ECAT Information

Platelet Light Transmission Aggregation Pilot Study

In Autumn 2023 a small pilot study was performed on Platelet Light Transmission Aggregation (LTA) testing in which 8 Dutch clinical laboratories participated.

Background

Light-transmission aggregometry (LTA) plays an important role in the detection of platelet disorders in the laboratory. Despite this role, assuring the quality of LTA-testing is a challenge for laboratories, due in part to the lack of standardised reference materials representing platelet disorders and the need to process platelets within hours of sample collection. Nanobodies targeting platelet proteins can be used to create standardised reference samples from fresh material collected by the participating laboratories.

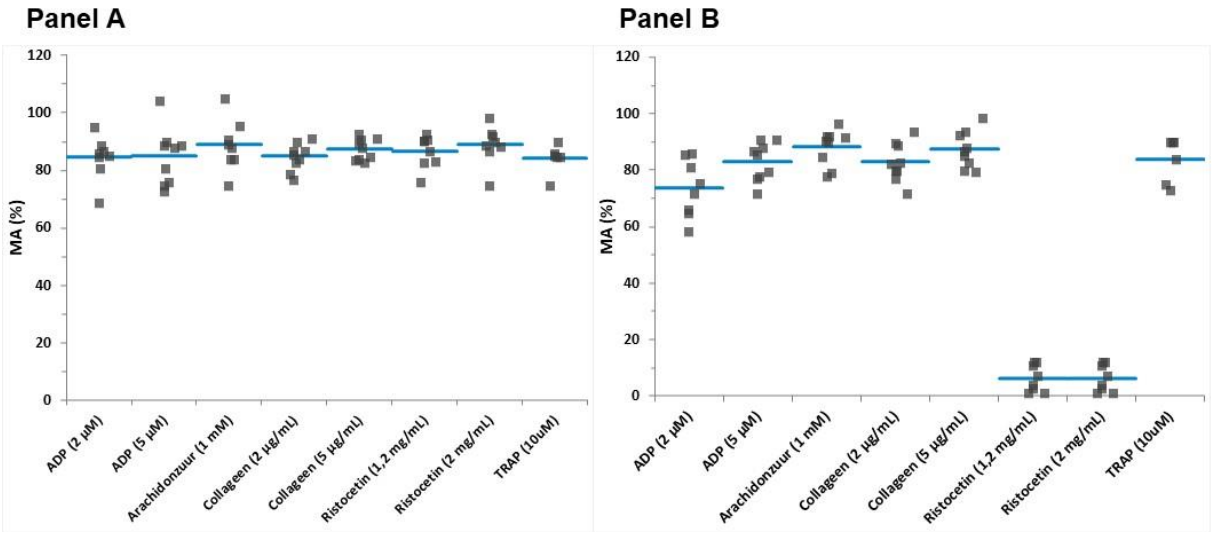
The aim of this pilot study was to investigate the feasibility of external quality control for LTA-testing with a nanobody that binds GPIb and blocks VWF-binding (GPIb clone 17), functionally mimicking the Bernard Soulier Syndrome [1].

Set-up

Test tubes without (normal control) and with 200 nM nanobody GPIb clone 17 (patient) was sent to 8 medical laboratories in The Netherlands. Participants were asked to prepare platelet-rich plasma (PRP) from a healthy donor according to their in-house procedure and to add 4 mL of PRP to each of the test tubes. They were asked to perform LTA-testing with ristocetin (1.2 and 2.0 mg/mL), collagen (2 and 5 µg/mL), ADP (2 and 5 µM) and arachidonic acid (1 mM). Maximum aggregation (MA) for each test was to be reported.

Results and Discussion

The figure below show the individual maximum aggregation (MA) results per frequently used agonist for the normal sample (panel A) and the sample with 200 nM nanobody GPIb clone 17 (panel B).



For the normal control sample, MA was normal for all agonists (panel A). The between-laboratory variation varied from 4–11.5%. In the patient sample (panel B), a low MA (5 – 7%) was found for both ristocetin concentrations, while MA was normal for all other agonists. The between-laboratory variation varied from 5.7–11.0% for the agonists with a normal MA. For both ristocetin concentrations the between-laboratory variation varied from 72.4 – 101.4%.

In this pilot study a nanobody was used that mimicks the Bernard Soulier Syndrome. This results in reduced maximum aggregation of the agonist ristocetin in the LTA. The results of this pilot study clearly confirm the expected results.

Despite the fact that all participants used their own local donor to prepare platelet-rich plasma, the observed between-laboratory variation (4 – 11.4%) observed is relatively low for a complex and non-standardised assay like the LTA. The high between-laboratory variation for ristocetin in the sample containing the nanobody is a consequence of the fact that the maximum aggregation is low.

For all tests with a normal MA in the two samples (ADP 2 µM; ADP 5 µM; Collagen 2 µg/mL; Collagen 5 µg/mL; Arachidonic acid 1 mM and TRAP 10uM) the within-laboratory variation could be assessed. The table below shows a summary of the within-laboratory variation.

Agonist	Mean (%)	Range
ADP (2 µM)	7.1	0.0 – 18.3
ADP (5 µM)	3.2	0.6 – 8.9
Arachidonic acid (1 mM)	2.9	0.5 – 10.6
Collagen (2 µg/mL)	2.5	0.0 – 9.3
Collagen (5 µg/mL)	2.4	0.0 – 5.0
TRAP (10uM)	2.3	0.0 – 8.2

On average the within-laboratory variation varied from 2.3–7.1%. Despite the complexity of LTA-testing, the observed within-laboratory variation is relatively low.

Conclusion

This pilot study demonstrates that external quality control surveys for LTA using nanobodies to mimic platelet disorders are feasible. In autumn 2024 the ECAT Foundation will organise a new pilot study on an international scale. Currently over eighty laboratories have registered to take part in this pilot study.