RESEARCH PAPER

A Study on Evaluation of Pre-Analytical Variables Influencing Coagulation Assays: A Cross-Sectional Study in a Tertiary Care Centre

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ABSTRACT

Background: Pre-analytical errors during collection, transport, and processing can significantly distort PT, aPTT, and INR results, leading to spurious coagulation abnormalities and potentially inappropriate clinical decisions.

Objectives: To evaluate the impact of pre-analytical variables on coagulation assay results (PT, aPTT and INR) and to compare these results across patient subgroups by age, sex, sample collection location, and clinical indication.

Methods: This single-centre, hospital-based analytical cross-sectional study was conducted in the Central Laboratory, in a Tertiary Care Hospital (November 2025–February 2026).

Results: In 320 patients (mean age 49.1 ± 16.9 years; 43.8% aged 40–59), males comprised 57.8%. Samples were mainly from OPD (33.1%) and IPD (28.7%); common indications were anticoagulant monitoring (24.1%) and pre-op screening (19.4%). Tube fill was adequate in 90.6%; hemolysis 6.2%, lipemia 5.0%, icterus 7.5%, microclots 0.9%. Most reached the lab within ≤60 min (60.3%), though 13.4% exceeded 120 min. Overall PT was 15.88 ± 1.91 s, aPTT 36.41 ± 5.70 s, INR 1.54 (1.23–1.95), with no age/sex differences. Values were higher in ICU/OT (e.g., ICU INR 1.74) and in anticoagulant monitoring (INR 1.87) or CLD (INR 1.80) (all $p<0.001$). Underfill increased PT/INR; delays increased aPTT or PT; suspected heparin contamination markedly prolonged aPTT (44.6 s).

Conclusion: Pre-analytical variables—particularly citrate tube underfilling, processing delays, transport delay, lipemia, and heparin/IV line contamination—significantly influenced PT/aPTT/INR results, underscoring the need for strict standardization of coagulation sample collection and handling to ensure reliable reporting.

Keywords: Pre-analytical variables, Coagulation assays, Prothrombin time, Activated partial thromboplastin time, International normalized ratio, Specimen handling

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Introduction

Coagulation screening and monitoring assays—prothrombin time (PT), activated partial thromboplastin time (aPTT), and the international normalized ratio (INR)—are among the most frequently requested hemostasis tests in hospital practice, supporting perioperative assessment, investigation of bleeding/thrombotic states, and monitoring of anticoagulant therapy.(1) INR was specifically developed to standardize PT reporting across thromboplastin reagents and remains the principal metric for vitamin K antagonist (warfarin) monitoring,(2) where inaccurate values can directly lead to inappropriate dose adjustment or unnecessary clinical intervention. PT/INR and aPTT are routinely used as first-line tests to triage suspected coagulopathy, including liver disease–related factor deficiency and sepsis-associated coagulopathy/DIC, where clinical decisions may be time-critical.(3, 4)

Despite their widespread use, clot-based coagulation assays are particularly vulnerable to the pre-analytical phase, and a substantial proportion of discordant results arise from specimen collection, transport, processing, or storage rather than true patient hemostatic status.(5) International Council for Standardization in Haematology (ICSH) guidance emphasizes that citrated coagulation testing depends on a tightly controlled blood-to-anticoagulant ratio (typically 9:1) using 3.2% sodium citrate tubes, and that tubes should generally be considered unacceptable when underfilled, because excess citrate can chelate more calcium during testing, delaying thrombin and fibrin formation, artifactually prolonging clotting time.(6) Similarly, markedly elevated hematocrit (commonly cited at ≥55%) can alter plasma volume relative to fixed citrate volume and may require citrate adjustment to prevent spurious prolongation. Pre-examination time/ temperature conditions also matter: delays in transport, delayed centrifugation to platelet-poor plasma, and prolonged holding before analysis can change factor stability and assay endpoints—often affecting aPTT earlier due to sensitivity to intrinsic pathway factor changes. Additional common interferences include visible hemolysis, lipemia, icterus, microclots, and heparin contamination from line draws, which can markedly prolong aPTT and generate diagnostic confusion if not recognized.(7) Against this background, the objective of the present study was to systematically evaluate the impact of pre-analytical variables on coagulation assay results (prothrombin time, activated partial thromboplastin time, and international normalized ratio) and to compare these results across patient subgroups by age, sex, sample collection location, and clinical indication.

Materials and Methods

This was a single-centre, hospital-based, analytical cross-sectional study conducted in the Central Laboratory, in a Tertiary Care Hospital over a period of four months between November 2025 and February 2026. The study was approved by the Institutional Human Ethics Committee (IHEC). All consecutive patients (nonprobability sampling technique) of either sex and any age (Neonates at risk of physiological vitamin K deficiency, including those without prophylactic vitamin K administration, preterm infants, and exclusively breastfed babies, were excluded to minimize bias in coagulation analysis) whose blood samples were received in the Central Laboratory during the study period with a clinician request for PT, aPTT and/or INR were included, provided that the sample yielded a reportable result and the requisition contained the minimum required clinical and demographic details (age, sex, location of collection, and clinical indication).

Sample size was estimated using the expected difference in aPTT attributable to delayed processing/extended storage. Patil et al. (2022) evaluating storage effects on routine coagulation assays reported a baseline aPTT mean (SD) of 30.45 ± 7.17 s, with an increase under non-ideal conditions (refrigerated delay) (34.14 ± 7.06 s at 12 h),(8) supporting an SD of 7 s. Using a conservative minimum detectable difference of $\Delta = 2.5$ s (smaller than the 3.7 s change observed in the parent study), with two-sided $\alpha = 0.05$, power = 80%, and equal group sizes, the minimum required sample size was rounded off to 320. Data were collected on a structured proforma with the test requisition, phlebotomy/sample-transport details, and analyzer output. For each specimen, patient subgroup variables (age, sex), location of collection (OPD/IPD/ICU/emergency/OT), and clinical indication as stated by the clinician were recorded.

Samples were collected into light blue-top tubes containing 3.2% sodium citrate with the recommended blood-to-anticoagulant ratio and were checked for adequacy of fill (underfilling being documented as a key pre-analytical variable because it alters the citrate ratio and can artifactually prolong clotting times). Markedly elevated haematocrit (≥55%) was noted as a pre-analytical factor because citrate volume adjustment is recommended in such samples to avoid excess anticoagulant effect. Pre-analytical handling timestamps were captured (time of collection, receipt, centrifugation, and analysis) to quantify transport delay and time-to-processing, since delays and storage conditions can influence PT/INR and especially aPTT stability. Sample processing variables documented included centrifugation conditions used to obtain platelet-poor plasma (as inadequate platelet removal can affect clot-based assays) and whether any repeat centrifugation was required. Each sample was inspected and graded for common pre-examination interferences—visible haemolysis, icterus, lipemia, and clots/microclots. Finally, the method of draw (peripheral venipuncture vs sampling from an indwelling line) and any suspicion of heparin/IV fluid contamination were captured, given the known potential for spuriously prolonged aPTT when collection is performed from heparinized lines or with inadequate discard/flush.

Statistical analysis: Data were entered in Microsoft Excel and analysed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for distributional normality using graphical methods (histograms and Q–Q plots) and the Shapiro–Wilk test. Normally distributed continuous variables were summarized as mean ± standard deviation (SD), while non-normally distributed continuous variables were summarized as median with interquartile range (IQR) and range, as appropriate. Categorical variables were summarized as frequency (n) and percentage (%). Comparisons of coagulation assay results (PT and aPTT) across patient subgroups and across pre-analytical variable categories were performed using the independent samples t-test (two groups) or one-way analysis of variance (ANOVA) (≥3 groups) for approximately normally distributed outcomes; where assumptions of normality or homogeneity of variances were not met, the Mann–Whitney U test or Kruskal–Wallis test was used, respectively. Because INR showed a skewed distribution, INR comparisons were primarily undertaken using non-parametric tests (Mann–Whitney U for two groups; Kruskal–Wallis for multiple groups), and results were presented as median (IQR). All tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

Results

Among the 320 participants, the mean age was 49.12 ± 16.94 years, with most patients in the 40–59 years group (140; 43.8%), followed by ≥60 years (87; 27.2%) and 18–39 years (83; 25.9%), while those <18 years comprised 10 (3.1%). Males predominated (185; 57.8%) compared with females (135; 42.2%). Samples were most commonly collected from the OPD (106; 33.1%) and IPD (92; 28.7%), with additional contributions from the ICU (52; 16.2%), Emergency (49; 15.3%), and OT (21; 6.6%). The major clinical indications were anticoagulant monitoring (77; 24.1%) and pre-operative screening (62; 19.4%), followed by bleeding evaluation (55; 17.2%), liver disease/CLD (50; 15.6%), sepsis/DIC evaluation (42; 13.1%), and thrombosis/PE evaluation (34; 10.6%).

Tube fill was adequate in 290 (90.6%) and mildly underfilled in 30 (9.4%); visible hemolysis was noted in 20 (6.2%), lipemia in 16 (5.0%), and icterus in 24 (7.5%), while microclots were rare (3; 0.9%). Most samples reached the lab within ≤60 minutes (193; 60.3%), though 43 (13.4%) had delays >120 minutes; time to centrifugation was ≤45 minutes in 186 (58.1%) and >90 minutes in 30 (9.4%), and time to analysis was ≤2 hours in 188 (58.8%) but >4 hours in 36 (11.2%), with holding mainly at room temperature (247; 77.2%). Repeat sampling due to pre-analytical issues occurred in 19 (5.9%), and suspected heparin/line contamination was documented in 9 (2.8%). Overall, coagulation results showed PT 15.88 ± 1.91 s, aPTT 36.41 ± 5.70 s, and INR 1.54 (IQR 1.23–1.95), with no significant differences by age (PT p=0.459; aPTT p=0.944; INR p=0.488) or gender (PT p=0.891; aPTT p=0.091; INR p=0.177). In contrast, results differed significantly by collection location, with higher values in acute care areas (ICU PT 16.53 ± 2.00 s, aPTT 38.05 ± 5.60 s, INR 1.74 [1.42–2.28]; OT INR 1.84 [1.43–2.27]) compared with OPD (PT 15.42 ± 1.97 s; INR 1.46 [1.16–1.77]) (PT p<0.001, aPTT p=0.013, INR p=0.001). Coagulation parameters also varied by clinical indication (all p<0.001), with the highest values in anticoagulant monitoring (PT 17.27 ± 1.71 s; INR 1.87 [1.57–2.28]) and liver disease/CLD (PT 16.89 ± 1.50 s; INR 1.80 [1.54–

2.25]), while pre-operative screening showed comparatively lower results (PT 14.34 ± 1.59 s; INR 1.23 [1.03–1.44]).

Across pre-analytical variables, tube underfilling (80–89%) was associated with significantly higher PT (16.58 ± 1.77 s vs 15.80 ± 1.91 s; p=0.029) and markedly higher INR (2.03 [1.62–2.29] vs 1.50 [1.22–1.87]; p<0.001), while the increase in aPTT (38.32 ± 6.22 s vs 36.22 ± 5.62 s) did not reach significance (p=0.085). Lipemia was linked to a significant prolongation of PT (17.49 ± 1.83 s vs 15.79 ± 1.87 s; p=0.002) without significant changes in aPTT or INR. Transport delay showed a significant effect on aPTT (≤60 min: 35.76 ± 5.58 s; 61–120 min: 37.12 ± 5.96 s; >120 min: 37.98 ± 5.38 s; p=0.028), whereas PT and INR were not significantly different. Delays in processing were also reflected in PT: longer time to centrifugation was associated with higher PT (≤45 min: 15.59 ± 1.87 s; 46–90 min: 16.23 ± 1.92 s; >90 min: 16.40 ± 1.81 s; p=0.007) and longer time to analysis similarly increased PT (≤2 h: 15.66 ± 1.81 s; 2–4 h: 16.10 ± 2.00 s; >4 h: 16.39 ± 2.03 s; p=0.042). Suspected heparin contamination was strongly associated with aPTT prolongation (44.59 ± 5.81 s vs 36.18 ± 5.53 s; p=0.002) without significant differences in PT or INR. In contrast, hemolysis, icterus, microclots, holding condition, and repeat sampling requirement showed no statistically significant associations with PT/aPTT/INR in this dataset (all p>0.05), though repeat sampling trended toward a higher INR (1.81 [1.51–2.07] vs 1.52 [1.23–1.92]; p=0.061).

Table 1: Baseline demographic and clinical profile of the study population (N = 320)

		Number	**Percent (%)**
Age (years), Mean ± SD		49.12 ± 16.94	
Age (years)	<18	10	3.1
	18-39	83	25.9
	40-59	140	43.8
	≥60	87	27.2
Gender	Male	185	57.8
	Female	135	42.2
Sample collection location	OPD	106	33.1
	IPD	92	28.7
	ICU	52	16.2
	Emergency	49	15.3
	OT	21	6.6
	Anticoagulant monitoring	77	24.1
Clinical indication	Bleeding evaluation	55	17.2
	Pre-operative screening	62	19.4
	Liver disease/CLD	50	15.6
	Sepsis/DIC evaluation	42	13.1
	Thrombosis/PE evaluation	34	10.6

Table 2: Distribution of pre-analytical variables observed in received coagulation samples (N = 320)

		Number	**Percent (%)**
Tube fill adequacy	Adequate (≥90%)	290	90.6
	Mild underfill (80-89%)	30	9.4
Hemolysis	No	300	93.8
	Yes	20	6.2
Lipemia	No	304	95.0
	Yes	16	5.0
Icterus	No	296	92.5
	Yes	24	7.5
Visible clots/microclots on inspection	No	317	99.1
	Yes (microclots; sample reprocessed)	3	0.9
Transport delay (collection to receipt)	≤60 min	193	60.3
	61-120 min	84	26.2
	>120 min	43	13.4
Time to centrifugation (collection to centrifugation)	≤45 min	186	58.1
	46-90 min	104	32.5
	>90 min	30	9.4
Time to analysis (post-centrifugation to analysis)	≤2 h	188	58.8
	2-4 h	96	30.0
	>4 h	36	11.2
Storage/holding condition prior to analysis	Room temperature	247	77.2
	Refrigerated (2-8°C)	73	22.8

Repeat sampling due to pre-analytical issue in initial specimen	No	301	94.1
	Yes	19	5.9
Suspected heparin/line contamination noted on requisition or by laboratory review	No	311	97.2
	Yes	9	2.8

Table 3: Comparison of PT, aPTT, and INR by age (years), gender, sample collection location and clinical indication

	PT (s) Mean ± SD	aPTT (s) Mean ± SD	INR Median (IQR)
Overall	15.88 ± 1.91	36.41 ± 5.70	1.54 (1.23-1.95)
Age (years)			
<18	16.46 ± 1.70	35.66 ± 7.59	1.44 (1.13-1.51)
18-39	15.93 ± 1.76	36.28 ± 6.13	1.50 (1.20-2.00)
40-59	15.71 ± 1.81	36.39 ± 5.51	1.57 (1.25-1.97)
≥60	16.03 ± 2.20	36.66 ± 5.43	1.62 (1.27-1.85)
p-value	0.459	0.944	0.488
Gender			
Male	15.89 ± 1.94	36.87 ± 5.89	1.59 (1.25-1.97)
Female	15.86 ± 1.86	35.78 ± 5.39	1.48 (1.22-1.83)
p-value	0.891	0.091	0.177
Sample collection location			
OPD	15.42 ± 1.97	35.67 ± 5.53	1.46 (1.16-1.77)
IPD	15.63 ± 1.70	35.39 ± 5.02	1.49 (1.18-1.87)
ICU	16.53 ± 2.00	38.05 ± 5.60	1.74 (1.42-2.28)
Emergency	16.34 ± 1.90	37.57 ± 6.24	1.59 (1.40-2.04)
OT	16.61 ± 1.40	37.91 ± 7.03	1.84 (1.43-2.27)
p-value	<0.001*	0.013*	0.001*
Clinical indication			
Anticoagulant monitoring	17.27 ± 1.71	37.97 ± 5.31	1.87 (1.57-2.28)
Bleeding evaluation	15.35 ± 1.37	37.34 ± 4.97	1.37 (1.15-1.74)
Pre-operative screening	14.34 ± 1.59	34.68 ± 6.20	1.23 (1.03-1.44)
Liver disease/CLD	16.89 ± 1.50	34.61 ± 5.78	1.80 (1.54-2.25)
Sepsis/DIC evaluation	16.21 ± 1.35	38.26 ± 5.68	1.69 (1.36-2.02)
Thrombosis/PE evaluation	14.49 ± 1.36	34.93 ± 4.88	1.28 (1.05-1.54)
p-value	<0.001*	<0.001*	<0.001*
*Statistically significant at p<0.05			

Table 4: Association between pre-analytical variables and coagulation results

Pre-analytical variable		PT (s) (Mean ± SD)	p (PT)	aPTT (s) (Mean ± SD)	p (aPTT)	INR (Median [IQR])	p (INR)
Tube fill	Adequate	15.80 ±	0.029*	36.22 ±	0.085	1.50 (1.22-	<0.001*

	(≥90%)	1.91		5.62		1.87)	
	Mild underfill (80-89%)	16.58 ± 1.77		38.32 ± 6.22		2.03 (1.62-2.29)	
Hemolysis	No	15.87 ± 1.91	0.721	36.25 ± 5.63	0.078	1.53 (1.23-1.96)	0.747
	Yes	16.03 ± 1.92		38.90 ± 6.25		1.56 (1.16-1.79)	
Lipemia	No	15.79 ± 1.87	0.002*	36.40 ± 5.68	0.812	1.52 (1.23-1.95)	0.382
	Yes	17.49 ± 1.83		36.79 ± 6.34		1.63 (1.44-1.87)	
Icterus	No	15.89 ± 1.90	0.698	36.55 ± 5.71	0.121	1.55 (1.23-1.92)	0.961
	Yes	15.72 ± 2.07		34.70 ± 5.45		1.46 (1.24-1.99)	
Microclots	No	15.86 ± 1.90	0.210	36.38 ± 5.70	0.316	1.53 (1.23-1.95)	0.502
	Yes	17.6		40.4		1.67	

		8 ± 1.74		3 ± 5.30		(1.54-1.91)	
Transport delay	≤60 min	15.74 ± 1.82	0.192	35.76 ± 5.58	0.028*	1.50 (1.19-1.92)	0.231
	61-120 min	15.98 ± 1.98		37.12 ± 5.96		1.56 (1.35-1.91)	
	>120 min	16.29 ± 2.09		37.98 ± 5.38		1.59 (1.30-2.15)	
Time to centrifugation	≤45 min	15.59 ± 1.87	0.007*	36.12 ± 5.47	0.482	1.49 (1.21-1.89)	0.291
	46-90 min	16.23 ± 1.92		36.70 ± 6.04		1.57 (1.23-1.96)	
	>90 min	16.40 ± 1.81		37.28 ± 5.93		1.62 (1.43-2.03)	
Time to analysis	≤2 h	15.66 ± 1.81	0.042*	36.18 ± 5.62	0.251	1.55 (1.20-1.97)	0.952
	2-4 h	16.10 ± 2.00		36.31 ± 6.15		1.53 (1.33-1.87)	

	>4 h	16.39 ± 2.03		37.90 ± 4.72		1.50 (1.22-2.01)	
Holding condition	Room temperature	15.84 ± 1.96	0.502	36.54 ± 5.59	0.495	1.52 (1.22-1.95)	0.413
	Refrigerated (2-8°C)	16.00 ± 1.72		36.00 ± 6.07		1.58 (1.36-1.90)	
Repeat sampling required	No	15.87 ± 1.86	0.852	36.40 ± 5.72	0.889	1.52 (1.23-1.92)	0.061
	Yes	15.99 ± 2.62		36.59 ± 5.59		1.81 (1.51-2.07)	
Suspected heparin contamination	No	15.88 ± 1.92	0.649	36.18 ± 5.53	0.002*	1.54 (1.23-1.93)	0.454
	Yes	15.66 ± 1.38		44.59 ± 5.81		1.36 (1.04-1.96)	
*Statistically significant at p<0.05							

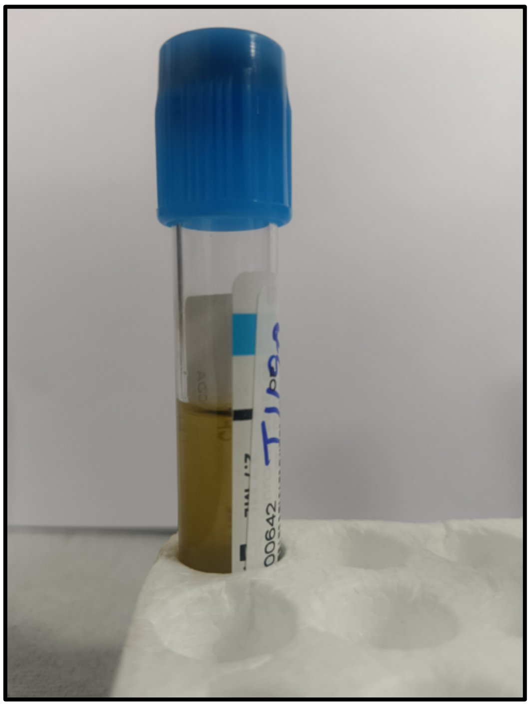

Figure 1: Citrate vacutainer without any visible pre-analytical variable. (Adequate sample)

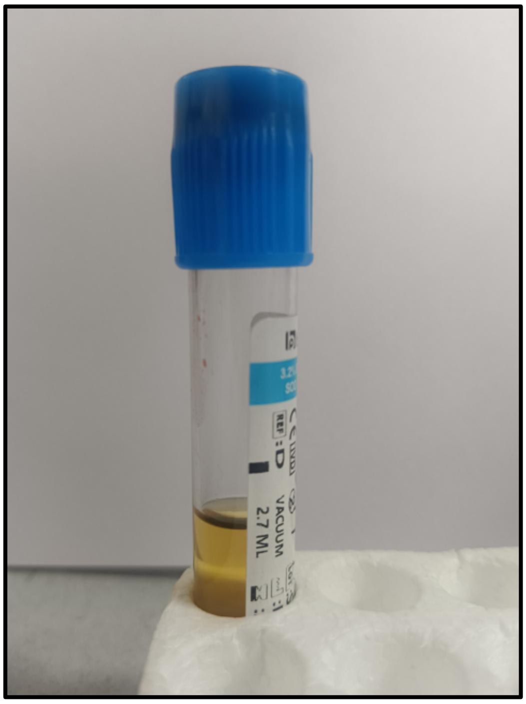

Figure 2: Underfilled citrate vacutainer

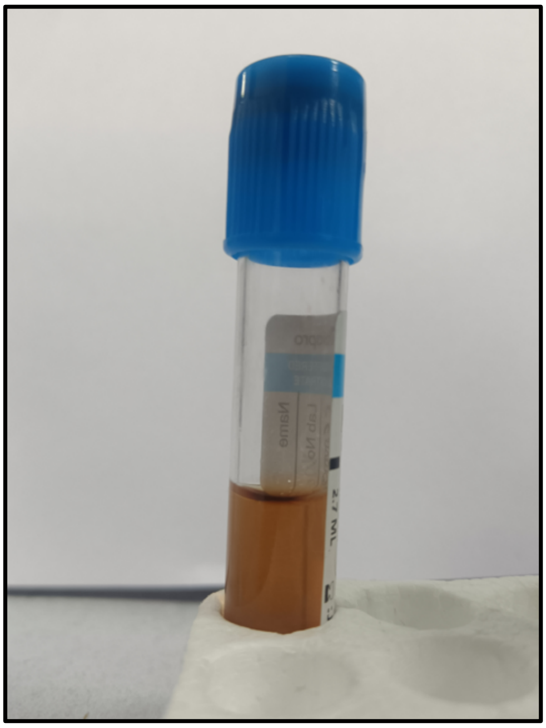

Figure 3: Citrate vacutainer with haemolysed sample

Discussion

The present cohort (N=320) represents a typical routine coagulation screening and monitoring, with a middle-aged predominance (mean age 49.12 ± 16.94 years; 43.8% aged 40–59 years) and a modest male preponderance (57.8%)(Table 1). The distribution of request settings (OPD 33.1%, IPD 28.7%, ICU 16.2%, Emergency 15.3%, OT 6.6%) and indications (anticoagulant monitoring 24.1%, pre-operative screening 19.4%, bleeding evaluation 17.2%, liver disease/CLD 15.6%, sepsis/DIC evaluation 13.1%) is clinically important because these contexts determine both the "true" biological signal (e.g., warfarin effect, cirrhosis-related factor deficiency, sepsis-associated coagulopathy) and the probability of pre-analytical stressors (line draws, transport delays, suboptimal fill) that can distort clot-based endpoints; as noted by Bozic et al. (2026), Mahto et al. (2024), Sonuga et al. (2016) and Tsantes et al. (2023).(9-12) PT and INR are the standardized metrics for monitoring vitamin K antagonists,(13) with INR specifically designed to harmonize PT across different thromboplastins, so the higher PT/INR observed in the anticoagulant-monitoring subgroup (PT 17.27 ± 1.71 s, INR 1.87 [1.57–2.28]) is directionally expected and reinforces that the indication mix meaningfully influences aggregate coagulation results. A notable quality-strength in this dataset is the generally acceptable specimen integrity profile, with 90.6% of tubes meeting the recommended citrate fill threshold (≥90% fill), low rates of gross interferences (hemolysis 6.2%(Figure 3), lipemia 5.0%, icterus 7.5%), and rare visible microclots (0.9%). This is relevant because ICSH guidance for citrated coagulation testing emphasizes maintenance of the 9:1 blood-to-anticoagulant ratio, typically operationalized as a minimum 90% tube fill; deviations alter the citrate-to-plasma balance and can artifactually prolong clotting times.(6, 14) In addition, nearly two-thirds of samples reached the laboratory within ≤60 minutes (60.3%), and most were centrifuged within ≤45 minutes (58.1%) and analyzed within ≤2 hours (58.8%)(Table 2; Table3), broadly aligning with Gosselin & Marlar (2019) that stresses timely transport, appropriate processing to platelet-poor plasma, and controlled pre-examination conditions to preserve analyte stability for plasma-based coagulation assays.(5)

Despite these overall acceptable process metrics, the analyses demonstrate several pre-analytical variables with statistically and clinically plausible associations with PT/aPTT/INR. The clearest signal was the effect of mild tube underfilling (80–89%)(Figure 2), which was associated with higher PT (16.58 ± 1.77 s vs 15.80 ± 1.91 s, p=0.029) and a markedly higher INR (2.03 [1.62–2.29] vs 1.50 [1.22–1.87], p<0.001). Underfilling increases the relative citrate concentration in the specimen; when calcium is reintroduced during testing, excess citrate chelates a larger proportion of calcium, delaying thrombin generation and fibrin formation and thereby prolonging clot-based endpoints—often more apparent in PT/INR, depending on reagent composition, the magnitude of underfill, and the patient's baseline factor reserves, as noted by Adcock et al. (2012).(15) The prominent INR shift in the mildly underfilled category is clinically meaningful because INR is frequently used to titrate anticoagulant dosing and to risk-stratify bleeding; therefore, even modest pre-analytical distortion can trigger unnecessary dose changes, repeat testing, or procedural delays; in corroboration with Favaloro et al. (2012) and Magnette et al. (2016).(1, 16)

The significant association between lipemia and PT prolongation (PT 17.49 ± 1.83 s vs 15.79 ± 1.87 s, p=0.002) is also biologically plausible, although its magnitude and direction can depend on platform-specific optical clot detection and the degree/type of turbidity. Gardiner et al. (2020) and Nikolac (2014) added that lipemia is a recognized pre-examination interference for photo-optical coagulation methods because turbidity alters light transmission/scattering, potentially shifting endpoint determination and yielding erroneous clotting times;(17, 18) its effect is often assay- and analyzer-dependent, which aligns with the present finding of selective PT impact without a consistent INR/aPTT change. This highlights the operational value of documenting visible lipemia and alternative detection strategies (e.g., mechanical clot detection) when results are discordant with the clinical picture.(1)

Time-related handling variables showed differential sensitivity across assays: transport delay significantly affected aPTT (≤60 min 35.76 ± 5.58 s, 61–120 min 37.12 ± 5.96 s, >120 min 37.98 ± 5.38 s, p=0.028), while PT/INR were comparatively stable across transport-delay strata (Table 4). This pattern is consistent with known assay biology: Do et al. (2022) noted that aPTT is more sensitive to pre-analytical degradation of labile intrinsic-pathway factors—particularly factor VIII—and to contact activation phenomena, whereas PT/INR (driven heavily by factor VII and common-pathway factors) is often more stable over short-term delays under appropriate conditions, as noted by Thiruvenkatarajan et al. (2014).(19, 20) In Feng et al. (2014) and Magnette et al. (2016) examining time/temperature effects, aPTT tends to drift earlier or more variably than PT/INR when samples are held or processed late, supporting the present finding that prolonged collection-to-receipt intervals preferentially impact aPTT in routine workflows.(16, 21) In contrast, time to centrifugation (p=0.007) and time to analysis (p=0.042) showed significant associations with PT, with progressively higher PT at longer delays (≤45 min centrifugation 15.59 ± 1.87 s vs >90 min 16.40 ± 1.81 s; ≤2 h analysis 15.66 ± 1.81 s vs >4 h 16.39 ± 2.03 s). These relationships may reflect a combination of factor consumption/activation in partially processed specimens, variable temperature exposure, and the practical reality that longer pre-analytical timelines cluster in higher-acuity settings (ICU/Emergency) where underlying coagulopathy is more common.(16) ICSH guidance emphasizes rapid generation of platelet-poor plasma because residual platelets and ongoing cellular metabolism can modify plasma composition and influence clot-based assays; thus, the observed PT trend across processing-time strata reinforces the importance of standardized timestamp capture and adherence to defined processing windows.(6)

The strongest single-variable "signal" for aPTT distortion was suspected heparin/line contamination, with aPTT 44.59 ± 5.81 s versus 36.18 ± 5.53 s (p=0.002), without a parallel rise in PT/INR. This is a classic pre-analytical phenomenon: samples drawn from heparinized vascular access devices can contain residual unfractionated heparin if discard volumes are inadequate or if line flushing is imperfect, and aPTT is particularly sensitive to heparin's inhibition of thrombin and factor Xa, producing spurious prolongation; as noted by Santoro et al. (2023).(22) Although many PT reagents include heparin neutralizers, heparin contamination can still disproportionately affect aPTT and downstream interpretations (e.g., "intrinsic pathway defect," lupus anticoagulant suspicion, unnecessary factor assays), making rigorous line-draw protocols and clear requisition documentation essential.(1) Patient subgroup comparisons provide additional interpretive context. The absence of significant differences in PT/aPTT/INR by age or sex suggests that, in this mixed real-world testing population, demographic variables were less influential than illness severity, indication mix, and pre-analytical handling. Conversely, the significantly higher PT/aPTT/INR in ICU and OT samples (e.g., ICU INR 1.74 [1.42–2.28], OT INR 1.84 [1.43–2.27]) likely reflects both higher baseline disease burden (sepsis-associated coagulopathy/DIC, liver dysfunction, anticoagulant exposure) and a greater probability of complex collection conditions (line draws, transport bottlenecks). Sepsis and DIC are well-recognized causes of prolongation of PT/INR and often aPTT due to consumption of clotting factors and dysregulated thrombin generation, and this biology fits the higher coagulation times seen in acute-care settings and in the "sepsis/DIC evaluation" indication group (PT 16.21 ± 1.35 s, aPTT 38.26 ± 5.68 s, INR 1.69 [1.36–2.02]).(23, 24) Similarly, liver disease/CLD was associated with higher PT/INR (PT 16.89 ± 1.50 s, INR 1.80 [1.54–2.25]), consistent with impaired hepatic synthesis of vitamin K–dependent and other clotting factors, where PT is often among the earliest routine assays to prolong.(25) In contrast, pre-operative screening showed comparatively lower PT/INR (PT 14.34 ± 1.59 s, INR 1.23 [1.03–1.44]), aligning with O'Connor et al. (2009) and Vries et al. (2018) that routine coagulation screening in unselected patients often yields normal results and has limited predictive value for bleeding without supportive clinical history.(26, 27)

This study has certain limitations. First, as a single-centre, hospital-based cross-sectional analysis, the results reflect local workflows, staffing patterns, transport logistics, and analyzer/reagent characteristics, which may limit generalisability to other laboratories with different pre-analytical systems and coagulation platforms. Second, the study relied on routine requisition information and observational documentation of pre-analytical factors; therefore, variables may have been subject to misclassification or under-reporting. Third, although subgroup comparisons were performed, the analysis did not fully adjust for clinical confounding, as patient severity and underlying diagnoses (e.g., liver dysfunction, sepsis-related coagulopathy, anticoagulant dose/timing, vitamin K status) can independently influence PT/aPTT/INR and may cluster with locations such as ICU/Emergency. Fourth, some pre-analytical events were infrequent (e.g., microclots, suspected heparin contamination), which reduces statistical power and may have obscured smaller but clinically relevant effects. Finally, because the study evaluated routine samples that yielded reportable results, severely compromised specimens that were rejected outright may be under-represented, potentially underestimating the overall burden and impact of pre-analytical errors in real-world practice.

Conclusion

In this single-centre laboratory-based study of 320 coagulation requests, pre-analytical variables were common and demonstrably influenced routine clot-based results, particularly PT/INR and aPTT. While PT, aPTT, and INR did not differ significantly by age or gender, values were consistently higher in acute care settings and in clinically high-risk indications such as anticoagulant monitoring, liver disease/CLD, and sepsis/DIC evaluation, underscoring the combined impact of patient acuity and testing context. Importantly, specific modifiable pre-analytical factors showed significant associations: mild citrate tube underfilling was linked to higher PT and markedly higher INR, lipemia was associated with PT prolongation, transport delay (>60 min) was associated with progressive aPTT prolongation, delays in centrifugation and analysis were associated with higher PT, and suspected heparin/line contamination produced pronounced aPTT prolongation. Overall, these findings highlight that systematic monitoring of specimen quality (especially tube fill), strict adherence to defined timelines for transport/processing, standardized plasma preparation, and robust protocols for line draws are essential to minimize spurious coagulation abnormalities, avoid unnecessary repeat sampling or inappropriate clinical decisions, and improve the reliability of PT, aPTT, and INR reporting in routine hospital practice.

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