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Evaluation of the fully automated Thrombomate® XRA for light transmission aggregometry

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Introduction

Light transmission aggregometry (LTA) in platelet rich plasma (PRP) against platelet poor plasma (PPP) is the gold standard for platelet function testing. However, LTA is laborious and requires specialized and experienced operators.

We investigated the performance of the novel fully automated Thrombomate® XRA for LTA in respect to precision, normal range and patient data and compared it to a manual device.

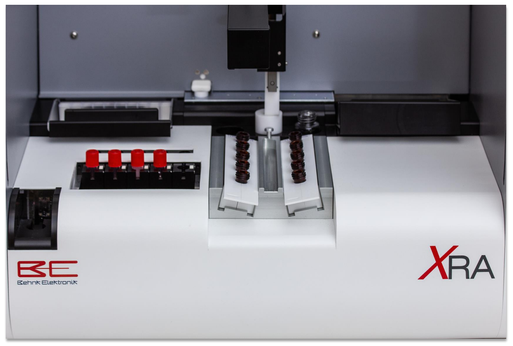
Material and methods

Citrate anticoagulated blood was obtained from healthy volunteers or from patients with VWS, other disorders and patients on ASA /and or ADR receptor antagonists.

Thrombomate® XRA ("TXRA", Behnk Elektronik), is a dedicated automate for LTA. It works with standardized system reagent combinations provided as a unit in coded racks (in this study ADP, arachidonic acid, ephinephrine, collagen, TRAP and ristocetin in the concentrations recommended by the SSC/ ISTH). (1). See table 1. TXRA- reagents have a stability of 3 weeks. TXRA data were compared against PAP-8 (BioData) using identical reagents.

PRP and PPP were centrifuged for 10 min/150g or 20 min/1500g). After sample identification and loading, TXRA controls the 30 min resting time of PRP after centrifugation. Before analysis the PRP is automatically inverted in a closed tube, next TXRA dispenses PRP by cap-piercing and adds reagents.

LTA is measured in cuvette strips by a high precision bichromatic LED optics against autologous PPP and mixing with a steel ball. Results are stored in the integrated PC and can be sent to an LIS or printed.



Results:

The investigation included the analysis of precision by 5-fold analysis of PRP from 5 different individuals with all reagents, the determination of normal ranges by analyzing PRP from 100 healthy donors, and a comparison of 29 patients who required LTA analysis for various reasons.

Precision

The precision with all reagents with TXRA was similar or better as compared to PAP-8 for the maximum aggregation (MA)-% value.

Note: All measurements at PAP-8 were performed by the same operator.

	Thrombomate® XRA	Manual System
Reagent	**Mean CV**	**Mean CV**
TRAP (10µM)	2.1	2.1
ADP (2.5µM)	1.6	3.7
COL (2 mg/ml)	1.4	3.0
ARA (1 mM)	4.6	3.3
EPI (5µM)	2.2	2.8
RIS (1.2 mg/ml)	2.1	1.7
ALL REAGENTS	**2.8**	**3.3**

Table 1: Precision analysis (5-fold determination, 5 individuals). Numbers represent the CV values in %.

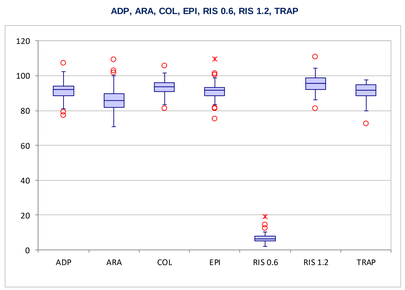
Normal Range

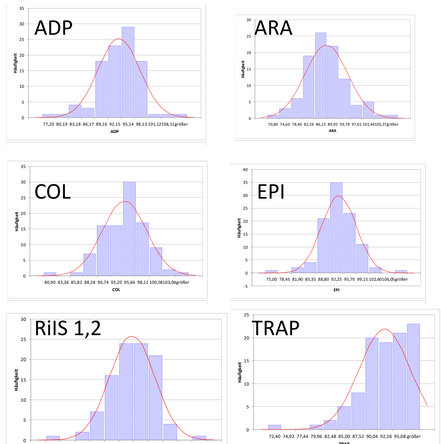
The normal ranges of 100 healthy subjects for both instruments is shown in the table. TXRA MA-% values in normal subjects were slightly higher than on PAP-8. Except TRAP and RIS 1,2 data are normally distributed on TXRA. (Anderson-Darling test).

Table 2: Normal range (both instruments)

Normal range (N= 100)		ADP, 2µM	ARA, 1 mM	COL, 2µg/ml	EPI, 5µM	TRAP, 10µM	RIST 1,2 mg/ml	RIST 0.6 mg/ml
Thrombomate XRA	Mean	91,6	86,1	93,5	91,2	91,1	95,4	6,7
	SD	4,7	6,9	4,1	4,6	4,6	4,5	2,4
	2-SD range	82- 101	72- 100	85- 102	82-100	82- 100	86- 105	2 -11
PAP-8	Mean	81,5	82,8	83,7	81,1	81,0	80,5	1,7
	SD	6,2	6,1	6,3	6,5	6,3	5,0	1,5
	2-SD range	69 - 94	70 -95	71- 96	68- 94	68 -94	71- 90	-1 -5

Figure 1: Normal range distribution (Thrombomate ® XRA)





Conclusion

The data demonstrate that TXRA provides similar results like traditional manual LTA, but with better precision and minimum handling.

Several aspects in the pre-analytical phase such as a controlled resting time of PRP and homogenizing the sample under defined conditions plus standardized stable reagents contribute to a better standardization of the LTA procedure and make results broadly user independent.

Automation and simple operation opens the possibility to implement LTA into the routine or even STAT program of the lab.

Patient Results

Patient results (MA-%, Bland-Altman) showed principal agreement for the majority of cases with no major difference (figure 2). A few data, primarily with EPI or TRAP showed individual differences in a limited number of patient samples. Individual differences between PAP-8 and a different aggregometry device PAP-4 have been reported earlier, (2).

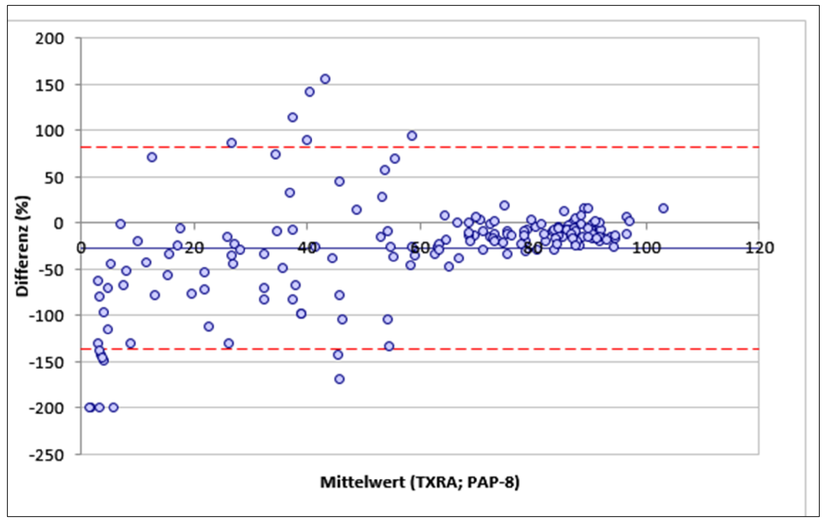


Figure 2: Comparison of TXRA and manual system (Bland-Altman)

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. Ringwald J, et al.. Paired comparsion of two light transmission platelet aggregation devices in healthy individuals. Hämostaseologie 2009;29(1): A61

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