

# Comparison of the Diagnostic Performance of Platelet Aggregation Test using Light Transmission Aggregation (LTA) Method

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Original Article

## Comparison of the Diagnostic Performance of Platelet Aggregation Test using Light Transmission Aggregation (LTA) Method

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### ABSTRACT

**Background:** Antithrombotic therapy, such as acetylsalicylic acid (ASA) and clopidogrel, may interfere with platelet aggregation in thrombotic strokes. Therefore, a platelet aggregation test is essential for monitoring therapy. A previous study observed a decrease in platelet aggregation using antithrombotics with a manual LTA analyzer. Further research is required to compare this with an automatic tool to determine whether both instruments have similar detection capabilities.

**Methods:** PPP and PRP samples were collected from 30 thrombotic stroke outpatients and 30 healthy controls. Platelet aggregation tests were performed using ADP 5 $\mu$ M and Collagen 2 $\mu$ g/mL on both instruments, Chronolog® Model 490, and Sysmex® CS-2500. The tests were then analyzed using the Wilcoxon test for differences, the Spearman test for correlation, and the Bland-Altman analysis for agreement.

**Results:** No differences were observed in MA values and numerical alignment between the two instruments for ADP and Collagen. If the MA result for the platelet aggregation test on Chrono-log® Model 490 was high, it was likewise high on Sysmex CS-2500 and vice versa. Although the values of LP and Vel differed, the numbers for ADP and Collagen in both instruments were consistent.

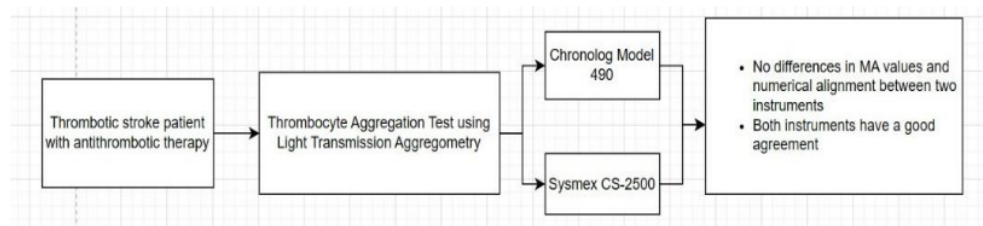
**Conclusion:** The comparison between both instruments indicates good agreement. Automated LTA demonstrates the same suitability as manual LTA and can be applied in clinical routines to assess platelet aggregation function. Due to many variations in the pre-analytics stage that might affect the results, it is vital to standardize both the pre-analytics, analytics, and post-analytics stages to compare platelet aggregation tests on the two instruments.

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### GRAPHICAL ABSTRACT



### Introduction

Stroke is currently considered a neurological emergency with a high mortality rate [1]. It stands as the primary cause of long-term morbidity [2, 3], trailing closely behind coronary heart disease [4, 5]. Thrombotic stroke is the most prevalent type [5]. It is caused by a decrease in cerebral blood flow, resulting in death and brain cell dysfunction. Various risk factors, including sex, age, hypertension, dyslipidemia, and smoking habits, are known to impact platelets and endothelial cells, thereby influencing the course of the disease [6-8]. Platelet aggregation can be affected by antithrombotic medications such as acetylsalicylic acid (ASA) and clopidogrel [9]. Clopidogrel, for instance, irreversibly inactivates the platelet P2Y12 receptor, inhibiting ADP stimulation of the GPIIb/IIIa receptor—an essential step in platelet activation. Meanwhile, acetylsalicylic acid has been observed to reduce platelet aggregation by blocking the thromboxane-mediated aggregation pathway. This blockade works by inhibiting the cyclooxygenase enzyme and preventing platelet aggregation [10, 11]. Consequently, a platelet aggregation test is essential for monitoring therapy [12, 13]. Previous studies of platelet aggregation utilizing manual Light Transmission Aggregometry (LTA), specifically with the Chrono-log® Model 490, in thrombotic stroke patients receiving acetylsalicylic acid have indicated a significant decrease in aggregation [14]. However, further research is necessary, especially employing automatic instruments for

comparison [15]. Currently, automatic coagulation analyzers can also assess platelet aggregation tests using the same LTA method. Thus, this study aims to compare the diagnostic performance of manual (Chrono-log® Model 490) and automatic (Sysmex® CS-2500) platelet aggregation testing.

### Materials and Methods

#### Sample collection

Four tubes of blood-fasted samples were collected from each of the 30 thrombotic stroke outpatients receiving acetylsalicylic acid (ASA) or clopidogrel, and 30 healthy controls from November 2022 to March 2023 at Dr. Soetomo Regional Public Hospital, Surabaya. The vacutainer tubes contained 3.8% sodium citrate with a blood-to-anticoagulant ratio of 9:1.

#### Ethics

This cross-sectional study received prior approval from the Health Research Ethics Committee of Dr. Soetomo Regional Public Hospital.

#### Platelet aggregation assay

Citrate tubes were centrifuged at 1500 G for 15 minutes to produce platelet-poor plasma (PPP) and other tubes at 200 G for 10 minutes to produce platelet-rich plasma (PRP). The samples were excluded if they exhibited hemolysis, icteric, or lipemic characteristics or if collected for more than 2 hours. Platelet aggregation tests were conducted on both instruments using ADP 5µM and Collagen (COL) 2µg/mL aggregators.

The research was carried out in the Clinical Pathology Central Laboratory of Dr. Soetomo Regional Public Hospital for automatic tools and in a non-government laboratory for manual tools. Maximum Aggregation (MA), Lagphase (LP), and Velocity (Vel) results from both instruments were recorded.

The results of the Maximum Aggregation (MA), Lagphase (LP), and Velocity (Vel) from both instruments were analyzed using SPSS version 26 software. The analysis included the Wilcoxon test for differences and the Spearman test for correlation. Medcall version 19.5 was employed for Bland-Altman analysis to assess the suitability of both instruments.

Statistical analysis

Table 1: Maximum aggregation (MA) statistical analysis results

Agonists	N		Sysmex® CS2500	Chrono-log® Model 490		Difference	Correlation	Sig.
			Median (%) (Percentile 5-95)	Median (%) (Percentile 5-95)				
HEALTHY	30							
ADP	30	75.1	73.2-76	75	72-80	0.233	0.307	0.048
COL	30	85.3	83.5-87.1	79	72.5-82	0.300	0.283	0.043
STROKE								
AAS	19							
ADP	19	35	28-53	31	25-41	0.280	0.915**	0.000
COL	19	27	17-35	15	10-37.9	0.402	0.890**	0.000
Clopidogrel	11							
ADP	11	32	20-52	28	17-52	0.624	0.867**	0.001
COL	11	89	87.2-92	90	83-91	0.408	0.678*	0.022
TOTAL	60							
ADP	60	62.4	53.5-71.8	68.5	42.5-71.5	0.400	0.893**	0.000
COL	60	83.5	82.5-85.7	74	71-80.5	0.342	0.790**	0.000

Note: \* indicates a strong correlation; \*\* indicates a very strong correlation

Table 2: Lagphase (LP) statistical analysis results

Agonists	N		Sysmex® CS2500	Chrono-log® Model 490		Difference	Correlation	Sig.
			Madian (%)(Percentile 5-95)	Madian (%)(Percentile 5-95)				
HEALTHY	30							
ADP	30	12	12-13	5.5	5-7	0.000	0.257	0.017
COL	30	12	11-13.5	4	4-5	0.000	0.306	0.046
STROKE								
AAS	19							
ADP	19	12	10-15	7	6-10	0.001	0.295	0.020
COL	19	8	7-10	2	0-4	0.004	0.582*	0.009
Clopidogrel	11							
ADP	11	15	12-16	8	7-10	0.003	0.652*	0.024
COL	11	8	6-12	4	3-5	0.003	0.675*	0.023
TOTAL	60							
ADP	60	12	12-13	7	6-7	0.000	0.741**	0.048
COL	60	10	10-12	4	3-4.5	0.000	0.608**	0.000

Note: \* indicates a strong correlation; \*\* indicates a very strong correlation

## Results and Discussion

Samples were collected from 19 patients receiving 1×100 mg AAS and 11 patients receiving 1×75 mg Clopidogrel, consisting of 61.7% male and 38.3% female patients, with an overall age range between 27 and 66 years.

The results of this study are further detailed in Table 1. For ADP and collagen agonists, the difference test between the Chronolog® Model 490 and Sysmex® CS-2500 yielded results of 0.400 and 0.342, with  $p > 0.05$ , indicating no significant difference in Maximum Aggregation (MA) values. However, the analysis of Lagphase (LP) and Velocity (Vel) values for both instruments is presented in Tables 2 and 3.

A significant difference in LP and Vel values was observed between the Chrono-log® Model 490 and Sysmex® CS-2500 for both ADP and collagen agonists, resulting in a p-value of 0.000 ( $p < 0.05$ ). The extent of this difference can be assessed by comparing the median LP and Vel values of both instruments. According to these data, the Sysmex® CS-2500 instrument, when evaluating LP, tends to be numerically higher compared to the Chrono-log® Model 490, with a difference of approximately 6 for both ADP and COL agonists.

Similarly, the difference in Vel is approximately 12 for ADP and 5 for COL agonists.

A significant numerical alignment correlation ( $p < 0.05$ ) between both instruments was identified in the MA, LP, and Vel correlation tests. This result indicates that if the MA result for the platelet aggregation test on Chrono-log® Model 490 was high, it would also be high on Sysmex® CS-2500.

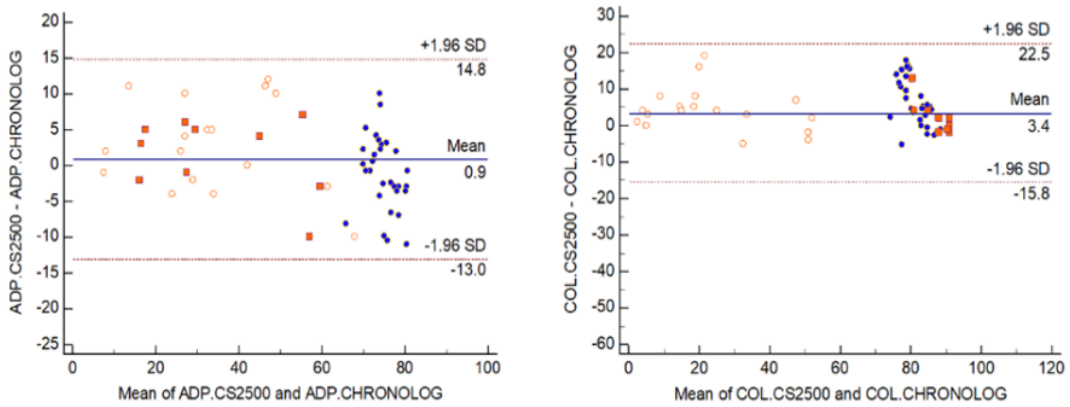
Overall, based on the Bland-Altman analyses presented in Figures 1, 2, and 3, a strong agreement is observed between the MA, LP, and Vel results for both instruments with ADP and Collagen agonists. This conclusion is drawn from the distribution of the samples near the midline, with no data points surpassing the maximum or lowest borders.

The platelet aggregation test utilizing Light Transmission Aggregation (LTA) stands as the gold standard for assessing platelet function, offering early detection capabilities for platelet disorders through the addition of a platelet agonist to platelet-rich plasma (PRP) [16]. The observed MA results reveal no significant difference between the manual and automatic instruments.

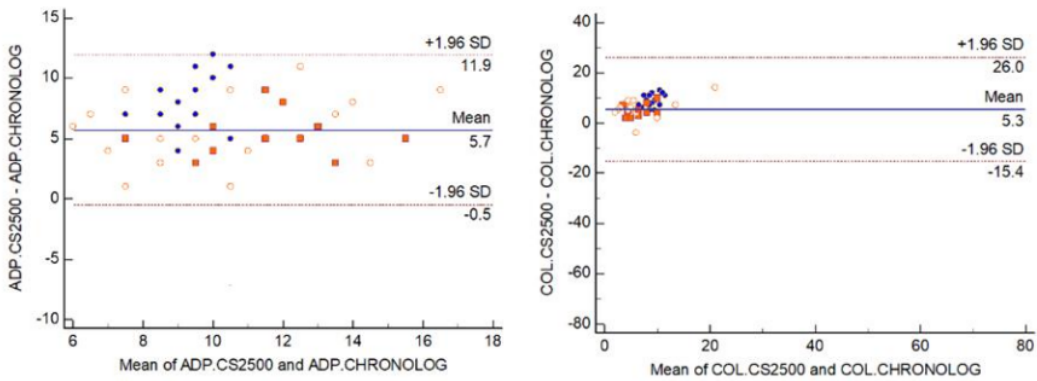
**Table 3:** Velocity (Vel) statistical analysis results

Agonists	N		Sysmex® CS2500	Chrono-log® Model 490		Difference	Correlation	Sig.
			Madian (%)(Percentil e 5-95)	Madian (%)(Percentile 5- 95)				
HEALTHY	30							
ADP	30	85	82-86.5	63	61.5-71.5	0.000	0.232	0.039
COL	30	132	131-134	95.5	92-100.5	0.000	0.315	0.042
STROKE								
AAS	19							
ADP	19	28	18-34	31	15-37	0.006	0.857**	0.000
COL	19	21	13-25	13	10-17	0.012	0.740**	0.000
Clopidogrel	11							
ADP	11	23	21-28	25	19-29	0.034	0.631*	0.046
COL	11	81	76-83	78	70-81	0.026	0.642*	0.042
TOTAL	60							
ADP	60	65	34-82	53	37-61	0.000	0.854**	0.000
COL	60	98.5	77.5-128	83.5	74-91	0.000	0.888**	0.000

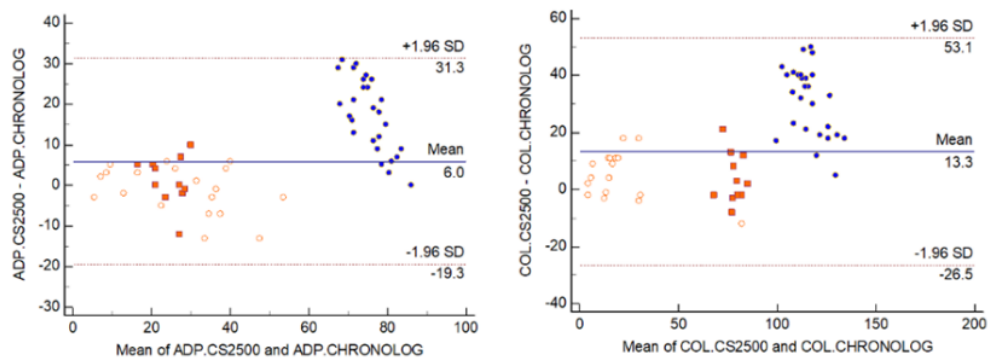
Note: \* indicates a strong correlation; \*\* indicates a very strong correlation



**Figure 1:** Bland-Altman analysis of maximum aggregation (MA) in ADP (right) and COL (left) (Three symbols are employed: a blue circle for the control group, an orange circle for the ASA therapy group, and an orange box for the clopidogrel therapy group)



**Figure 2:** Bland-Altman analysis of lagphase (LP) in ADP (right) and COL (left) (Three symbols are employed: a blue circle for the control group, an orange circle for the ASA therapy group, and an orange box for the clopidogrel therapy group)



**Figure 3:** Bland-Altman analysis of velocity (VEL) in ADP (right) and COL (left) (Three symbols are employed: a blue circle for the control group, an orange circle for the ASA therapy group, and an orange box for the clopidogrel therapy group)



This aligns with findings in the study by Stratmann *et al.* [17]. However, the LP and Vel difference tests on both instruments reveal a numerical distinction. The Chronolog® Model 490 tends to exhibit a shorter duration than the Sysmex® CS-2500, attributed to differences in agonist characteristics. These differences impact the waiting time from agonist addition to the change in transmission light caused by aggregated platelets. LP differences were also noted in Stratmann *et al.*'s study. It is important to define separate reference values for automatic coagulation analyzers [17]. In addition, the LP and Vel differences between both instruments do not affect clinical utility in diagnosing platelet aggregation diseases or assessing antithrombotic therapy. This is because platelet aggregation test results primarily rely on the MA value. If the MA value is less than the reference value, it is concluded as hypo aggregation. If it is between the reference values, it is concluded as normo-aggregation, and if it is above the reference value, it is concluded as hyperaggregation [18].

Thus, the results of this study are likely applicable only to identical sample preparation procedures, instruments, agonist types, concentrations, and characteristics.

Several factors could influence the results of this study, including the platelet aggregation test, which has a complicated and different procedure for each laboratory. Therefore, it requires standardized procedures for both pre-analytical, analytical, and post-analytical levels [17]. It is essential to standardize sample collection procedures, PPP and PRP preparation techniques, and agonist concentrations, especially for patients with abnormal platelet function or those on antiplatelet medications frequently prescribed post-thrombotic strokes [19]. Achieving uniformity is challenging due to the wide range of principles, reporting units, and criteria employed by analysts [20, 21]. Another limitation of this study that may impact the sample's quality is the difference in location between the sampling sites and both instruments, which can affect platelet aggregation assay results.

## Conclusion

The comparison of platelet aggregation tests between both instruments demonstrates good agreement. However, it is essential to standardize the pre-analytical, analytical, and post-analytical stages due to various pre-analytical differences that may impact the results. Despite these considerations, the automatic analyzer exhibits suitability equivalent to the manual analyzer and can be seamlessly integrated into clinical routines for the assessment of platelet aggregation function and monitoring of antithrombotic therapy.

This study did not explore the influence of different agonist concentrations on MA, LP, and Vel measurements. Previous research indicates that different ADP concentrations can significantly affect MA levels in the Sysmex CS-2X00 system [22]. Consequently, further investigations are required to evaluate the performance of automated LTA analyzers across a broader spectrum which might include the examination of other concentrations or types of agonists, as well as investigations involving patients with rare inherited platelet disorders.

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## Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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