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COMPARISON OF PT AND aPTT RESULTS IN ICTERIC SAMPLES USING OPTICAL AND ELECTROMECHANICAL METHODS

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ABSTRACT

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests are important screening tests for assessing coagulation pathway function and monitoring anticoagulant therapy. These tests use a coagulation analyzer with different detection methods, namely optical and electromechanical methods. The working principles of the two methods can cause variations in test results, especially in samples with icteric interference. Therefore, it is necessary to evaluate the comparison of PT and aPTT values in icteric samples using both methods. This study aimed to evaluate the differences in PT and aPTT values in icteric samples using optical and electromechanical methods. This study used a prospective analytical design with a cross-sectional approach, conducted at Adam Malik Hospital, Medan, from July to August 2025. A total of 69 samples that met the inclusion criteria were selected using sequential sampling techniques. Each sample is tested for PT and aPTT using optical and electromechanical methods. The results of each test were analyzed using the Mann-Whitney, Kappa, correlations tests, and Bland-Altman plots. Significant differences were observed in PT and aPTT values between the optical and electromechanical methods ($p<0.001$), while INR values showed no significant difference ($p=0.426$). Interpretation agreement was moderate for PT ($\kappa=0.599$; $p<0.001$) and strong for aPTT ($\kappa=0.661$; $p<0.001$). Despite numerical differences, both methods provided comparable clinical interpretations, and total bilirubin showed no association with PT or aPTT values. The optical and electromechanical methods produced comparable clinical interpretations despite differences in PT and aPTT values, and total bilirubin levels were not associated with PT or aPTT results.

Keywords: aPTT; electromechanical method; icteric sample; optical method; PT

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INTRODUCTION

Hemostasis testing is routinely performed in clinical laboratories to identify abnormalities in blood coagulation and to support the monitoring of anticoagulant therapy. Among the available assays, prothrombin time (PT) and activated partial thromboplastin time (aPTT) are the cornerstone tests for assessing the coagulation cascade. Prothrombin time reflects the functional integrity of clotting factors within the extrinsic and common pathways, whereas aPTT evaluates factors associated with the intrinsic and common pathways. To reduce inter-laboratory variation in PT reporting, the World Health Organization (WHO) recommends the use of the International Normalized Ratio (INR) as a standardized expression of PT results. (DeLoughery, 2019; Patologi Klinik et al., 2012). Modern coagulation testing is predominantly performed using automated coagulation analyzers, which have largely replaced manual techniques due to improved precision and workflow efficiency. These analyzers differ mainly in how clot formation is detected and are generally classified as optical or electromechanical systems. Optical analyzers identify clot formation by monitoring alterations in light transmission or scattering that occur during fibrin polymerization. In contrast, electromechanical analyzers detect clot formation by sensing changes in plasma viscosity, typically

through the movement of a steel ball within the reaction cuvette under magnetic control. (Hernaningsih & Akualing, 2017; Hernaningsih & Butarbutar, 2019)

In routine laboratory settings, particularly in referral hospitals, laboratories may operate more than one coagulation analyzer using different detection principles. This situation provides an opportunity to compare results across methods when discrepancies arise. Such comparisons are clinically relevant because coagulation assays are known to be highly sensitive to pre-analytical interferences, especially those related to abnormal plasma appearance, including hemolysis, icterus, and lipemia. These interferences can introduce analytical bias and reduce the reliability of coagulation test results, potentially affecting patient management. (Hernaningsih and Butarbutar,2019; Nougier et al.,2020). Each detection principle has specific analytical limitations. Optical coagulation assays are particularly vulnerable to interference from colored substances in plasma, as variations in absorbance caused by hemoglobin, bilirubin, or lipids may disrupt optical signal detection. Electromechanical assays are generally considered less susceptible to color interference because clot detection does not depend on light-based measurements. However, guidance from the Clinical and Laboratory Standards Institute (CLSI) indicates that hemolyzed samples may promote premature clot formation or unintended activation of coagulation factors, suggesting that analytical interference is not exclusively limited to optical systems. The extent of interference depends on both instrument performance and reagent composition. (Ramadhani,2021; Nougier et al., 2020)

Interfering samples remain common in everyday laboratory practice. Several studies have demonstrated that hemolysis, icterus, and lipemia account for a substantial proportion of rejected or flagged coagulation specimens. Among these, icteric samples represent a particular analytical challenge due to elevated bilirubin concentrations. Bilirubin exhibits strong absorbance within the visible spectrum, particularly between 400 and 520 nm, with a peak near 456 nm. This spectral characteristic may overlap with the detection wavelengths used in optical coagulation analyzers, thereby affecting clot detection. Beyond its optical properties, bilirubin can undergo oxidative conversion into biliverdin and bilipurpurin, compounds that may interact with reactive components in plasma. Clinically, hyperbilirubinemia is defined as a total bilirubin concentration exceeding 1.5 mg/dL and may be related to conjugated or unconjugated bilirubin fractions. Previous investigations have suggested that optical analyzers operating at longer wavelengths, typically at or above 650 nm, demonstrate improved resistance to bilirubin-related interference and may provide acceptable results at moderate bilirubin concentrations. (Lippi, Plebani and Favaloro, 2013; Woolley, Golmard and Kitchen, 2016).

The effect of icteric interference on coagulation testing has been evaluated in several studies, although the reported outcomes are inconsistent. Some investigations have shown minimal or no clinically significant differences in PT and aPTT results between icteric and non-icteric samples when advanced optical detection systems are used. In contrast, other studies have identified measurable bias in coagulation parameters across both optical and electromechanical analyzers, particularly at higher bilirubin concentrations. These discrepancies indicate that bilirubin interference is influenced by multiple factors, including analyzer design, detection principle, reagent sensitivity, and the degree of hyperbilirubinemia. (Nougier et al., 2020) Ongoing technological developments have led to the introduction of coagulation analyzers with enhanced detection capabilities, such as multi-wavelength optical systems, which aim to reduce the impact of sample-related interferences. Despite these advances, direct comparisons between optical and electromechanical methods in icteric samples remain limited, and the relationship between bilirubin concentration and coagulation test results has not been fully clarified. (Médica Boliviana, 2020; Nougier et al., 2020)

In view of the heterogeneity of previous reports, the fundamental differences in coagulation detection principles, and the high prevalence of icteric specimens encountered in routine laboratory

practice, further investigation is warranted. Accordingly, the present study was designed to compare PT and aPTT measurements obtained from icteric plasma samples using optical and electromechanical coagulation systems, with the aim of elucidating the extent of bilirubin-related analytical interference and improving the accuracy of coagulation result interpretation in patients with hyperbilirubinemia.

METHOD

This study used a prospective analytical design with a cross-sectional approach, conducted at Adam Malik Hospital, Medan, from July to August 2025. The study population consisted of all patients who underwent blood testing at the Clinical Pathology Laboratory of Adam Malik General Hospital, Medan. The study sample consisted of icteric plasma specimens that met the predefined inclusion and exclusion criteria. Samples were consecutively enrolled using a non-probability sampling method until the minimum required sample size was reached. Each specimen was analyzed once using both optical and electromechanical coagulation methods. The minimum sample size, calculated using the Lemeshow formula for a cross-sectional study, was 68 specimens. This study employed a non-probability sampling method using a consecutive sampling technique, through which a total of 69 icteric plasma samples with total bilirubin levels greater than 1.5 mg/dL that met the inclusion criteria were collected. Exclusion criteria included samples that showed hemolysis or lipemia after collection and centrifugation. Coagulation tests were performed on plasma samples collected in 3.2% sodium citrate tubes (9 : 1 blood-to-anticoagulant ratio), while total bilirubin was measured from serum obtained in plain tubes. Prothrombin time and activated partial thromboplastin time were analyzed using a fully automated coagulation analyzer with optical and electromechanical detection principles, while total bilirubin concentration was measured by the colorimetric diazo method.

Optical Methods

Optical coagulation analysis was performed using a fully automated analyzer based on the detection of changes in plasma turbidity after reagent activation by transmitted light. For PT testing, 50 µL of plasma and 100 µL of Dade Innovin reagent were used, while aPTT testing employed 50 µL of plasma with Dade Actin reagents (Actin FS and $CaCl_2$). Measurements were carried out automatically using multi-wavelength detection, and results were reported in seconds.

Electromechanical Methods

Electromechanical coagulation testing was performed using a fully automated analyzer based on viscosity-dependent detection of oscillation changes in a steel ball within the cuvette via an electromagnetic sensor. For PT testing, 50 µL of plasma with STA-NeoPTimal reagent was used, while aPTT testing employed 50 µL of plasma with STA-CK Prest 5 reagent. Results were automatically generated and reported in seconds.

Quality control procedures were implemented to ensure analytical validity. Total bilirubin was monitored using lyophilized commercial control materials at two concentration levels, while PT and aPTT were controlled using lyophilized plasma controls for both optical and electromechanical analytical systems. Quality control performance was displayed graphically for optical methods and indicated by an in-range status marker for electromechanical methods.

This study has obtained ethical approval from the Health Research Ethics Committee of the University of North Sumatra (No. 14888/KEPK/USU/2024) and research permission from the Human Resources, Education, and Research Department of Adam Malik Hospital (No. DP. 04.03/D.XXVIII/241/2025). Statistical analysis was performed using appropriate software. Demographic characteristics were summarized descriptively. Differences in PT and aPTT values between optical and electromechanical methods were analyzed using the Mann–Whitney U test, while agreement between methods was assessed using Cohen's kappa. Spearman's correlation, one-

sample t-test, Bland–Altman analysis, and linear regression were applied for method comparison. A p-value < 0.05 was considered statistically significant.

RESULT

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) measurements were performed on 69 jaundiced samples with hyperbilirubinemia. The median age of the study subjects was 51 years, with ages ranging from 1 to 74 years. The study population consisted of 56.5% male participants and 43.5% female participants. The mean total bilirubin level was 10.5 mg/dL, with values ranging from 1.64 mg/dL to 34.5 mg/dL. The optical method yielded lower median values for PT (14.3 s), aPTT (29.7 s), and INR (1.3) compared with the electromechanical method, which showed medians of 19.8 s, 35.9 s, and 1.4, respectively. (Table 4.1)

Table 1.
Analysis of Differences in PT, aPTT, and INR Results Between Optical and Electromechanical Methods

Variable	Optical Method	Electromechanical Method	P Value*
	Median (Min-Max)	Median (Min-Max)	
PT	14,3 (10,3 – 80)	19,8 (11,1 – 86,4)	<0,001
aPTT	29,7 (19,6 – 65,8)	35,9 (26,3 – 82,1)	<0,001
INR	1,3 (0,91 – 7,69)	1,4 (0,75 – 7,84)	0,426

The Mann–Whitney test showed significant differences in PT and aPTT between the optical and electromechanical methods (p < 0.001), whereas no significant difference was found for INR (p = 0.426). This may reflect differences in method-specific reference ranges, although the clinical interpretation was consistent.

A high level of interpretative agreement was observed between the two methods. Both methods classified PT as normal in 13 samples and prolonged in 45 samples, INR as normal in 9 samples and increased in 45 samples, and aPTT as normal in 40 samples and prolonged in 19 samples. However, discrepant interpretations were noted in 11 PT samples and 10 aPTT samples. (Table 4.2)

Table 2. Agreement Analysis of PT and aPTT Interpretation Between Optical and Electromechanical Methods

Variable		Electromechanical Method		κ	P Value
		Normal	Abnormal		
Optical Method					
PT	Normal	13	9	0,599	< 0,001
	Abnormal	2	45		
aPTT	Normal	39	0	0,661	< 0,001
	Abnormal	11	19		

The agreement analysis yielded a Kappa value of 0.599 (p < 0.001) for PT, indicating a moderate and statistically significant level of agreement between the optical and electromechanical methods in classifying normal and abnormal results. For aPTT, the Kappa value was 0.661 (p < 0.001), reflecting strong agreement and consistent interpretation between the two methods, suggesting that both methods provide comparable and reliable assessments.

Table 3.
Correlation Between Bilirubin Levels and PT and aPTT Measurements Using the Optical Method

Variable	Optical Method			
	PT		aPTT	
	r	P Value*	r	P Value*
Total Bilirubin	0,237	0,05	0,221	0,068

Weak and non-significant correlations were observed between total bilirubin levels and PT (r=0.237; p=0.05) as well as aPTT (r=0.221; p=0.068) measured by the optical method, indicating no meaningful association between bilirubin concentration and coagulation parameters.

Table 4.
Correlation Between Bilirubin Levels and PT and aPTT Measurements Using the Electromechanical Method

Variable	Electromechanical Method			
	PT		aPTT	
	r	Nilai p*	R	Nilai p*
Total Bilirubin	0,209	0,085	0,039	0,750

As shown in able 4, total bilirubin exhibited a weak, non-significant correlation with PT measured by the electromechanical method (r = 0.209; p = 0.085) and a very weak, non-significant correlation with aPTT (r = 0.039; p = 0.750), indicating no meaningful effect of bilirubin levels on these parameters. Agreement between PT measurements obtained by the optical and electromechanical methods was assessed using a one-sample t-test and Bland–Altman analysis. A significant mean difference of −5.532 ± 4.584 was observed (p < 0.001), reflecting systematic differences related to the different measurement ranges of the two methods. However, despite these numerical differences, the clinical interpretation of PT results remained consistent between methods.

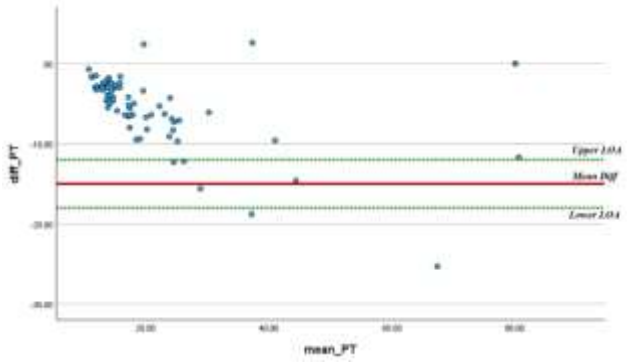

Figure 1. Bland–Altman Plot of PT Measurements Using Optical and Electromechanical Methods

The upper and lower limits of agreement for PT were 3.453 and −14.517, respectively. The Bland–Altman plot (Figure 4.1) demonstrated that most differences lay outside the limits of agreement, indicating significant measurement differences between methods. Linear regression revealed a proportional bias, with a beta coefficient of −0.109 (p = 0.002).

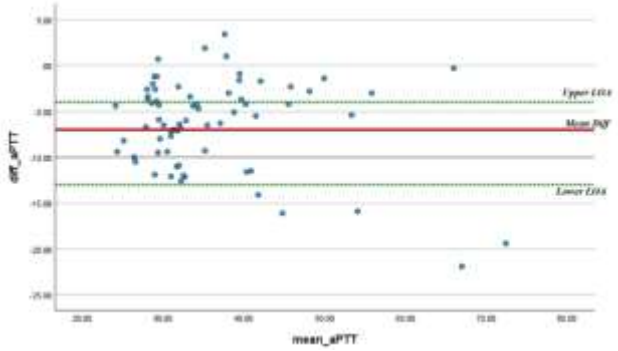

Figure 2. Bland-Altman Plot of aPTT Measurements Using Optical and Electromechanical Methods

The mean difference in aPTT between the optical and electromechanical methods was −6.346 ± 4.917 (p < 0.001). The limits of agreement ranged from 3.293 to −15.985, with many values lying outside these limits on the Bland–Altman plot (Figure 4.2), indicating significant measurement differences. Linear regression showed no proportional bias (β = −0.110; p = 0.067).

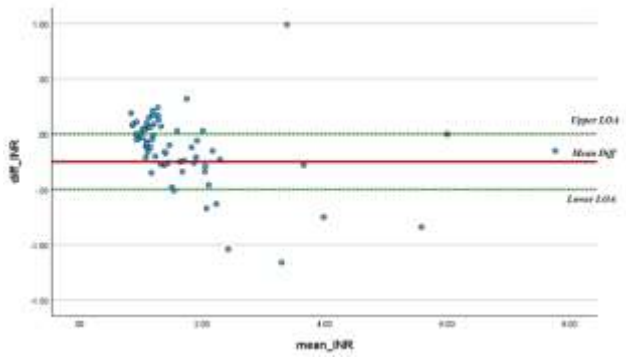

Figure 2. Bland-Altman Plot of aPTT Measurements Using Optical and Electromechanical Methods

The mean difference in INR between the optical and electromechanical methods was −0.134 ± 0.316 ($p < 0.001$). The limits of agreement ranged from 0.486 to −0.753, with many values lying outside these limits on the Bland–Altman plot (Figure 4.3), indicating significant inter-method differences. Linear regression identified proportional bias ($\beta = -0.061$; $p = 0.035$).

DISCUSSION

Coagulation testing is essential for diagnosing thromboembolic and bleeding disorders, assessing hemorrhagic and thrombotic risk, and monitoring anticoagulant or coagulation factor replacement therapy. Ensuring analytical accuracy is critical, as erroneous coagulation results may lead to inappropriate clinical decisions. This concept is well established in the literature and underlines the importance of method evaluation in routine coagulation testing. (Kristoffersen et al., 2025)

Prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR) are among the most frequently requested coagulation assays in routine laboratories. PT reflects the extrinsic and common pathways, whereas aPTT assesses the intrinsic and common pathways, and INR provides standardized PT reporting. The clinical relevance of these assays has been consistently highlighted in previous studies, and the present study is in agreement with this established framework. (Ülfer, 2025)

Interference from hemolysis, icterus, and lipemia (HIL) has been widely reported to affect coagulation testing, with the degree of interference depending on the clot detection principle. Optical methods are theoretically more susceptible to color interference, while electromechanical methods are considered less affected by plasma discoloration. This understanding is consistent with previous reports, although the relative impact of icterus remains controversial. (Kristoffersen et al., 2025; Ülfer, 2025)

In this study, significant numerical differences were observed for PT and aPTT between the optical and electromechanical methods ($p<0.001$), whereas INR values did not differ significantly. These findings are partially consistent with previous studies by Nagant et al. (2016), who reported that PT and aPTT may vary between analytical systems, while INR remains relatively stable due to standardization. Thus, the present findings are largely in agreement with earlier observations regarding INR robustness. (Nagant et al., 2016)

However, the direction of PT prolongation observed in this study differs from some previous reports. While several studies have suggested that optical methods are more affected by bilirubin interference and may yield longer PT values, the present study demonstrated longer PT results with the electromechanical method. This finding is not fully aligned with the study by Nagant et al. (2016), but is consistent with the mechanistic explanation proposed by Aggarwal et al. (2014), who described prolonged clotting times in mechanical detection systems due to delayed endpoint recognition in samples with weak fibrin formation. (Aggarwal et al., 2014; Nagant et al., 2016)

The observed PT prolongation with the electromechanical method may be attributed to reagent sensitivity rather than bilirubin interference. The STA-Neoptimal reagent used in this study has a low ISI and high sensitivity to reductions in factor VII and fibrinogen. Carta et al. (2021) reported similar findings, demonstrating that STA-Neoptimal exhibits greater responsiveness to coagulation factor deficiencies compared with other thromboplastins. Therefore, the present results are consistent with studies emphasizing reagent sensitivity as a key determinant of PT variability. (Carta et al., 2021)

Conversely, the optical method used Dade Innovin reagent, which contains recombinant human tissue factor and is known for rapid clotting response and lower sensitivity to mild factor deficiencies. Nagant et al. (2016) reported that bilirubin interference in optical methods becomes significant primarily at very high bilirubin concentrations (20–30 mg/dL). As most samples in the present study had bilirubin levels below this threshold, the lack of excessive PT prolongation with the optical method is consistent with previous findings. (Nagant et al., 2016)

For aPTT, the present study demonstrated longer clotting times with the electromechanical method compared with the optical method. This finding is consistent with multiple previous studies reporting that aPTT variability is predominantly reagent-dependent. Yuzaqi et al. (2018) showed that CK Prest reagent, which uses kaolin as an activator, is highly sensitive to intrinsic pathway factor deficiencies. Thus, the present findings are in agreement with earlier reports highlighting the influence of reagent composition on aPTT results (Yuzaqi, Halimah and Novianti, 2018). In contrast, the Dade Actin reagent used in the optical method contains ellagic acid, which is a less aggressive activator and confers lower sensitivity to mild factor deficiencies. Sommer et al. (2014) and Wong et al. (2023) similarly reported that kaolin-based reagents tend to produce longer aPTT values than ellagic acid-based reagents, even in samples with near-normal coagulation activity. Therefore, the present findings are consistent with the existing literature regarding aPTT reagent sensitivity. (Sommer et al., 2014; Wong et al., 2023)

Despite statistically significant numerical differences, agreement analysis showed that PT and aPTT interpretations were largely concordant between methods. Moderate agreement was observed for PT and strong agreement for aPTT, indicating that most results were classified similarly as normal or abnormal. This observation aligns with studies by Clarisse et al. (2020), which emphasized that inter-method numerical differences do not necessarily translate into different clinical interpretations when method-specific reference ranges are applied. (Jean et al., 2020)

INR values showed no significant difference between the optical and electromechanical methods, a finding that is fully consistent with previous studies. Nagant et al. (2016) and Clarisse et al. (2020) both reported that INR remains stable across different analytical platforms, even when PT values differ. This confirms that INR standardization effectively compensates for variability arising from reagent characteristics and detection principles. (Jean et al., 2020; Nagant et al., 2016)

Correlation analysis between total bilirubin levels and PT or aPTT values revealed weak and non-significant associations for both methods. These results are consistent with studies by Nougier et al. (2020), who demonstrated that bilirubin interference becomes clinically relevant mainly at very high concentrations. Thus, the present findings support previous evidence that moderate hyperbilirubinemia does not substantially influence coagulation measurements. (Nougier et al., 2020c)

Similarly, weak and non-significant correlations were observed between bilirubin levels and coagulation parameters measured using the electromechanical method. This finding is in agreement with previous reports by Nagant et al. (2016) and Woolley et al. (2016), who concluded that

mechanical detection methods are largely unaffected by bilirubin interference and that observed variability is more closely related to reagent characteristics than plasma color. (Nagant et al., 2016; Woolley et al., 2016)

Bland–Altman analysis demonstrated significant systematic differences between the two methods for PT and aPTT, with wide limits of agreement indicating limited numerical interchangeability. These findings are consistent with studies by Aggarwal et al. (2014) and Bellio et al. (2022), which reported negative bias and longer clotting times with mechanical detection due to delayed endpoint recognition.(Aggarwal et al., 2014; Bellio et al., 2022) For INR, Bland–Altman analysis revealed a smaller but significant mean difference and proportional bias, indicating increasing inter-method differences at higher INR values. This finding is not fully aligned with Nougier et al. (2020) and Bellio et al. (2022), who reported minimal INR bias. However, it is consistent with Scalambrino et al. (2023), who demonstrated that electromechanical methods may yield higher INR values in icteric samples due to greater sensitivity to reduced coagulation factor activity. (Bellio et al., 2022; Nougier et al., 2020; Scalambrino et al., 2023)

Overall, although significant numerical differences were observed between optical and electromechanical methods, these differences were largely attributable to differences in analytical range, reagent sensitivity, and detection principles rather than bilirubin interference alone. Importantly, these numerical discrepancies did not alter clinical interpretation. When method-specific reference ranges were applied, both methods provided comparable and clinically meaningful interpretations. This conclusion is consistent with several previous studies emphasizing that analytical differences should be interpreted within the appropriate methodological context. (Nougier et al., 2020c; Woolley et al., 2016)

CONCLUSION

In conclusion, despite statistically significant differences in PT and aPTT values between the optical and electromechanical methods, both approaches yielded comparable clinical interpretations when method-specific reference ranges were applied. The numerical discrepancies therefore did not translate into clinically relevant differences in result interpretation. Moreover, total bilirubin levels showed no meaningful association with PT or aPTT values in either method, supporting the reliability of both methods for interpreting coagulation results in icteric samples.

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