

Disclosures of: Yvonne Henskens

Employment	No conflict of interest to disclose
Research support	No conflict of interest to disclose
Scientific advisory board	No conflict of interest to disclose
Consultancy	No conflict of interest to disclose
Speakers bureau	No conflict of interest to disclose
Major stockholder	No conflict of interest to disclose
Patents	No conflict of interest to disclose
Honoraria	No conflict of interest to disclose
Travel support	No conflict of interest to disclose
Other	No conflict of interest to disclose

LTA standardisation in the Netherlands

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VHL Society for Hematological Laboratories
&
WHD-NVTH Working group Hemostasis Diagnostics
Dutch Society for Thrombosis and Hemostasis

Inspired by:



- SSC Platelet session LTA in Boston 2009
- The worldwide survey on LTA in 2009
- The large number of “advises” on LTA in literature
- The lack of an alternative for LTA

We were convinced that The Netherlands could be the first country with 100 % standardization by a national LTA protocol

A practical approach to reach 100 % standardization:



What is the Golden standard LTA Guideline ?

•SSC-ISTH Cattaneo et al

- Draft SSC Egypt, 2010 → JTH april 2013

•CLSI

- Clinical and Laboratory Standards Institute/2008/Platelet Function testing by aggregometry/

•Dawood and Watson /GAPP

- Genotyping and Platelet Phenotyping)/UK/Blood 2012 and JTH 2013

•C. Hayward et al.

- Development of North American Consensus Guidelines for Medical Laboratories That Perform and Interpret Platelet Function Testing Using Light Transmission Aggregometry) USA/Am J Clin Pathol 2010

•P. Harrison et al

- Guidelines for the laboratory investigation of heritable disorders of platelet function/ British Committee for Standards in Haematology/ BJH 2011

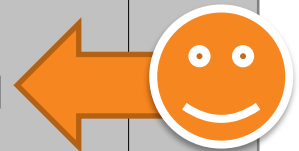
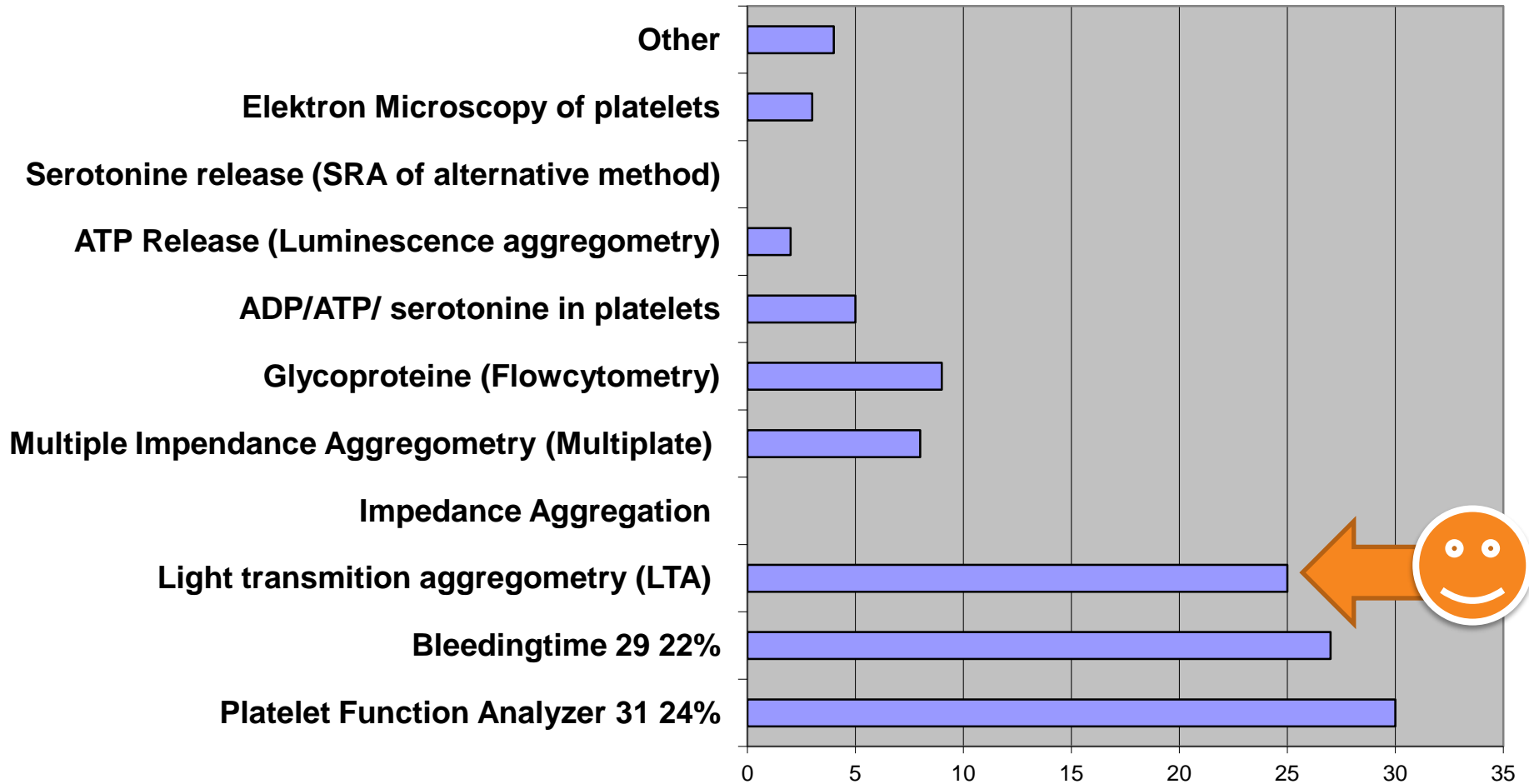
GOALS LTA standardization project NL

PLAN A (2011-2013)

1. Questionnaire
2. Show current differences in MA (%) between laboratories
3. Influence of brands of agonists on one analyzer
4. Influence of different analyzers

Plan A-1: Questionnaire on platelet function tests

n=46






n=25/SSC guideline	Yes	No
Patient resting	8	17
Fasting ??	3	22
Vacutainers	16	9
Citrate 109 or 129 mM	18	7
Discard tube	15	10
Sample resting	18	7
Centrifugal force 200 g	4	21
Centrifugal time 10 min	12	13
Use brake	5	20
Reference values	6	19
Normal subject	11	14
Adjustment	17	8

n=25/SSC guideline	Yes	No
Patient resting	8	17 😊
Fasting ??	3	22
Vacutainers	16	9
Citrate 109 or 129 mM	18	7 😊
Discard tube	15	10 😊
Sample resting	18	7 😊
Centrifugal force 200 g	4	21 😊
Centrifugal time 10 min	12	13 😊
Use brake	5 😊	20
Reference values	6	19
Normal subject	11	14
Adjustment	17	8

Questionnaire: results on agonist concentrations



	End conc	SSC-ISTH	NL	% (n=25) Used by
ADP	μM	2 and higher	0,4-100	100 % 
Collagen	μg/ml	2 and higher	1-10	100 % 
Arachidonic Acid	mM	1 and higher	0,5-3,0	76 %
Epinephrine	μM	5 and higher	0,025-200	60 %
Ristocetin	mg/ml	1,2 and higher	0,5-12,5	96 % 
TRAP-Par-1 SFLLRN	μM	10 and higher	15	12 %
U46619	μM	1 and higher	1	8 %

Plan A-2

**To determine differences in MA (%)
between the current procedures in Dutch
hospital laboratories**

**n= 9 laboratories shared their results of
healthy volunteers (used as quality
control) with us**

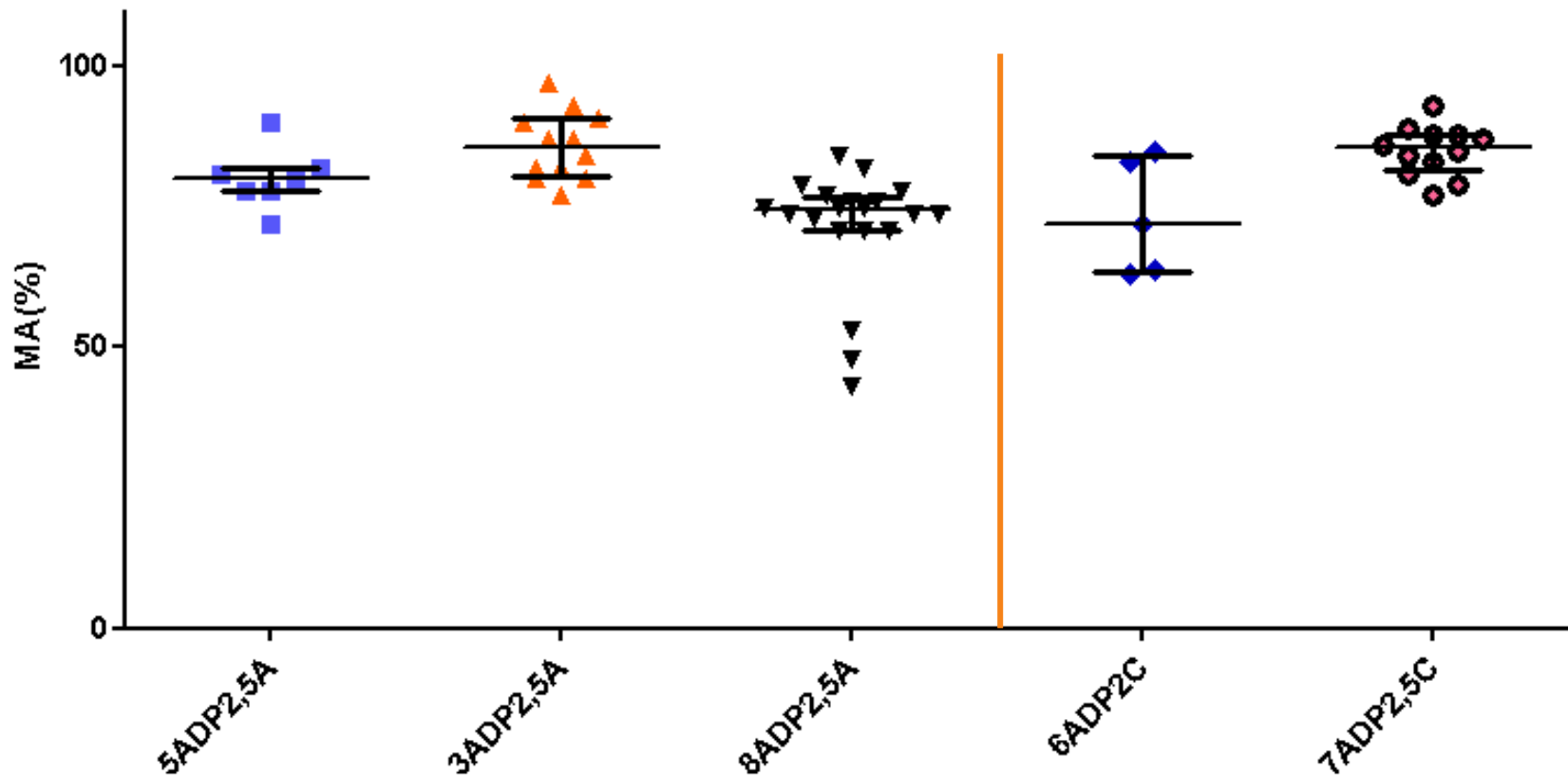
This means: 9 different procedures !

5: chronolog, 1: pap8, 3: apact

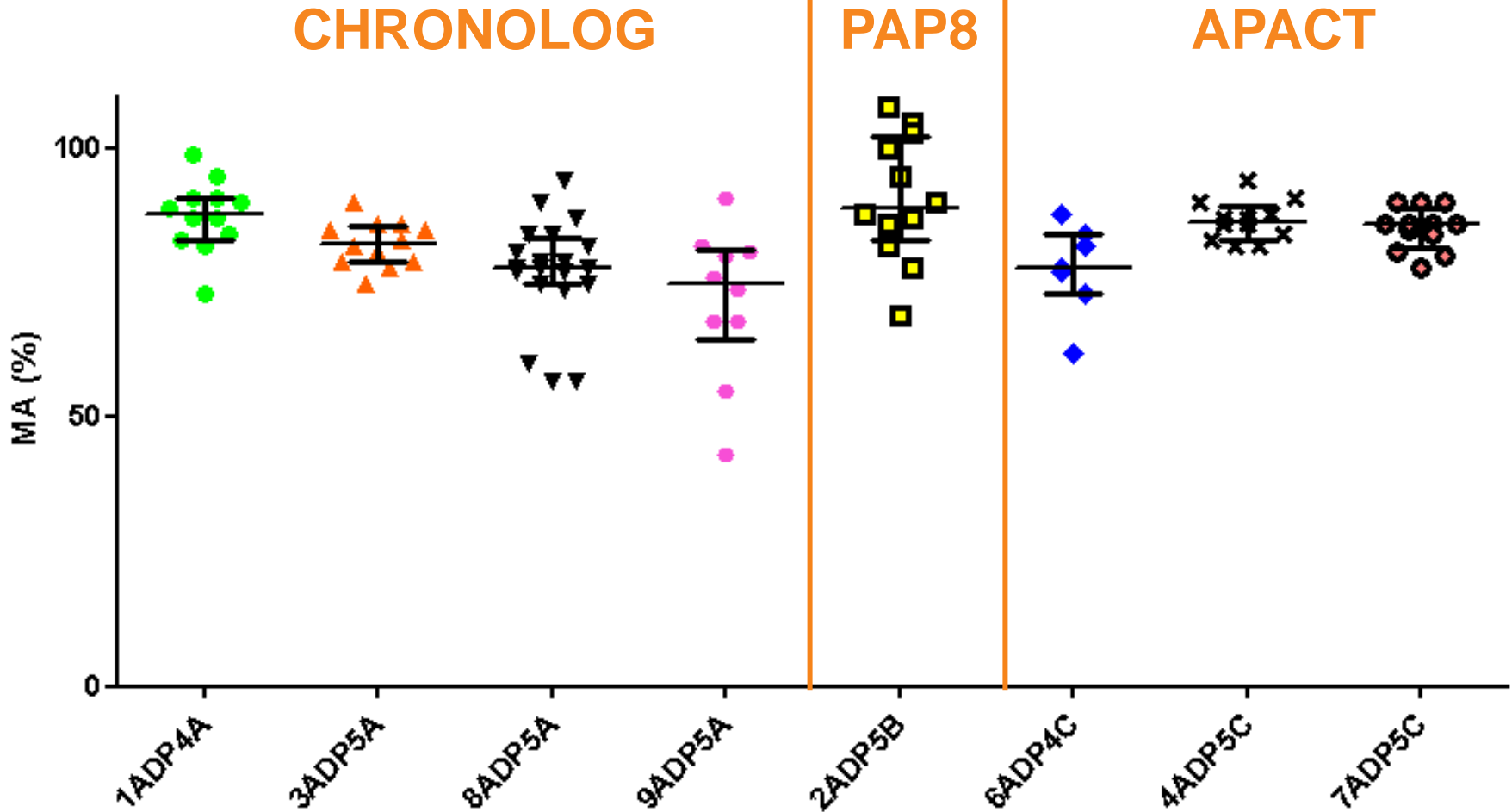
ADP range 2 to 2,5 μM

CHRONOLOG

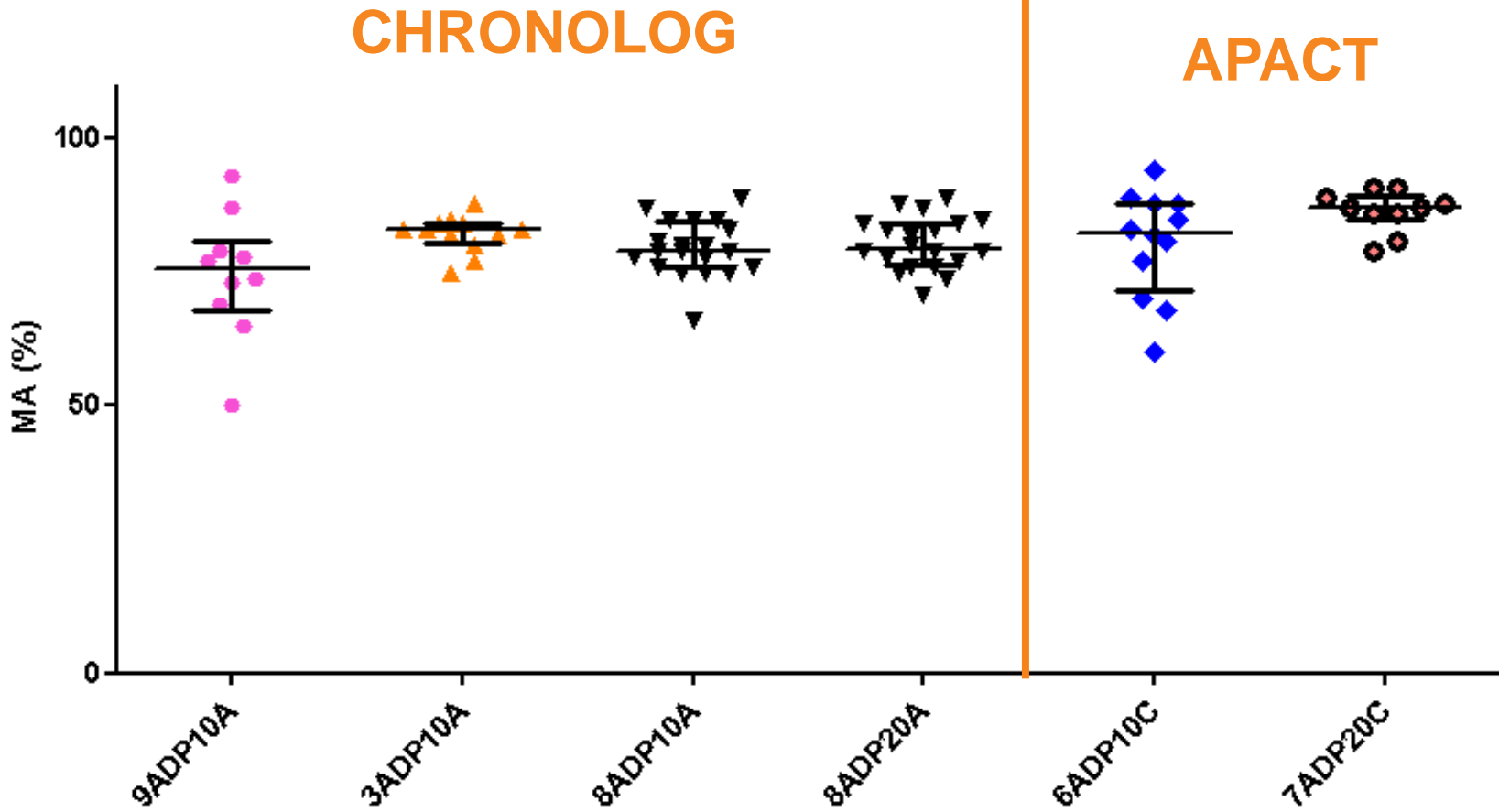
APACT



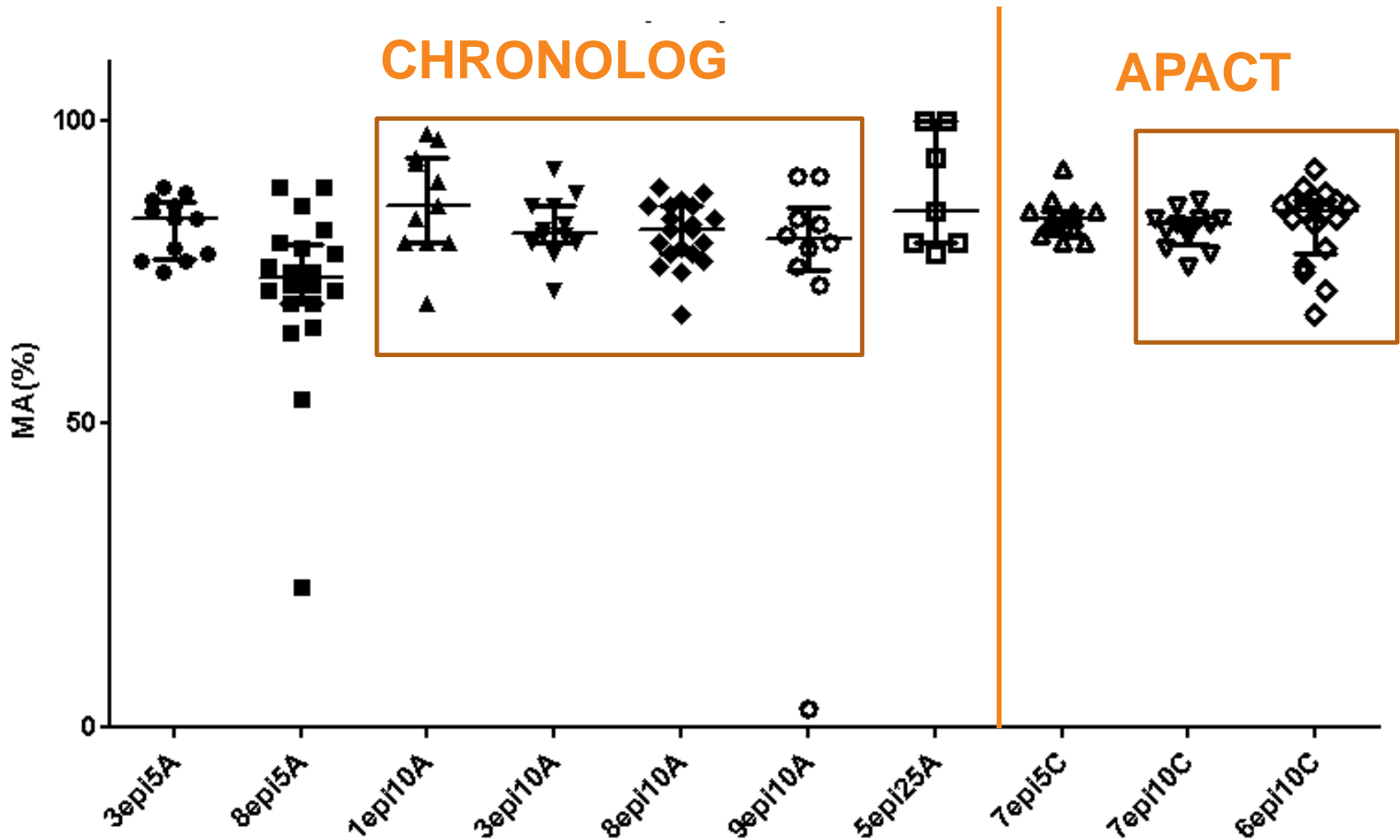
ADP range 4 to 5 μM



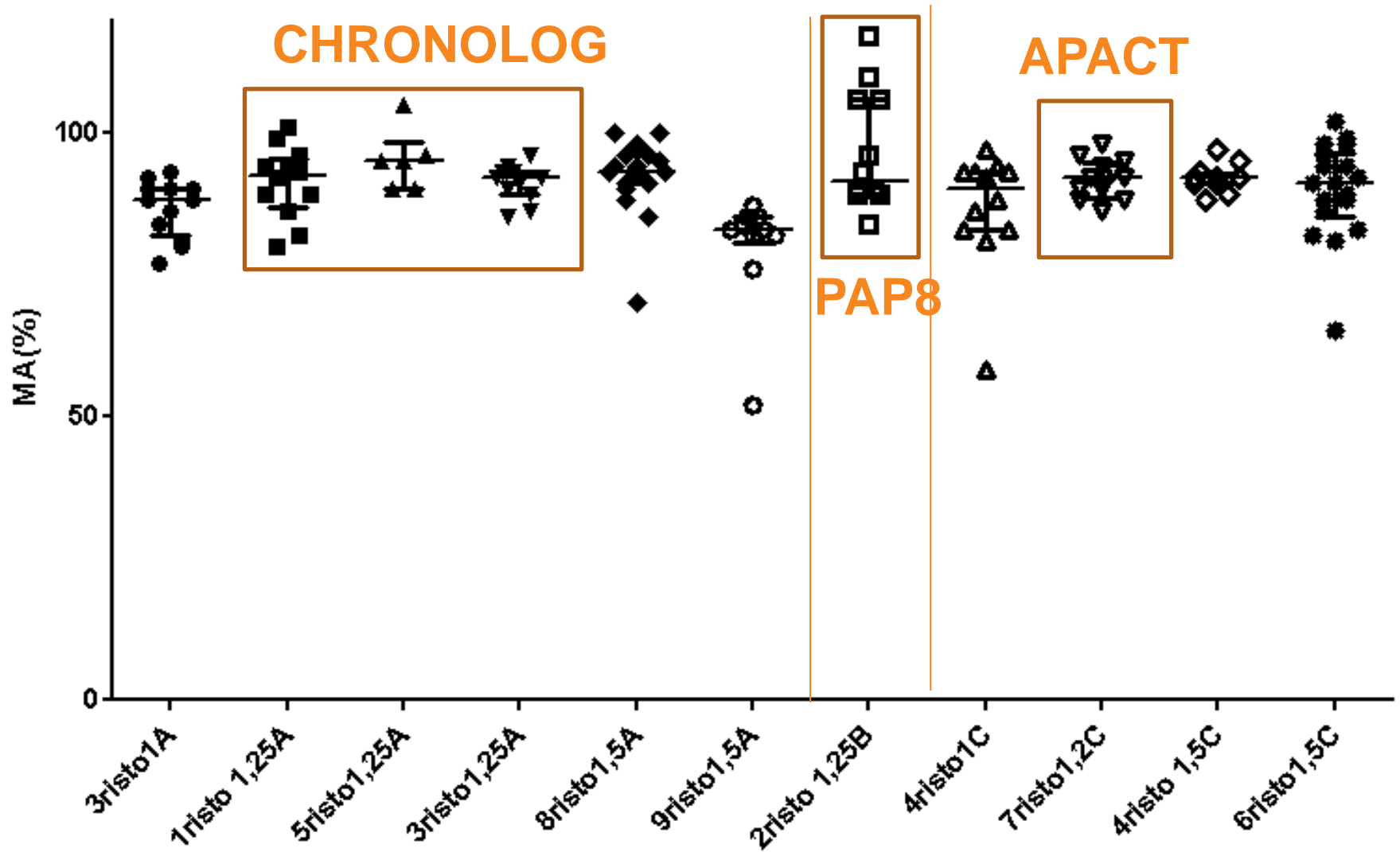
ADP range 10 to 20 μM



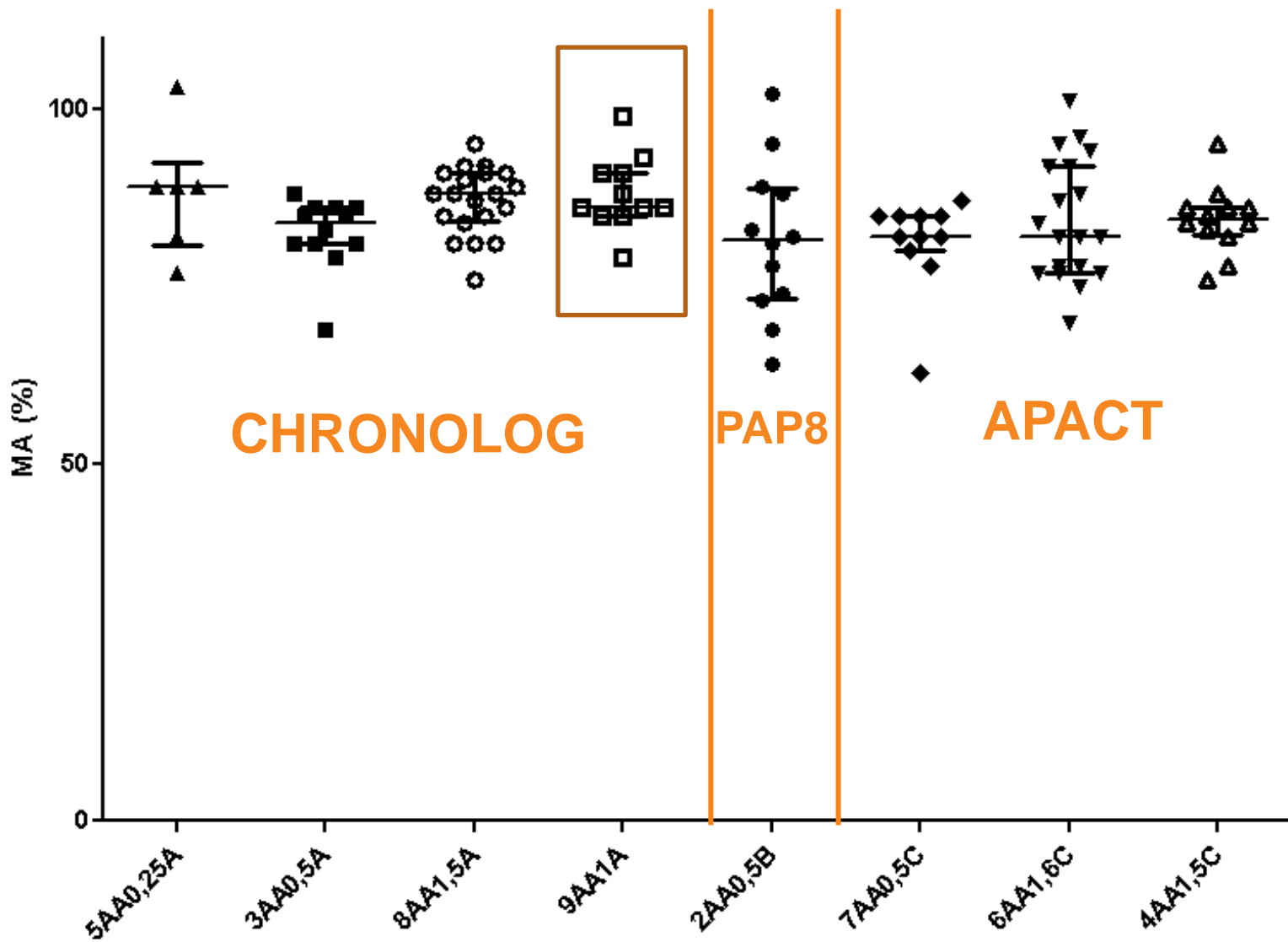
Epinephrine range 5, 10, 25 μM



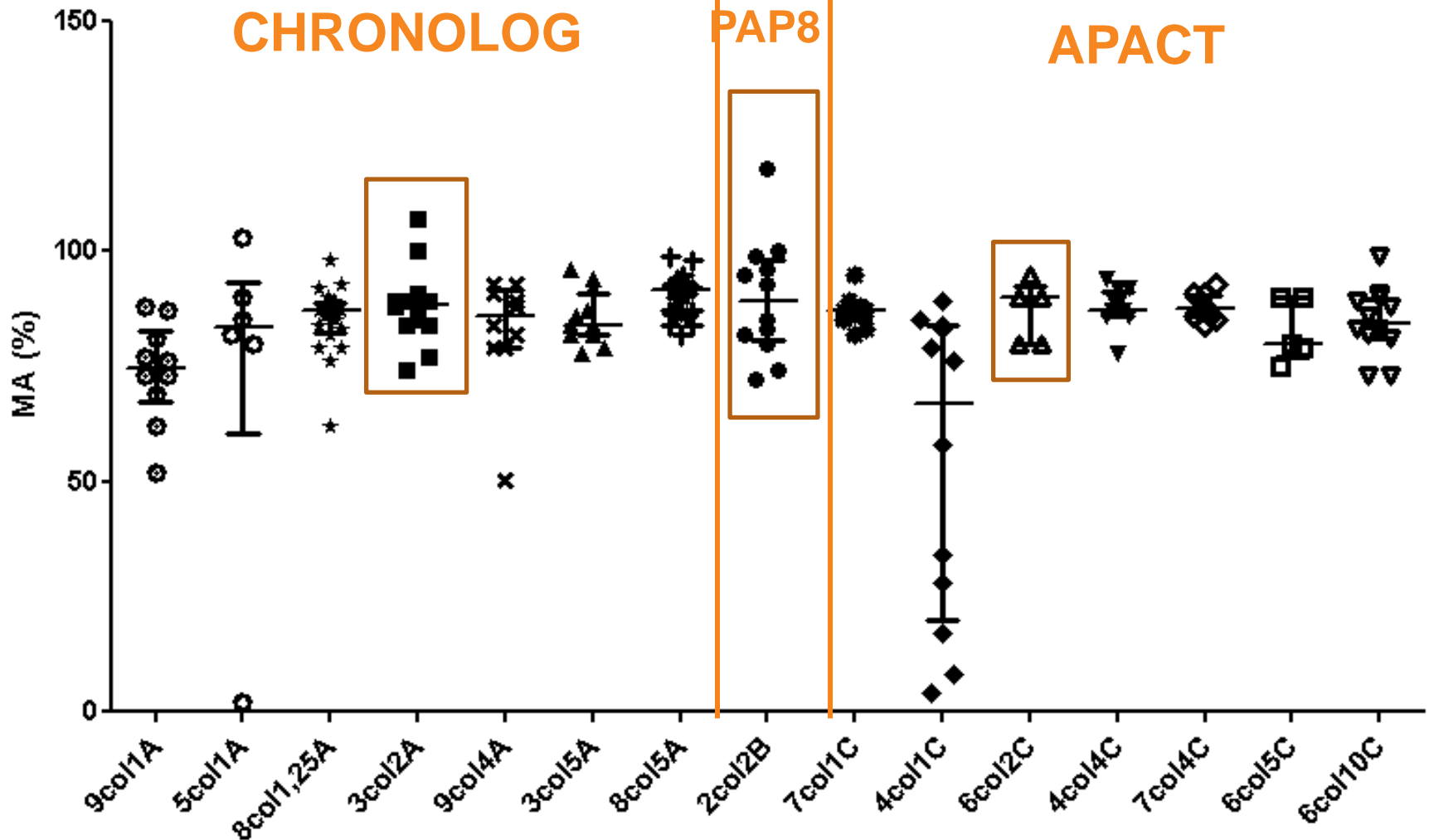
Ristocetin 1-1,2-1,25-1,5 mg/mL



Arachidonic acid 0,25-0,5-1-1,5-1,6 mM



Collagen 1-2-4-5-10 mM



Plan A-3

To determine differences using the same healthy volunteers between:

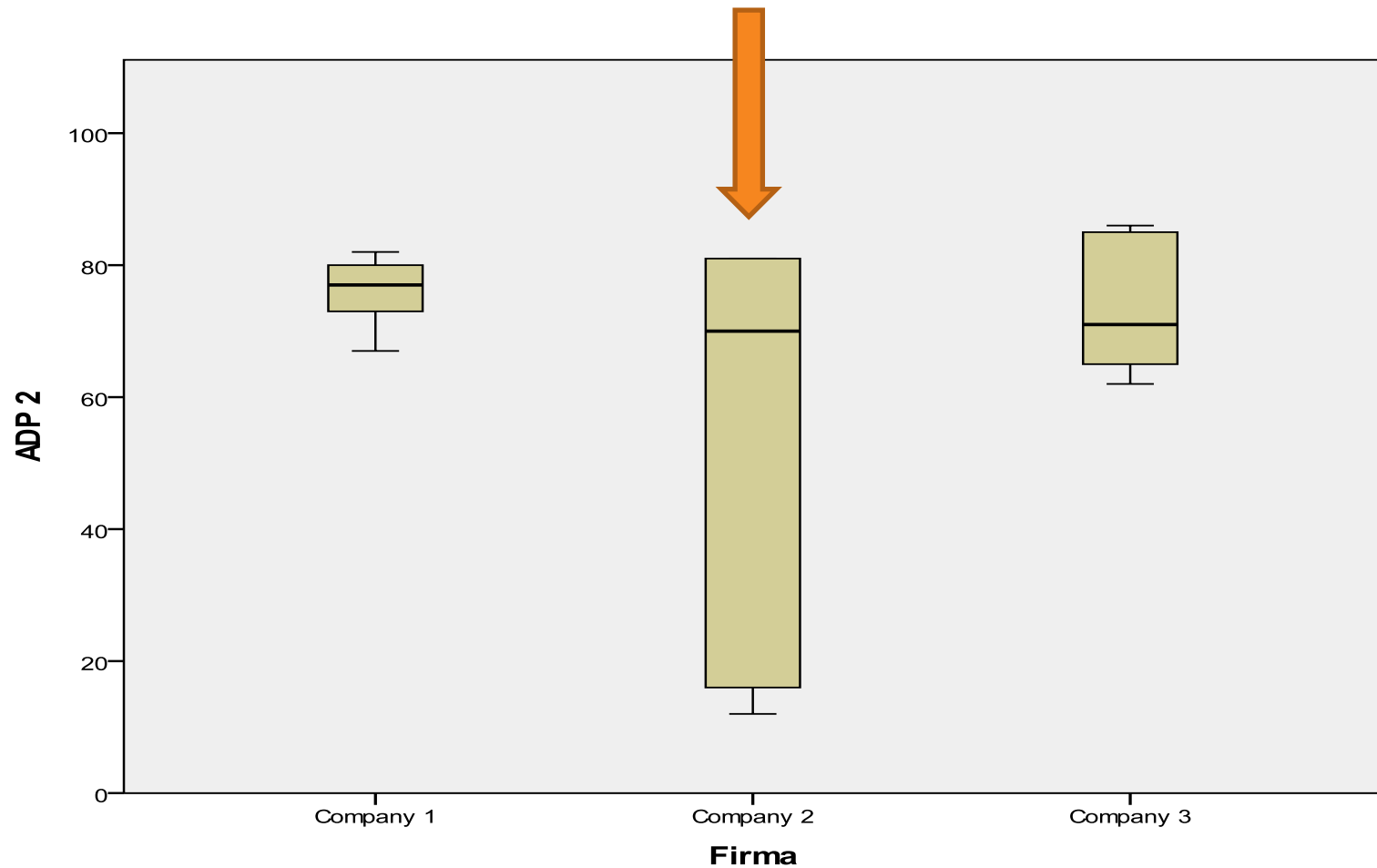
- most used reagents brands in NL on one analyzer**
- most used analyzers in NL**
- (non) adjustment of PRP on three analyzers**



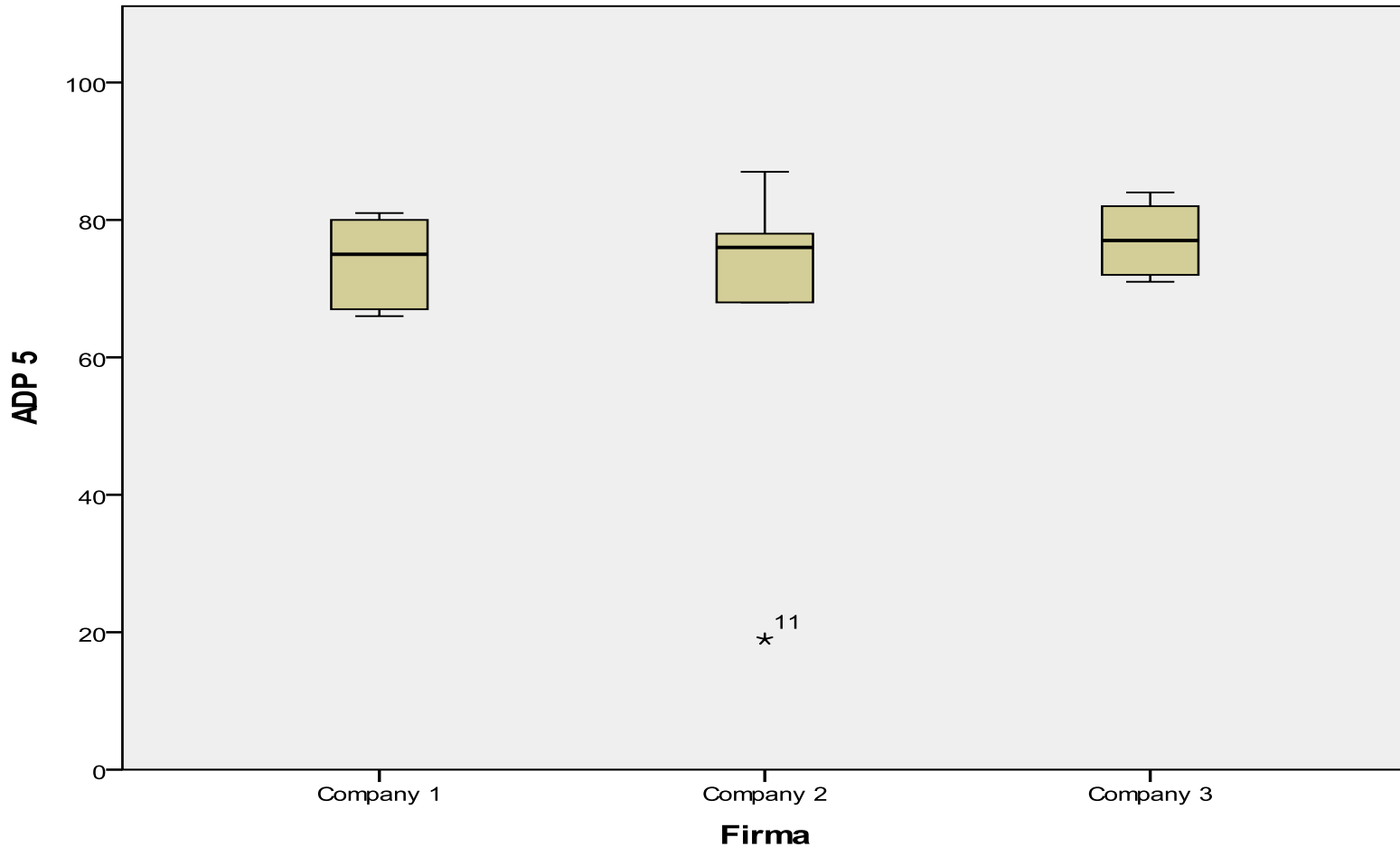
One analyser (PAP8)
Healthy volunteers (n=6)
Different reagent brands
SSC advised concentration

Agonist	Concentration	Company 1	Company 2	Company 3
Epinephrine	5 μ M	BioData	ABP	Chronolog
ArachidonicA	1 mM	BioData	Hart	Sigma
Ristocetin	1.2 mg/mL	Biopool	ABP	Chronolog
ADP	2 en 5 μ M	BioData	Chronolog	Sigma
Collagen	2 en 5 mg/L	BioData	Chronolog	Horm

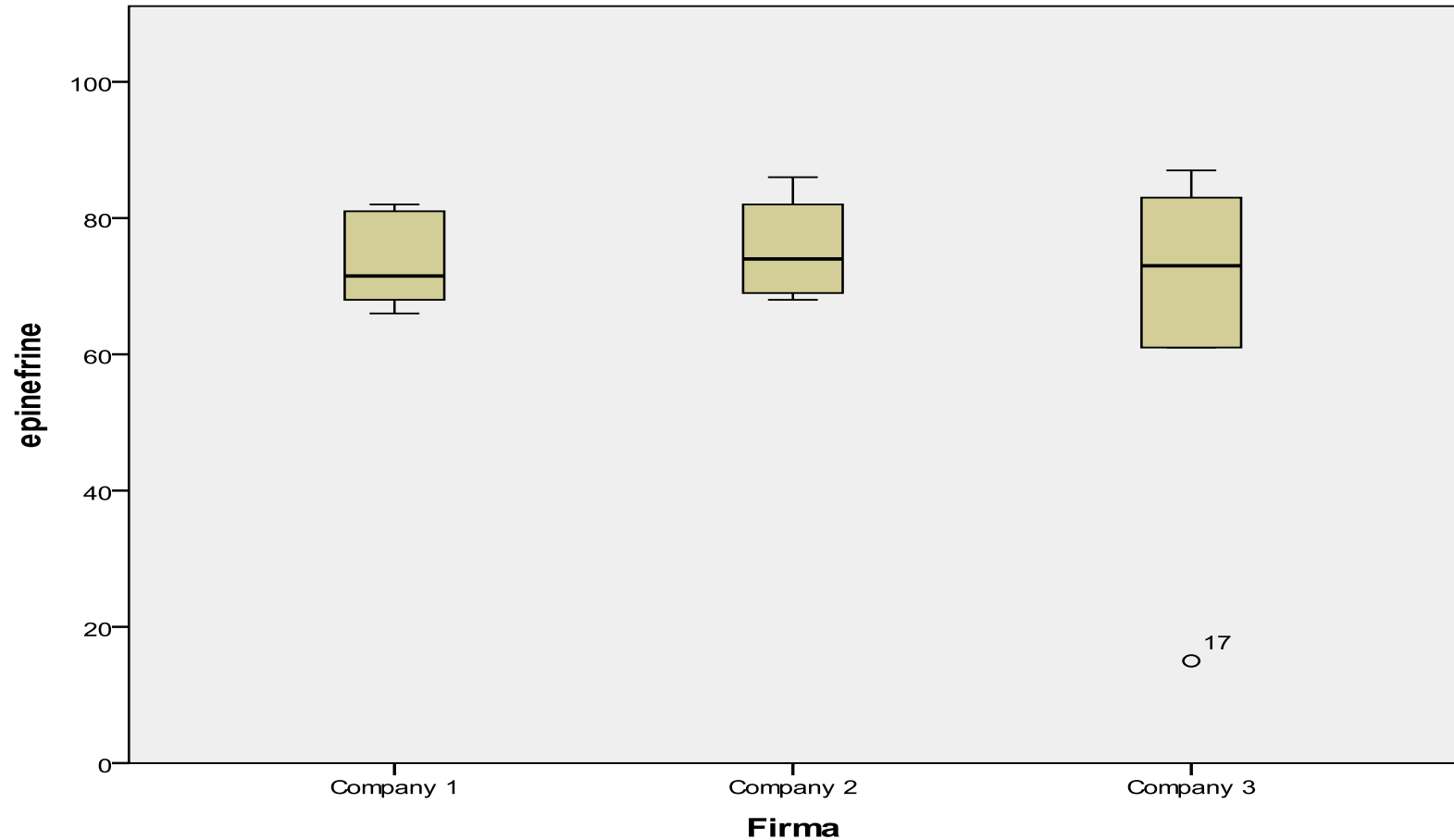
Maximal aggregation (%) on PAP8 ADP 2 μ M



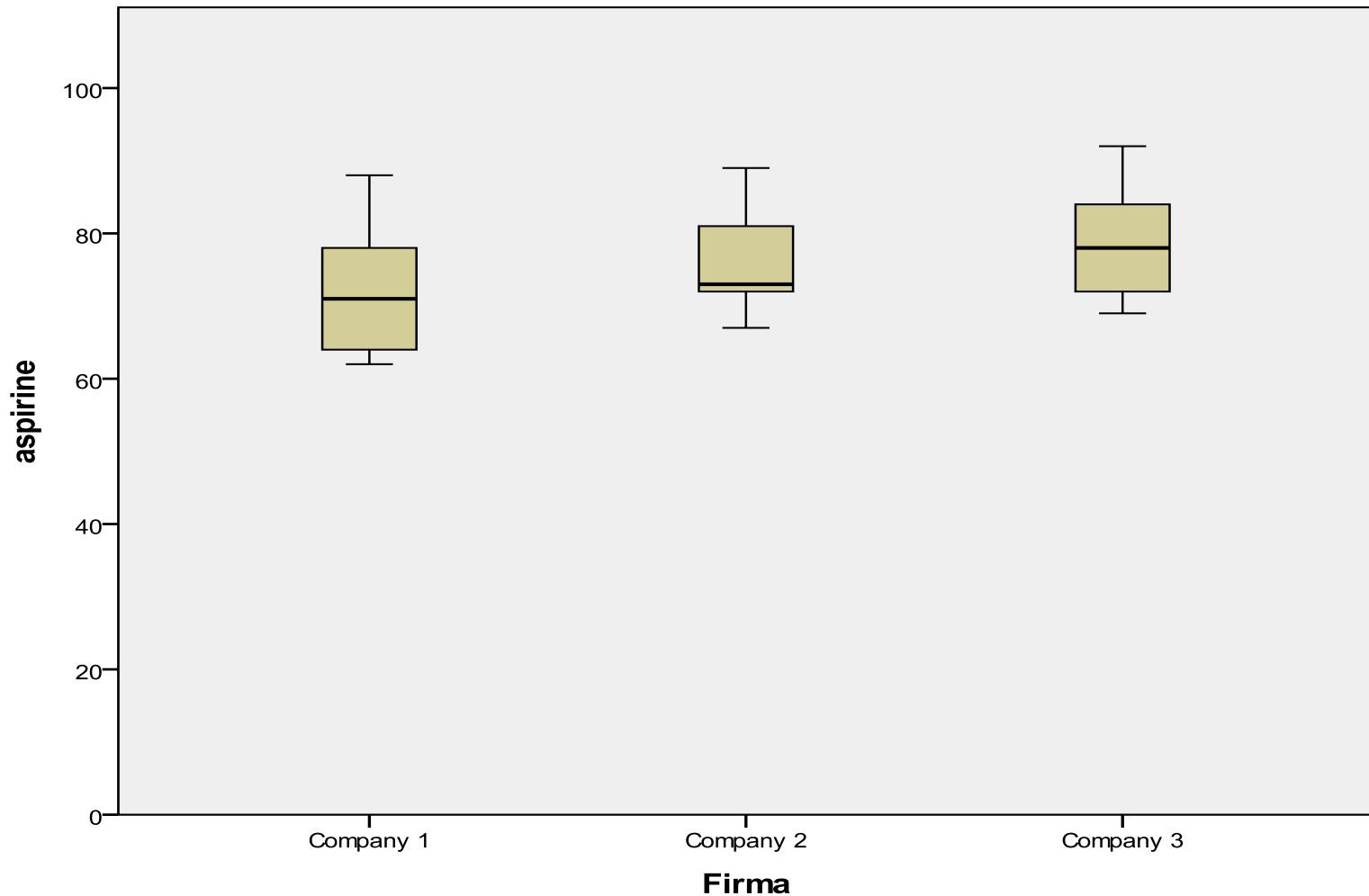
Maximal aggregation (%) on PAP8 ADP 5 μ M



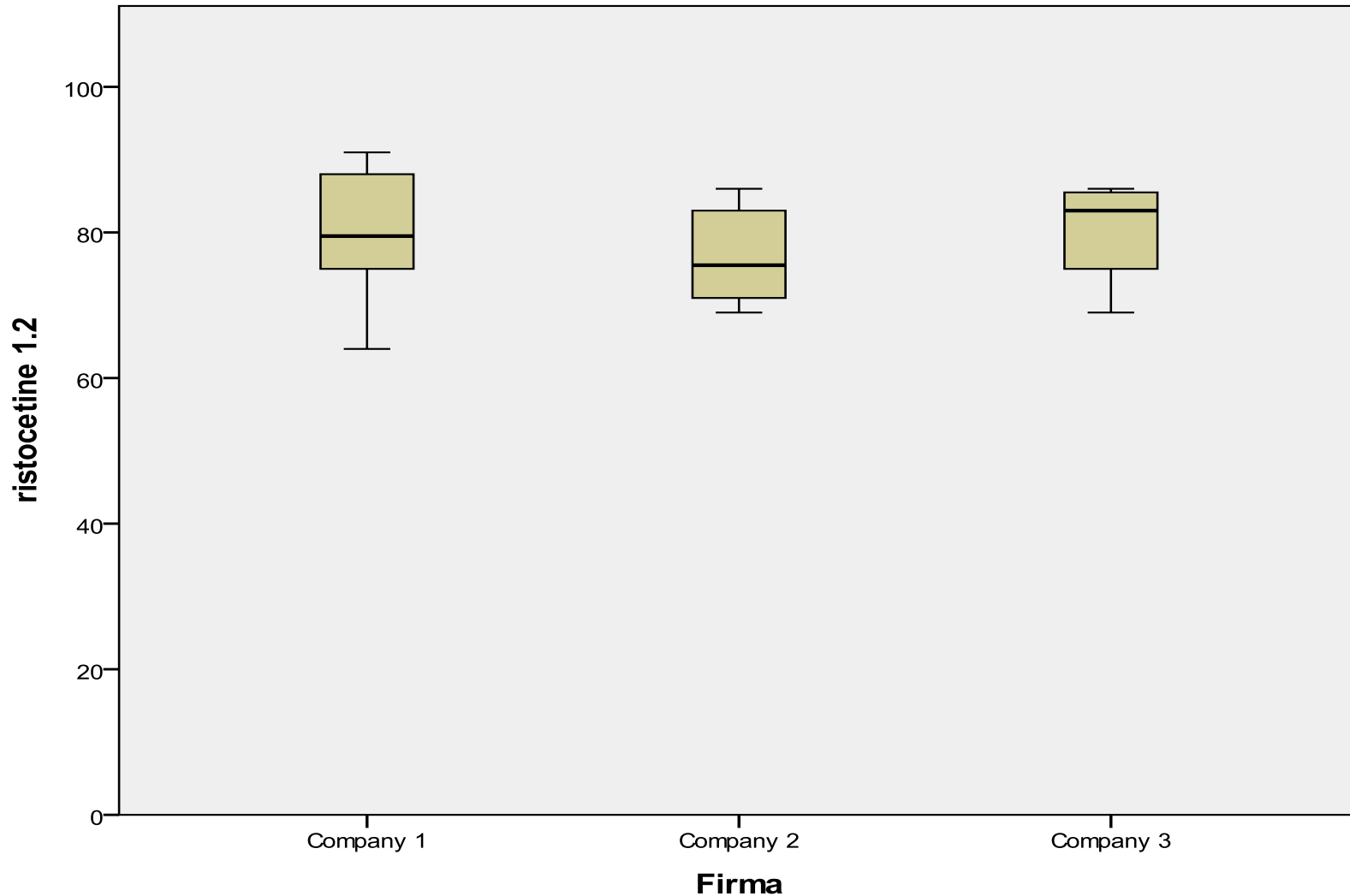
Maximal aggregation (%) on PAP8 Epinephrine 5 $\mu\text{mol/L}$



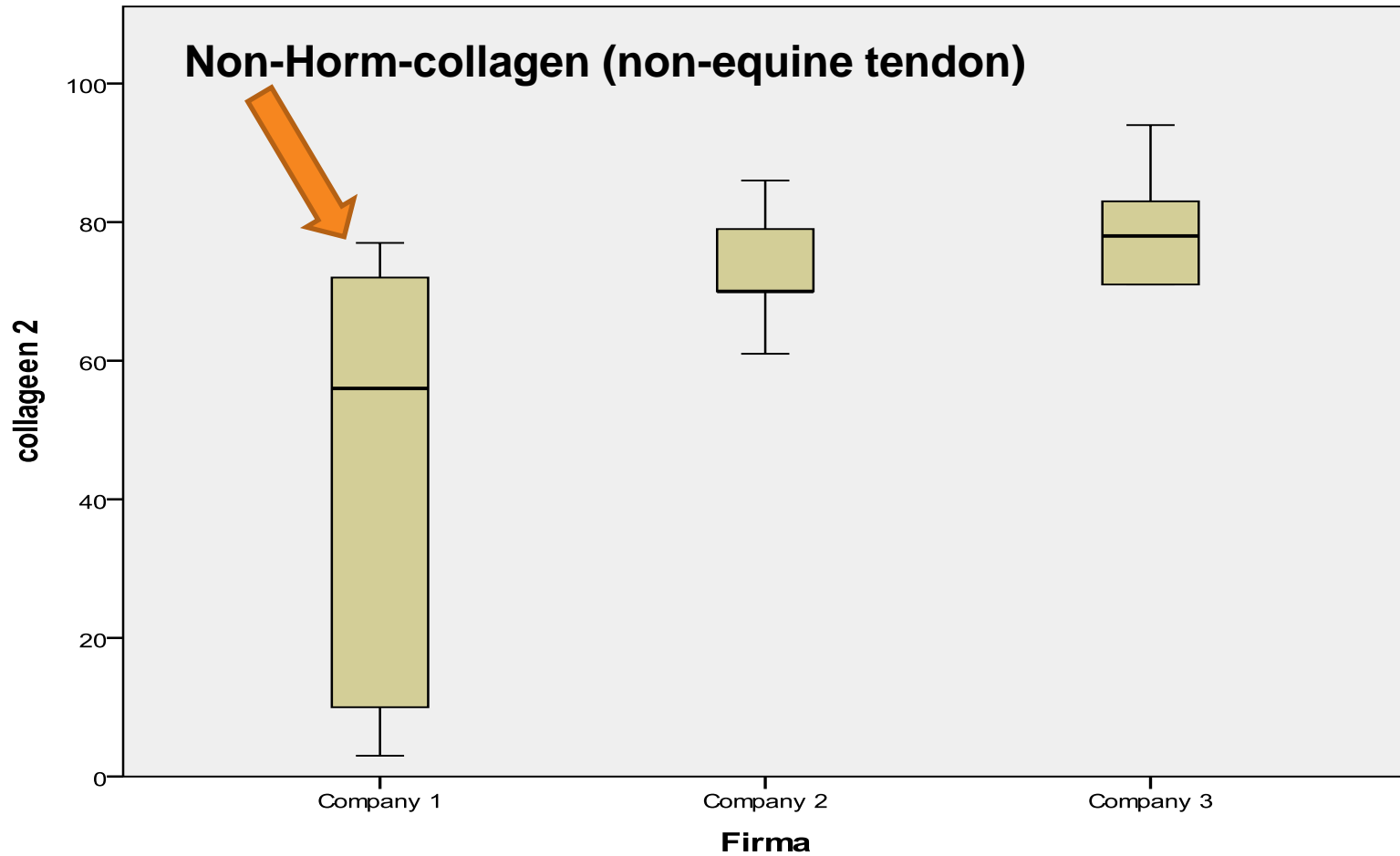
Maximal aggregation (%) on PAP8 Arachidonic acid 1 mmol/L



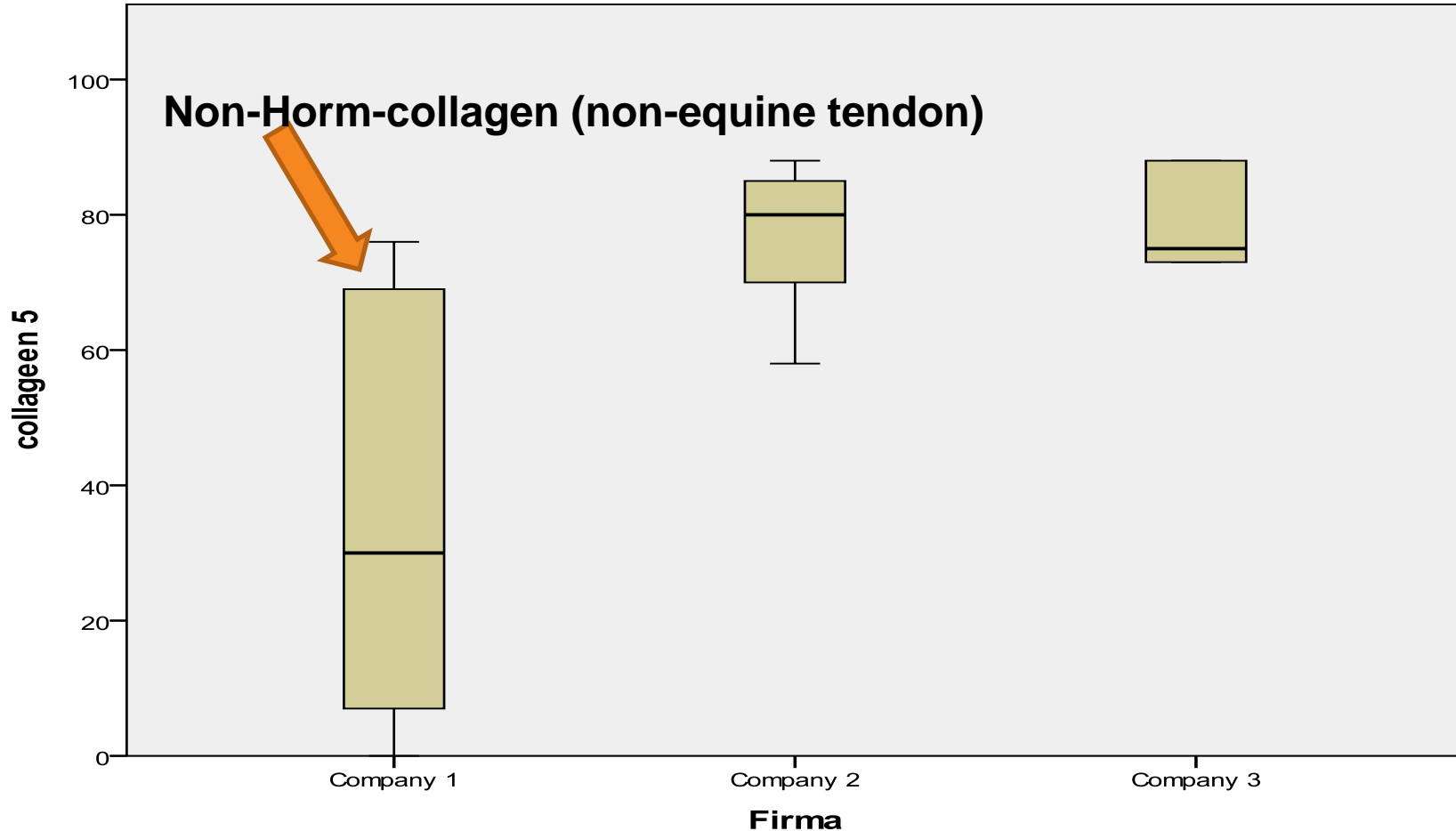
Maximal aggregation (%) on PAP8 Ristocetin 1,2 mg/mL



Maximal aggregation (%) on PAP8 Collagen 2 mg/L



Maximal aggregation (%) on PAP8 Collagen 5 mg/L

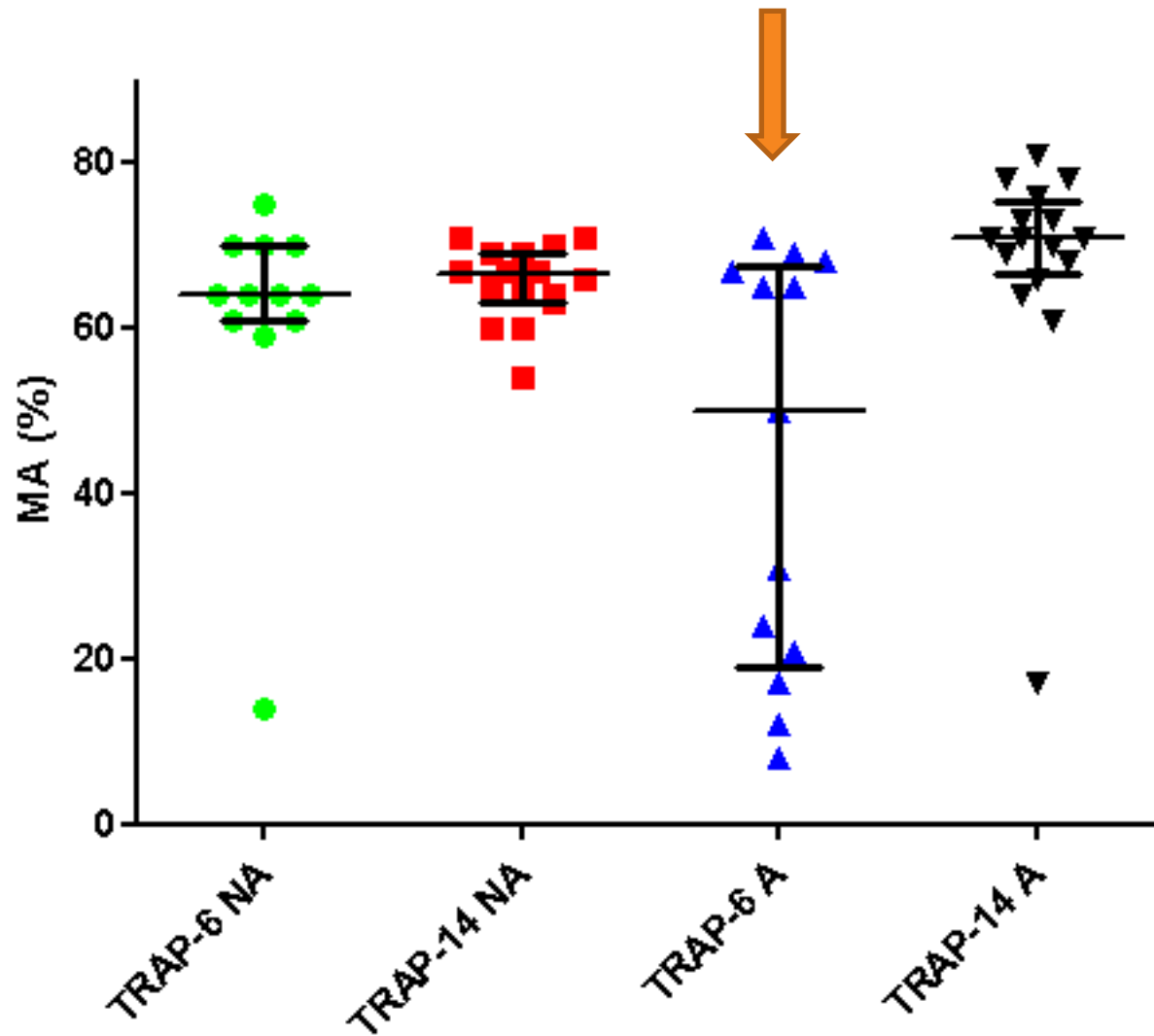




**One analyser (PAP8)
Healthy volunteers (n=30)
Different TRAP peptides (PAR-1)
SSC advised concentration
Adjusted vs non-adjusted**

Agonist	Company	Concentration
TRAP 6 (SFLLRN)	Hart	10 μM
TRAP 14 ((SFLLRNPNDKYEPF)	Bachem	10 μM

TRAP



Three different analysers

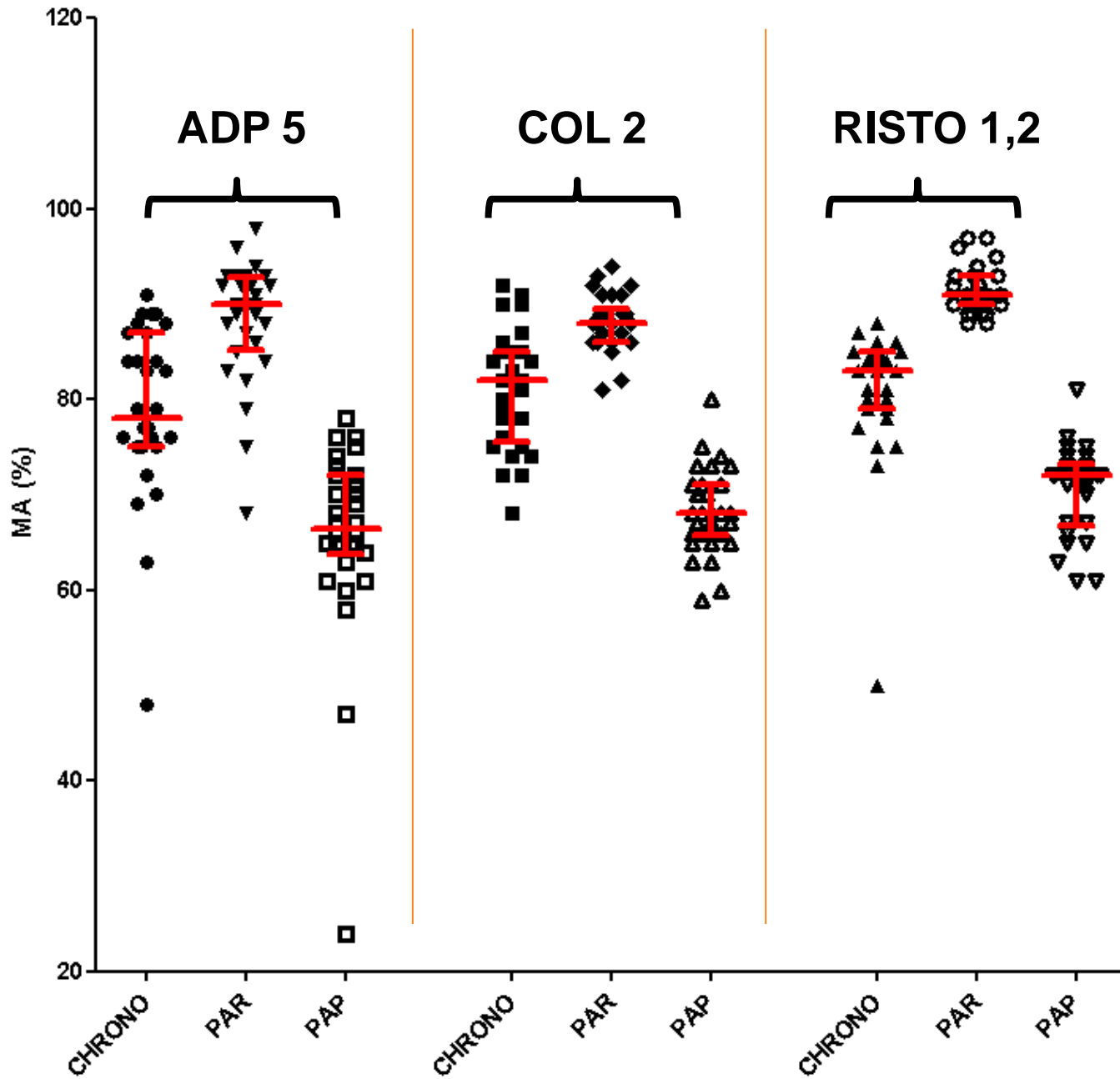
Healthy volunteers (n=30)

Adjusted and non-adjusted PRP

Agonist	Chrono-log Number 1 NL	PAR8	PAP8e Number 2 NL
ADP (5 μ M)			
Collagen (2 μ g/mL)			
Ristocetine (1.2 mg/mL)			

PO 332 Irene Keularts (Thursday)

NON-adjusted PRP on 3 analyzers'



MA % (n=30): adjusted vs non-adjusted PRP

Agonist	End Conc	Chrono-log	PAR8	PAP8e
		Median (25 – 75 %)		
ADP NA	5 μ M	78 (75 – 86)	90 (86 – 92)	67 (64 – 72)
ADP A		78 (73 – 82)	90 (86 – 92)	70 (67 – 73)
Collageen NA	2 μ g/mL	82 (76 – 85)	88 (86 – 90)	68 (66 – 71)
Collageen A		83 (79 – 86)	88 (86 – 90)	74 (71 – 77)
Ristocetine NA	1.2 mg/mL	83 (79 – 85)	90 (90 – 92)	72 (67 – 73)
Ristocetine A		83 (78 – 87)	92 (90 – 94)	75 (71 – 78)

In conclusion

- Adherence to the SSC guideline in the NL requires a lot of changes for the individual hospitals (25 hospitals = 25 procedures) !
- There are some prominent differences in MA (%) between brands (reagents, equipment) and they will remain after standardization !
- Lower concentrations of agonists (ADP, collagen) than recommended show the largest variations between hospitals.
- Reference ranges (reagent-analyzer combination) will always be needed
- No differences between adjusted vs non-adjusted PRP were found using three different analyzers (exception TRAP-6)

GOALS LTA standardization project NL

PLAN B (2013-2015)

1. Investigate the last questions in the guideline (fasten)
2. Make a definite choice for a national protocol → implement
3. Repeat differences in MA (%) between hospital laboratories and define reference ranges per reagent-analyzer combination
4. Start a national registry (LTA results and diagnosis)
5. Start a national quality control program



Thanks to

- Paul Verhezen, Irene Keularts (MUMC+, Maastricht)
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- VHL and WHD-NVTH
- The 9 laboratories who shared the healthy volunteers results with us
- The 56 participants in the questionnaire