Platelet count dependency of the fully automated Thrombomate® XRA for light transmission aggregometry

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Background

Light transmission aggregometry (LTA) is still perceived as the gold standard in platelet function testing, though the method is very laborious and therefore expensive. Guidelines recommend the use of LTA only at platelet count (PlC) of ≥ 150/nl. The literature refers to a PlC dependency in (LTA). (1, 2). However, concrete data are limited and may be instrument and reagent dependent. Therefore we investigated the PlC dependency of the novel Thrombomate® XRA analyzer, a fully automated LTA system using a modified Born technology with minimum hands-on time.

Methods

Citrate anticoagulated blood from normal volunteers (N=10, PlC in EDTA blood 166/nl to 322/nl) was centrifuged to get platelet rich plasma (PRP) or platelet poor plasma (PPP). In order to lower the PlC while maintaining the sample matrix, PRP was mixed with autologous PPP (1:2, 1:3, and 1:4). This results in samples with a PlC (in PRP) ranging from 88/nl to 590/nl.

System reagent sets for Thrombomate XRA come in disposable racks with a multi-functional QR-code. We used the recommended concentrations of the SSC/ISTH. (Kit LTA-1: collagen 2 µg/ml, ADP 2.5 µM, epinephrine 5µM, TRAP 10 µM, arachidonic acid, 1mM, and ristocetin 1,2 mg/ml from Kit LTA 3). (1) The stability of these reagents is 3 weeks.

Thrombomate XRA reagent rack and cuvette

After identification, PRP or its dilutions with PPP are loaded in closed tubes into Thrombomate® XRA. The instrument counts down a resting time. Then PRP is homogenized by automated inversion. Next, PRP and reagents are dispensed by cap-piercing. The reaction takes place in optimized cuvette strings with steel balls for mixing and generation of shear forces. The change in turbidity is monitored with bichromatic LED optics. The software calculates parameters such as maximum aggregation (MA-%), slope, disintegration, and others. Data are stored or can be sent to an LIS.

Thrombomate XRA instrument and a typical printout

Literature:

1. Cattaneo M.,, et al. Recommendations for the standardization of light transmission aggregometry, a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH. J. Thromb. Haemost. 2013;11:1183–1189
2. Boknäs N, et al.Platelet function testing at low platelet counts: When can you trust your analysis? Res Pract Thromb Haemost. 2019;3:285-29

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Results

The effect of PlC was more pronounced on slope and area under curve (AUC) as compared to maximum aggregation (MA). Reactivity was different between individual samples and clearly reagent dependent. MA was almost independent from platelet count for arachidonic acid and ristocetin, moderate for ADP and collagen, and more pronounced for epinephrine and especially for TRAP (figure 1 and 2). In general, the effect of PlC for MA is low at PlC ≥200/nl in PRP for all reagents.

AUC, similar to MA, shows a reagent dependent and individual effect of PlC at with saturation at about 300/nl in PRP. Disintegration gets dependent in most cases at PlC <200/nl in PRP.

In contrast, slope shows a clear dependency of PlC up to about 400/nl in PRP for all reagents. When calculated back to the platelet count in whole blood, a major decrease of MA takes place when platelets are lower than approximately 100/nl in whole EDTA-blood with all reagents. Individual samples show a quite variable influence of platelet count (figure3).

Figure 1: Effect of platelet count (in PRP) with various reagents on MA-% (left), slope (right), AUC (bottom, left), and disintegration (bottom right)

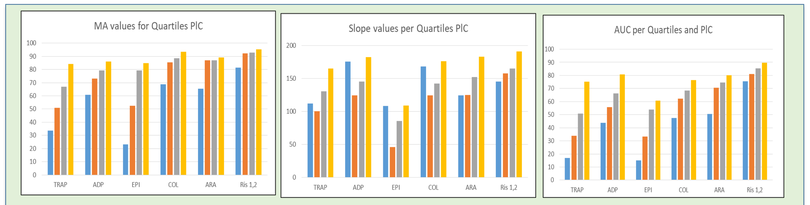

Figure 2: Results of platelet counts in PRP (in quartiles) on MA-%, slope and AUC values

	Platelet Count	
	Mean	Range
Q1	112,1	88-129
Q2	160,0	133-180
Q3	242,3	199 -307
Q4	473,8	350- 613

Conclusion:

The results validate principal function of LTA at Thrombomate® XRA even at low platelet counts. For the MA-% parameter, the effect of PlC is moderate and reagent specific as long as the PlC in whole blood is >100/nl or in PRP is >200/nl, or even lower.

For MA-%, the PlC effect differs depending on the agonist, but also from sample to sample, probably influenced by other variables. In contrast to MA-%, the effect on slope is clearly PlC dependent, while the effect on AUC is intermediate.

Inspection of the curve and its derived parameters is essential for full characterization of reactivity with different reagents also in a fully automated LTA instrument.

A limitation of this approach is that dilution of PRP with PPP may lead to alterations of the sample. The results shown for Thrombomate® XRA and its standard reagent panel may not be applicable for other LTA instruments and reagents.

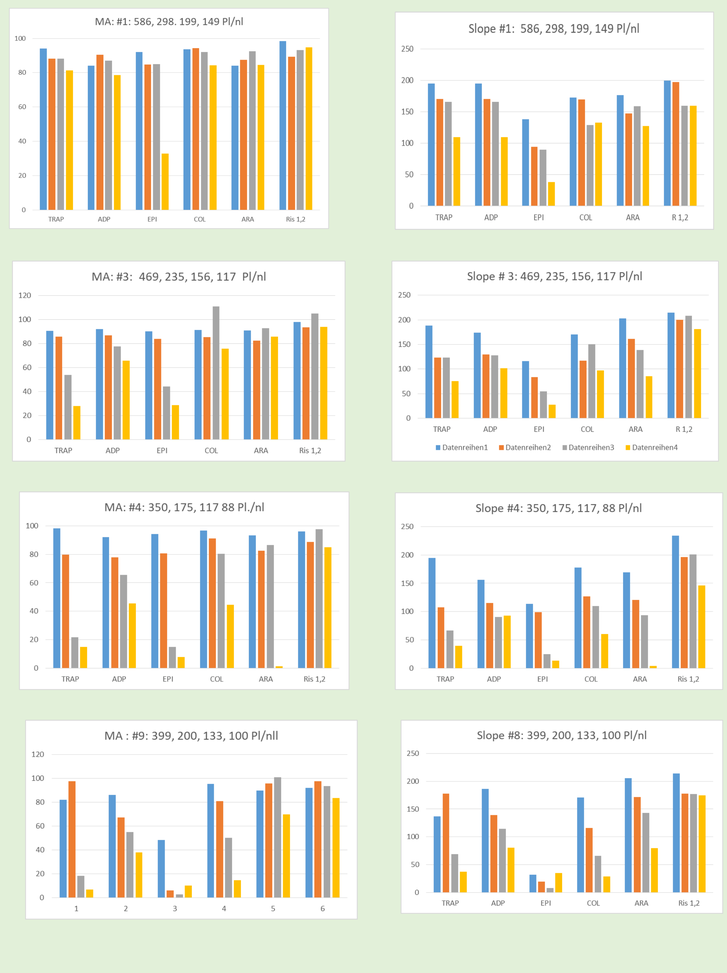

Figure 3: Example of MA (left) and slope values (right) 3 individual donors with all reagents. The bars represent different platelet counts (see headlines).