ECAT FOUNDATION

EXTERNAL QUALITY ASSESSMENT PROGRAMME

IN HAEMOSTASIS AND THROMBOSIS

Platelet Light Transmission Aggregation International Pilot Study

2024

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GENERAL INFORMATION

In Autumn 2024 an international pilot study was performed on testing for Light Transmission Platelet Aggregation (LTA). The purpose of this pilot study was to investigate the feasibility of international external quality assessment surveys for LTA.
This report shows the results of this pilot study. Because of the scope of this pilot study no performance assessment is yet performed. Where appropriate your results are indicated.

PARTICIPATION

Number of participants: 87
Number of responders: 66

SAMPLES

The following samples were used in this pilot study (see table below):

Sample	Description
24.LTA1	Tube with 15 µg/mL Abciximab (ReoPro) (final concentration)
24.LTA2	Normal control sample
24.LTA3	Tube with 20 nM nanobody GPIb clone 17 (final concentration) *

Note: Our intention was to distribute a sample with a final concentration of 200 nM nanobody GP1b clone 17. Unfortunately the concentration in the tube provided was 10 times lower.

INSTRUMENTS

The table below shows the different instruments used to measure light transmission aggregometry.

Instrument	**No.**
AggRAM	7
APACT	8
Behnk	2
Biodata	11
Chronolog	18
Other	3
Sysmex CN-serie	5
Sysmex CS-serie	5
TA8V Stago	6

AGONISTS

The following agonist were recommended to test:

- Ristocetin : 1.2 mg/mL and 2.0 mg/mL
- Collagen : 2 µg/mL and 5 µg/mL
- ADP : 2 µM and 5 µM
- Arachidonic Acid : 1 mM

If sufficient PRP was available, participants were asked to test thrombin receptor agonist peptide (TRAP) SFFLRN (10 µM) as well.

Because locally used agonist concentrations may differ from the recommended list above, below an overview is given from the agonists used by the participants.

The table below shows the different agonists used by the participants. The maximum number of responders may differ per sample.

Agonist	No.
ADP (1 µM)	1
ADP (2 µg/mL)	1
ADP (2 - 3 µM) *	54
ADP (5 µg/mL)	1
ADP (4 - 5 µM) *	62
ADP (10 µM)	3
ADP (20 µM)	1
Arachidonic acid (0.5 mg/mL)	3
Arachidonic acid (1 mM) *	54
Arachidonic acid (1.5 / 1.6 mM)	9
Collagen (0.2 µg/mL)	5
Collagen (1 / 1.25 µg/mL)	3
Collagen (2 µg/mL) *	50
Collagen (4 - 5 µg/mL) *	53
Collagen (10 µg/mL)	2
Epinephrine (1 µM)	1
Epinephrine (5 µM)	4
Epinephrine (10 µM)	2
Epinephrine (1000 µM)	1
Ristocetin (0.5 / 0.6 mg/mL)	16
Ristocetin (1.2 / 1.25 mg/mL) *	58
Ristocetin (1.5 mg/mL)	7
Ristocetin (2.0 mg/mL) *	36
TRAP (5 µM)	1
TRAP (10 µM)	20
TRAP (30 µM)	1
TRAP (100 µM)	4
U46619 (1 / 1.6 µM)	3

* recommended agonists

GENERAL INFORMATION

Platelet count in Platelet-Rich Plasma

Median (*10^9/l)	318
Range (*10^9/l)	162 - 565

Correction platelet count in Platelet-Rich Plasma

	No correction	Correction
Number	48	18
Median (*10^9/l)	340	300
Range (*10^9/l)	162 - 565	200 - 400

Quality Platelet-Rich Plasma

	No	Yes
Hemolytic	66	0
Lipemic	66	0

RESULTS

Sample 24.LTA1 (15 µg/mL Abciximab)

The table below shows a summary of the maximum aggregation results (%) for sample 24.LTA1.

Agonist	No.	Median	Range	CV (%)
ADP (1 µM)	1	**0.0**	-	-
ADP (2 µg/mL)	-	**-**	-	-
ADP (2 - 3 µM) *	54	**0.9**	0 – 23	183
ADP (5 µg/mL)	-	**-**	-	-
ADP (4 - 5 µM) *	62	**1.0**	0 – 45	204
ADP (10 µM)	3	**0.0**	0 – 6	-
ADP (20 µM)	1	**2.0**	-	-
Arachidonic acid (0.5 mg/mL)	3	**0.0**	0 - 24	-
Arachidonic acid (1 mM) *	54	**13.4**	0 – 90	115
Arachidonic acid (1.5 / 1.6 mM)	9	**5.0**	0 - 31	118
Collagen (0.2 µg/mL)	5	**50.0**	1 – 71	78
Collagen (1 / 1.25 µg/mL)	3	**1.3**	1 - 2	-
Collagen (2 µg/mL) *	50	**4.0**	0 – 86	163
Collagen (4 - 5 µg/mL) *	53	**6.0**	0 - 99	139
Collagen (10 µg/mL)	2	**35.3**	34 - 36	-
Epinephrine (1 µM)	-	**-**	-	-
Epinephrine (5 µM)	4	**1.5**	0 - 5	-
Epinephrine (10 µM)	2	**0.5**	0 - 1	-
Epinephrine (1000 µM)	1	**0.0**	-	-
Ristocetin (0.5 / 0.6 mg/mL)	14	**0.9**	0 - 4	99
Ristocetin (1.2 / 1.25 mg/mL) *	58	**66.5**	7 - 103	42
Ristocetin (1.5 mg/mL)	6	**80.5**	65 - 89	10.1
Ristocetin (2.0 mg/mL) *	36	**86.0**	26 - 110	18.4
TRAP (5 µM)	-	**-**	-	-
TRAP (10 µM)	19	**4.5**	0 - 70	153
TRAP (30 µM)	1	**34.0**	-	-
TRAP (100 µM)	4	**37.5**	32 - 67	-
U46619 (1 / 1.6 µM)	1	**10.0**	-	-

Comment:

Abciximab binds to the GPIIb/IIIa receptor to inhibit platelet aggregation. The responses for the most frequently used agonists are as can be expected for a sample with a final concentration of 15 µg/mL Abciximab.
Because of the low maximum aggregation for most of the agonists the between-laboratory variation (CV) is high.

For details on the effect of Abciximab on platelet aggregation, see: Gold, H.K., L.W. Gimple, T. Yasuda, R.C. Leinbach, W. Werner, R. Holt*, et al.*, Pharmacodynamic study of F(ab')2 fragments of murine monoclonal antibody 7E3 directed against human platelet glycoprotein IIb/IIIa in patients with unstable angina pectoris. J Clin Invest, 1990; 86: 651-9.

Sample 24.LTA2 (Normal Control Sample)

The table below shows a summary of the maximum aggregation results (%) for sample 24.LTA2.

2Agonist	No.	Median	Range	CV (%)	No. of outliers **
ADP (1 µM)	1	**65.0**	-	-	-
ADP (2 µg/mL)	1	**32.0**	-	-	-
ADP (2 - 3 µM) *	49	**78.0**	43 - 96	18.9	7
ADP (5 µg/mL)	1	**79.0**	-	-	-
ADP (4 - 5 µM) *	58	**83.7**	62 - 105	11.7	3
ADP (10 µM)	3	**99.0**	91 - 99	-	-
ADP (20 µM)	1	**76.0**	-	-	-
Arachidonic acid (0.5 mg/mL)	3	**83.0**	79 - 85	-	-
Arachidonic acid (1 mM) *	50	**84.0**	62 - 112	10.8	4
Arachidonic acid (1.5 / 1.6 mM)	8	**78.0**	68 - 93	9.7	-
Collagen (0.2 µg/mL)	5	**75.0**	72 - 94	9.9	-
Collagen (1 / 1.25 µg/mL)	3	**84.4**	67 - 90	-	-
Collagen (2 µg/mL) *	50	**84.8**	62 - 106	11.1	2
Collagen (4 - 5 µg/mL) *	53	**84.8**	44 - 110	14.1	-
Collagen (10 µg/mL)	2	**86.9**	78 - 96	-	-
Epinephrine (1 µM)	-	**-**	-	-	-
Epinephrine (5 µM)	5	**84.0**	82 - 90	3.4	-
Epinephrine (10 µM)	3	**87.0**	82 - 89	-	-
Epinephrine (1000 µM)	2	**75.0**	75 - 75	-	-
Ristocetin (0.5 / 0.6 mg/mL)	16	**3.0**	0 - 24	137	-
Ristocetin (1.2 / 1.25 mg/mL) *	58	**88.0**	47 - 115	15.0	1
Ristocetin (1.5 mg/mL)	7	**85.4**	72 - 93	7.2	-
Ristocetin (2.0 mg/mL) *	36	**91.0**	64 - 125	12.5	-
TRAP (5 µM)	1	**61.0**	-	-	-
TRAP (10 µM)	20	**86.0**	62 - 100	10.1	2
TRAP (30 µM)	1	**95.0**	-	-	-
TRAP (100 µM)	3	**80.0**	80 - 85	-	-
U46619 (1 / 1.6 µM)	3	**85.0**	84 - 94	-	-

** For some agonists outlying results were observed. These results were excluded from the statistical evaluation. In most cases these outliers were also observed for sample 24.LTA3 for the same agonists and for the same participants.

For the Normal Control Sample the effect of platelet count adjustment for the most frequently used agonists was investigated. The table below shows the maximum aggregation %, median and range).

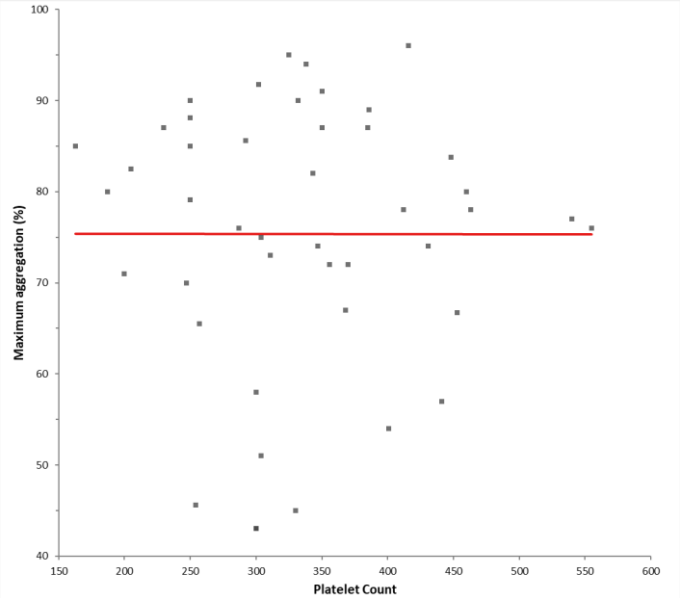
Agonist	No correction	Correction	P-value
ADP (2 - 3 µM)	78 (45 – 96)	77 (43 – 90)	0.5574
ADP (4 - 5 µM)	83 (66 – 105)	85 (62 – 103)	0.9455
Arachidonic acid (1 mM)	84 (68 – 102)	85 (62 – 112)	0.6476
Collagen (2 µg/mL)	85 (65 – 97)	84 (62 – 106)	0.9295
Collagen (4 - 5 µg/mL)	84 (44 – 110)	87 (56 – 110)	0.6486
Ristocetin (1.2 / 1.25 mg/mL)	89 (47 – 104)	83 (49 – 115)	0.2485
Ristocetin (2.0 mg/mL)	91 (76 – 103)	91 (64 – 125)	0.7666

From the table above it is clear that there is no difference between the group without and with correction for the platelet count.

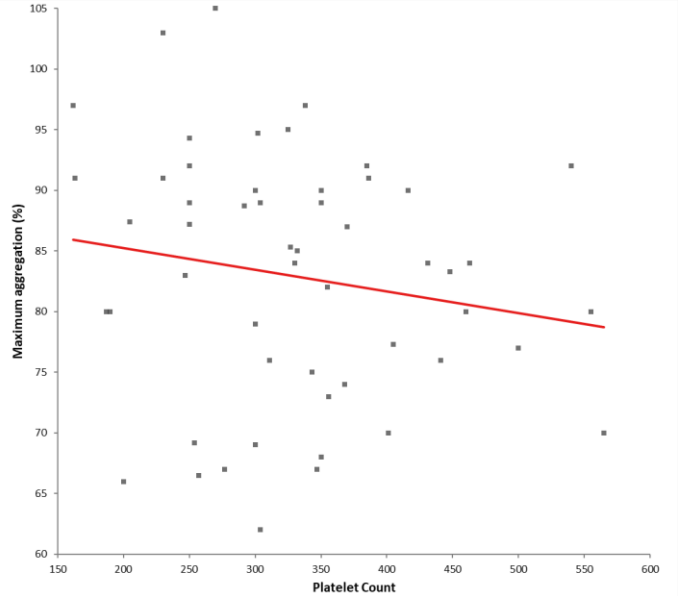
Maximum aggregation vs platelet count

For the Normal Control Sample the relationship between the maximum aggregation (%) and the platelet count was evaluated for the recommended agonists.

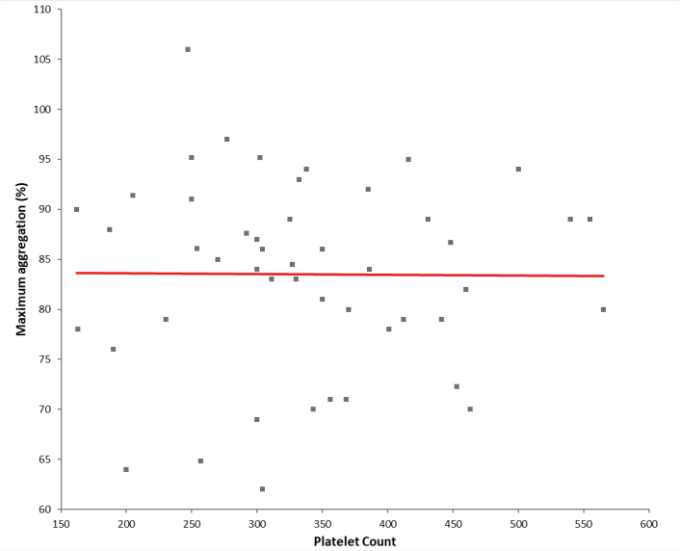
ADP (2 / 2.5 / 3.0 µM)



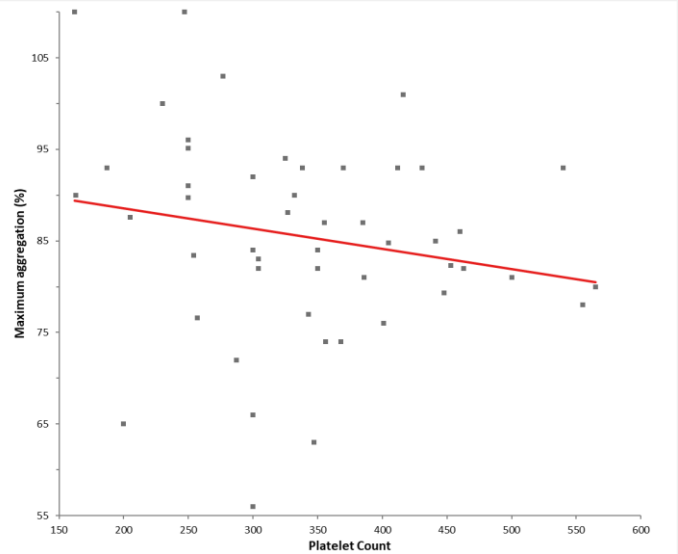
ADP (4 / 5 µM)



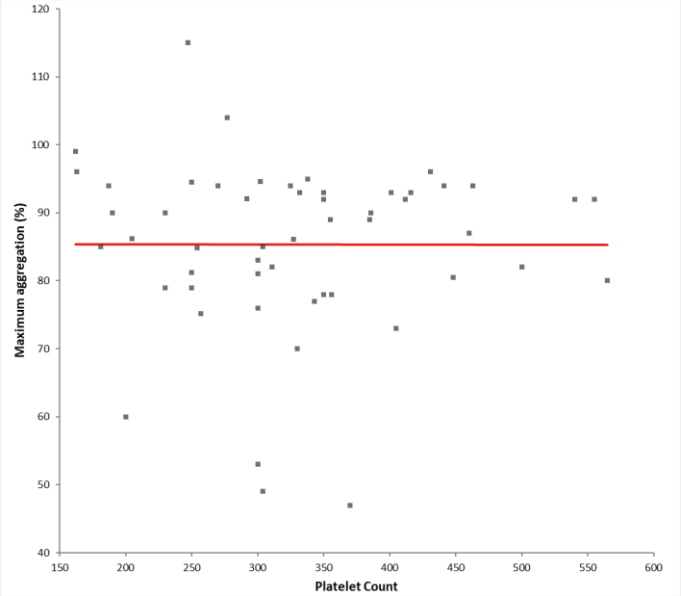
Collagen (2 µg/mL)



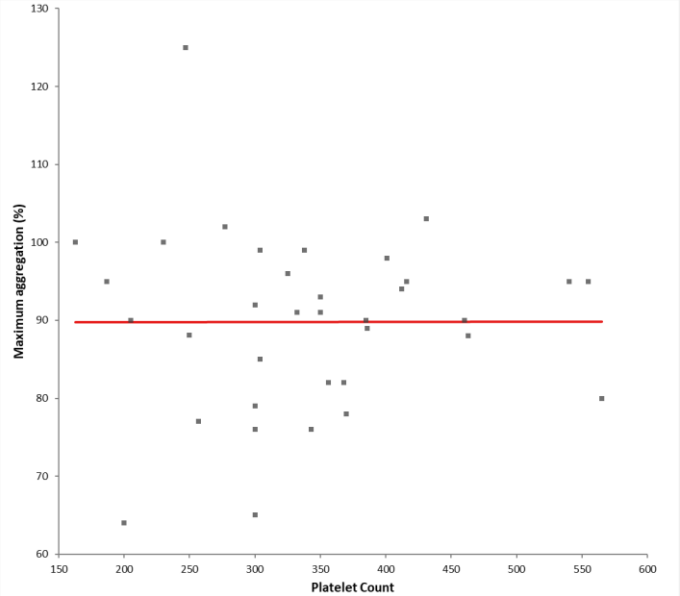
Collagen (5 µg/mL)



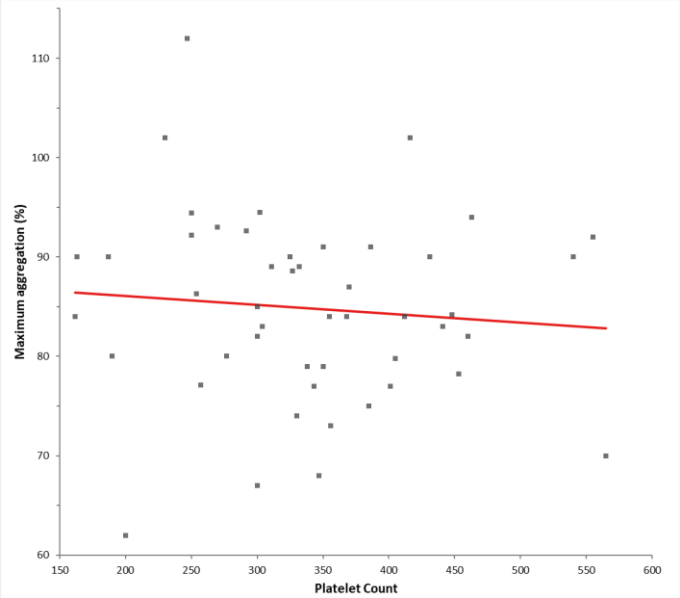
Ristocetin (1.2 / 1.25 mg/mL)



Ristocetin (2 mg/mL)



Arachidonic Acid (1 mM)

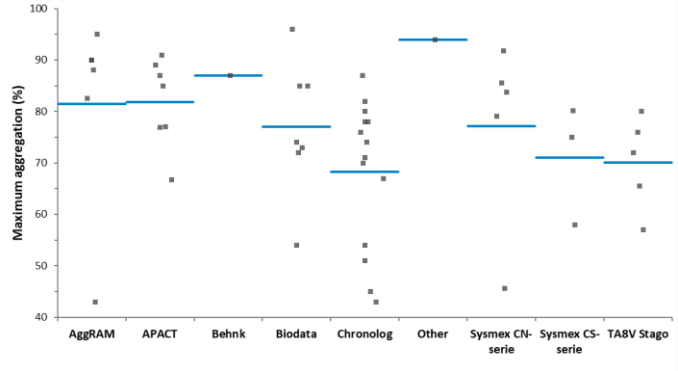


It can be concluded that for the Normal Control Sample no correlation between the maximum aggregation and the platelet count in the platelet-rich plasma is observed for the recommended agonists.

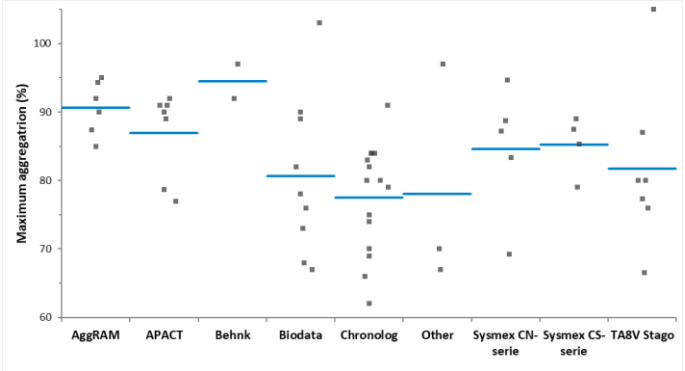
Maximum aggregation vs equipment

For the Normal Control Sample and the recommended agonists the maximum aggregation (%) versus the brand of instrument used was evaluated.

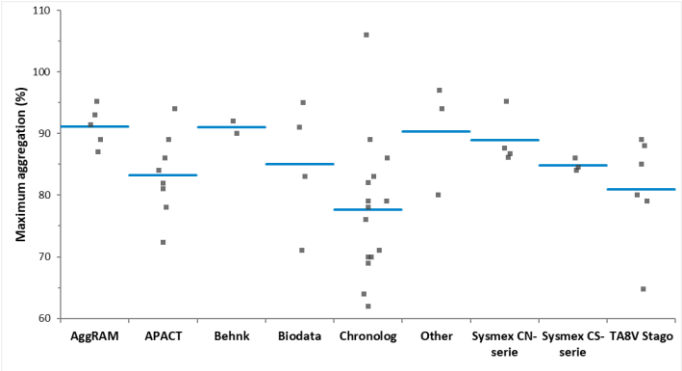
ADP (2 / 2.5 / 3.0 µM)



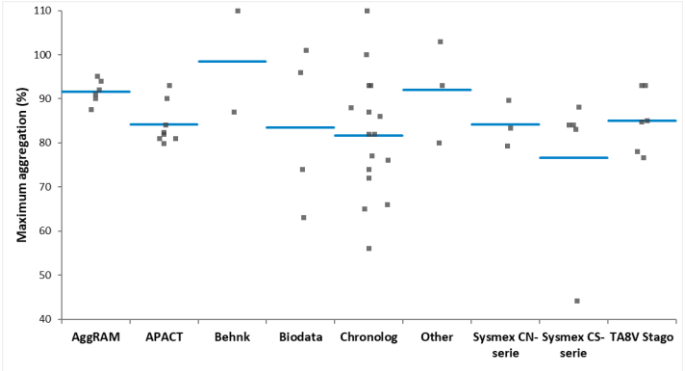
ADP (4 / 5 µM)



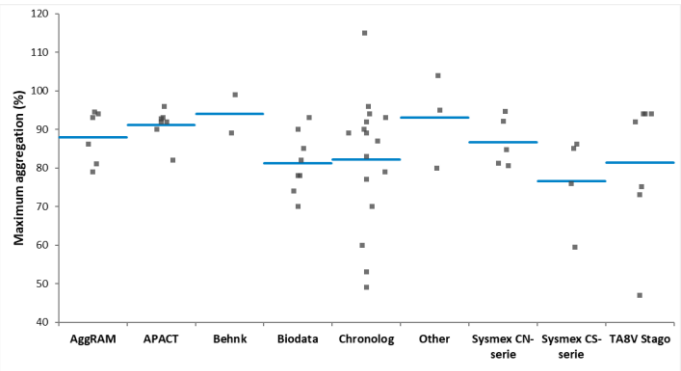
Collagen (2 µg/mL)



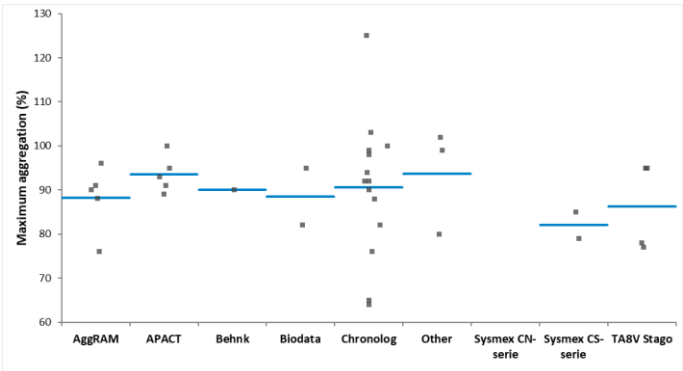
Collagen (5 µg/mL)



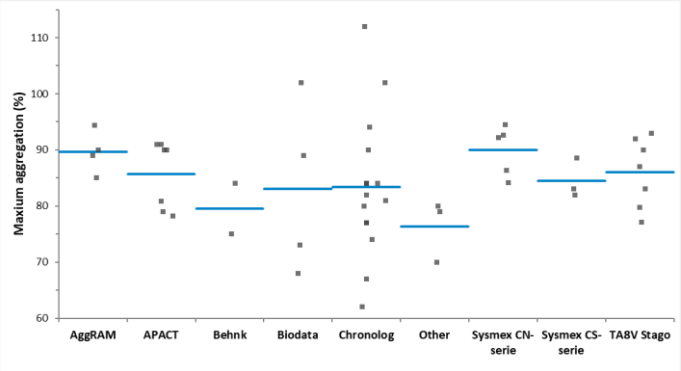
Ristocetin (1.2 / 1.25 mg/mL)



Ristocetin (2 mg/mL)



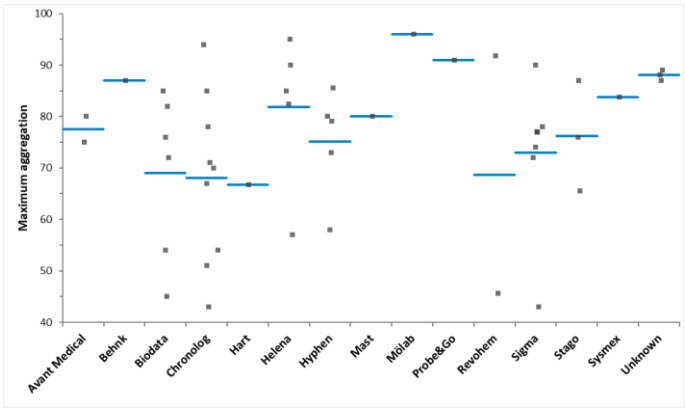
Arachidonic Acid (1 mM)



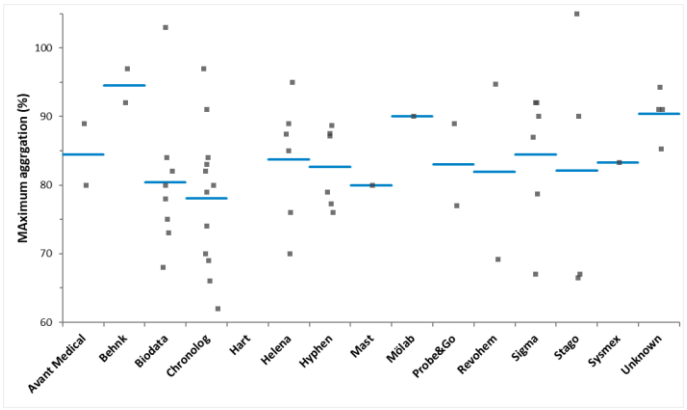
Maximum aggregation vs brand agonist

For the Normal Control Sample and the recommended agonists the maximum aggregation (%) versus the brand of agonists used was evaluated.

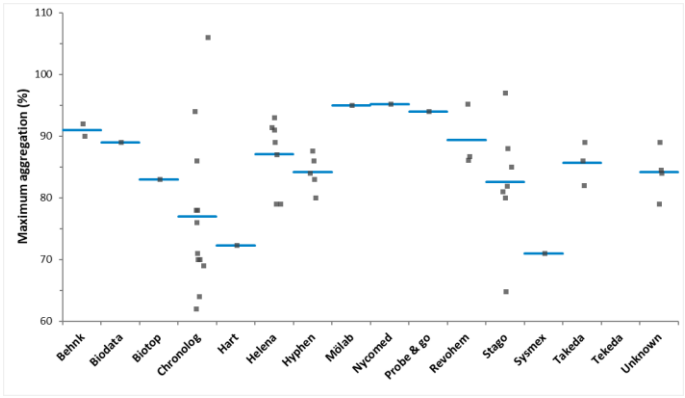
ADP (2 / 2.5 / 3.0 µM)



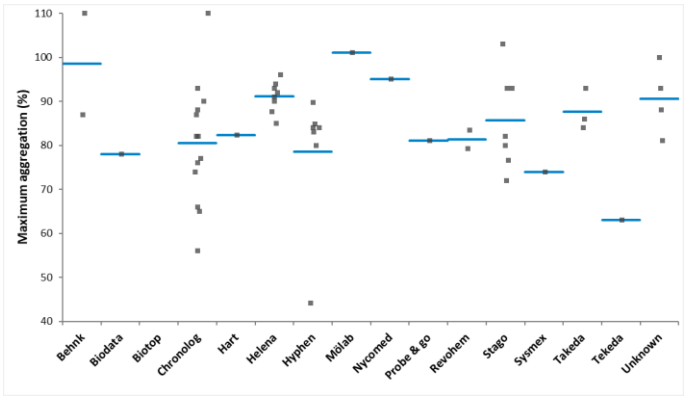
ADP (4 / 5 µM)



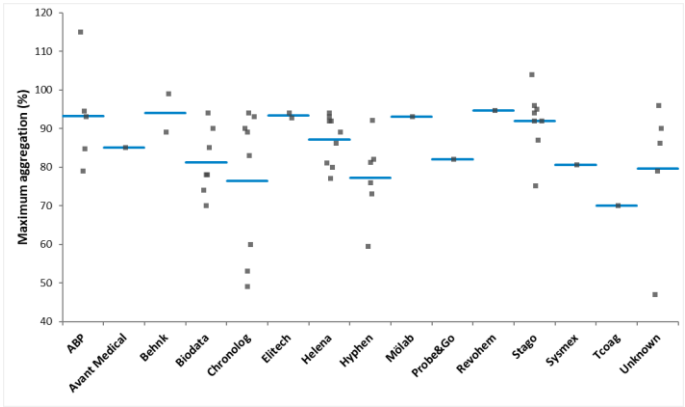
Collagen (2 µg/mL)



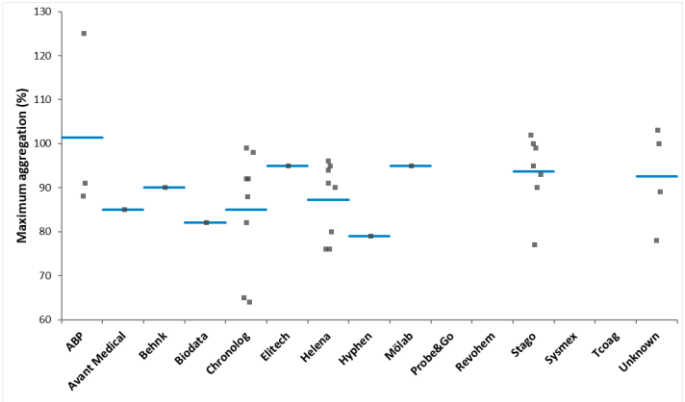
Collagen (5 µg/mL)



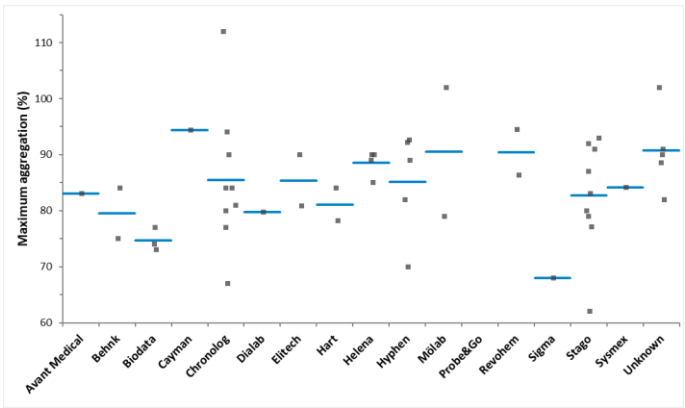
Ristocetin (1.2 / 1.25 mg/mL)



Ristocetin (2 mg/mL)



Arachidonic Acid (1 mM)



Sample 24.LTA3 (20 nM nanobody GPIb clone 17)

The table below shows a summary of the maximum aggregation results (%) for sample 24.LTA3.

2**Agonist**	**No.**	**Median**	**Range**	**CV (%)**	**No. of outliers ****
ADP (1 µM)	1	**51.0**	-	-	-
ADP (2 µg/mL)	1	**36.0**	-	-	-
ADP (2 - 3 µM) *	46	**79.9**	35 - 100	26.3	9
ADP (5 µg/mL)	1	**72.0**	-	-	-
ADP (4 - 5 µM) *	57	**82.0**	56 - 98	12.8	3
ADP (10 µM)	3	**91.0**	85 - 105	-	-
ADP (20 µM)	1	**77.0**	-	-	-
Arachidonic acid (0.5 mg/mL)	2	**82.0**	78 - 86	-	-
Arachidonic acid (1 mM) *	53	**86.0**	69 - 106	9.9	6
Arachidonic acid (1.5 / 1.6 mM)	2	**89.5**	84 - 95	-	-
Collagen (0.2 µg/mL)	5	**87.0**	73 - 105	12.4	-
Collagen (1 / 1.25 µg/mL)	3	**83.0**	83 - 90	-	-
Collagen (2 µg/mL) *	49	**85.0**	58 - 108	12.0	2
Collagen (4 - 5 µg/mL) *	52	**87.0**	46 - 107	12.8	-
Collagen (10 µg/mL)	2	**85.0**	78 - 92	-	-
Epinephrine (1 µM)	1	**85.0**	-	-	-
Epinephrine (5 µM)	5	**81.0**	48 – 89	21.0	-
Epinephrine (10 µM)	3	**84.0**	79 - 85	-	-
Epinephrine (1000 µM)	1	**77.0**	-	-	-
Ristocetin (0.5 / 0.6 mg/mL)	16	**3.0**	0 – 7	59.0	-
Ristocetin (1.2 / 1.25 mg/mL) *	58	**86.0**	45 - 105	14.3	-
Ristocetin (1.5 mg/mL)	6	**86.4**	85 - 95	4.3	-
Ristocetin (2.0 mg/mL) *	36	**92.0**	72 - 106	9.9	-
TRAP (5 µM)	1	**6.0**	-	-	-
TRAP (10 µM)	18	**86.7**	60 - 97	11.0	3
TRAP (30 µM)	1	**91.0**	-	-	-
TRAP (100 µM)	3	**97.0**	85 - 100	-	-
U46619 (1 / 1.6 µM)	3	**93.0**	78 - 96	-	-

** For some agonists outlying results were observed. These results were excluded from the statistical evaluation. In most cases these outliers were also observed for sample 24.LTA2 for the same agonists and for the same participants.

Note: Due to an error during the preparation of this samples the final concentration of nanobody GP1b clone 17 was 10 times lower as originally planned. With this low nanobody concentration this sample behaved like a normal control sample.

During a small pilot study in The Netherlands in 2023 a final concentration of 200 nM nanobodies was used. This resulted in the following responses:

	200 nM nanobody GPIb clone 17		
Agonist	**Mean**	**Range**	**CV (%)**
Epinephrine 5 µM	89.4	85.7 – 93.0	-
Epinephrine 10 µM	87.1	-	-
Ristocetin (0.6 mg/mL)	6.1	0.0 – 14.0	-
Ristocetin (1.0 mg/mL)	4.6	-	-
Ristocetin (1.25 mg/mL)	8.2	-	-
TRAP (5 µM)	55.4	33.0 – 77.8	-
TRAP (30 µM)	84.7	-	-
U46619 (1 µM)	89.9	-	-

As expected, with a concentration of 200 nM nanobody GPIB clone 17 a reduced maximum aggregation with ristocetin was observed. The nanobody GPIB clone 17 binds GPIbα and blocks VWF binding, functionally mimicking the Bernard Soulier Syndrome[1]

Within-laboratory variation

For tests with an expected normal maximum aggregation in sample 24.LTA2 and 24.LTA3 for the recommended agonists (ADP 2 µM; ADP 5 µM; Collagen 2 µg/mL; Collagen 5 µg/mL and Arachidonic acid 1 mM) the within-laboratory variation could be assessed. The table below shows a summary of the within-laboratory variation.

Agonist	Median (%)	Range (%)
ADP (2 µM)	3.2	0.0 – 36.9
ADP (5 µM)	2.8	0.0 – 16.0
Arachidonic acid (1 mM)	2.4	0.0 – 12.6
Collagen (2 µg/mL)	2.1	0.0 – 22.0
Collagen (5 µg/mL)	2.2	0.1 – 11.1

GENERAL REMARKS

- The sample containing Abciximab (24.LTA1) behaves like expected in the light-transmission aggregometry assay.
- The concentration of nanobody GPIb clone 17 in sample 24.LTA3 was, unfortunately, too low to demonstrate the effect of a Bernard Soulier Syndrome.
- Despite the fact that all participants have used their own local donor to prepare platelet-rich plasma, the observed between-laboratory variation for the recommended agonists in samples 24.LTA 2 and 24.LTA3 (9.9 – 26.3%) is relatively low for a complex and non-standardized assay like the LTA. The high between-laboratory variation in sample 24.LTA1 for most of the agonists is a consequence of the low maximum aggregation.

- The observed median within-laboratory variation is relatively low (2.1 – 3.2%).

- This pilot study has demonstrated that external quality control surveys for LTA using antibodies to mimic platelet disorders are feasible.

[1] Sanrattana, W., S. Smits, A.D. Barendrecht, N.D. van Kleef, H. El Otmani, M. Zivkovic, *et al.*, Targeted SERPIN (TaSER): A dual-action antithrombotic agent that targets platelets for SERPIN delivery. J Thromb Haemost, 2022; 20: 353-365.