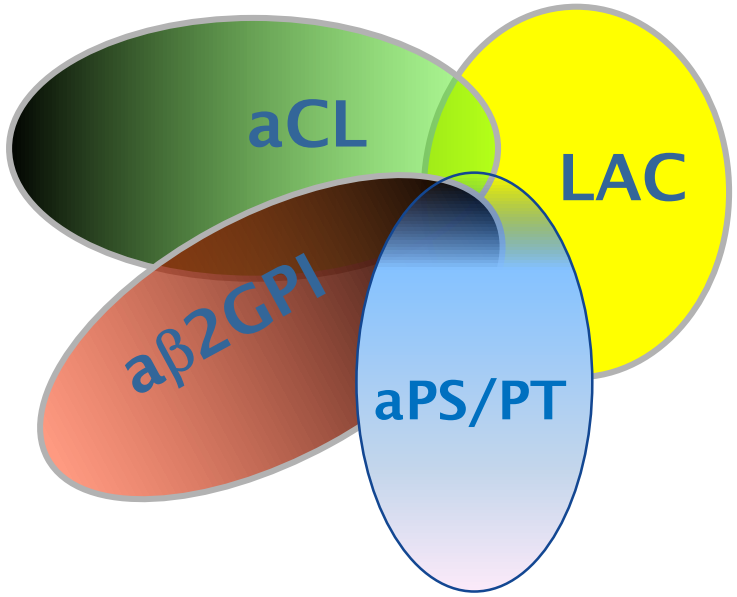
65 individuals over the three groups, all positive for IgG aβ2GP1



PENGO ET AL, J THROMB HAEMOST 2015, 14:1779-87

Non-criteria aPL: Anti-prothrombin/phosphatidyl serine

- Only ELISA (in-house, commercial)
- Good agreement between two commercial assays (MBL, INOVA)
- Promising results
- Often associated with LAC
- Few studies in animal models
- Unknown epitope on prothrombin to which antibodies are directed
- Clinical significance is further investigated



Amengual et al, Lupus 2017; 26: 266-276
Sciascia, S. et al.. Thromb. Haemost. 2014; 111, 354–364
Vega-Ostertag M, et al. Br J Haematol 2006;135:214-209
Haj-Yahia S et al. Lupus 2003;12:364-369

CONCLUSIONS

- Automated systems are easy to apply with less variation in local working conditions
- Detecting several aPL (aCL, aβ2GPI IgG/IgM) simultaneously in one sample
- More rapid and less labor intensive: alternative to running multiple ELISAs

- Between-run imprecision and inter-lab variation of newer systems is good and lower compared to ELISA

- New (automated) assays perform well (sensitivity, specificity) but differences between platforms
  - This may hamper uniformity in the classification of aPL positive samples
- Moderate (aCL IgM)/ (very) good qualitative (aCL IgG, aβ2GPI IgG and aβ2GPI IgM) agreement for positivity

- No comparison between numerical values of titers, but correlation is good for aCL/aβ2GPI IgG and aβ2GPI IgM
  - ➢Opportunity to classify into low-medium-high titers

CONCLUSIONS

- The number of triple positive patients depends on the platform
  - However, independently of the platform, triple positivity is a very sensitive biomarker to identify patients with thrombotic APS
  - The strength of the thrombotic association depends on the platform used

- Diagnostic accuracy is comparable: Odds ratio for clinical complications considering all aPL together were concordant.
  - Measurement of the four aPL with one system

- Classification of APS patients and follow-up is preferable performed with the same system

- **One step closer towards harmonisation**

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