

*Full Length Research Paper*

# **Effect of haemolysis, icterus and lipemia on prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen measured using Sysmex CA50 Hemostasis Analyzer**

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Plasma prothrombin time (PPT) and activated partial thromboplastin time (aPTT) are coagulation tests routinely performed in laboratories to evaluate the function of the coagulation system. Generally, samples with haemolysis, icterus and lipaemia are rejected in different laboratories which delays medical care and cause discomfort to patients. This was a cross sectional study conducted to determine the effect of haemolysis, icterus and lipaemia on PT, aPTT and fibrinogen as measured by Sysmex CA-50. Included samples were those that were showing one or more of the following characteristics: hemolysis, icterus, or lipemia. A purposive sampling technique was used to select the study participants. Venous blood were collected from the participants using Tri-Sodium Citrate anticoagulated tubes, centrifuged and visually inspected for hemolysis, icterus and lipemia. aPTT, PT and fibrinogen of the selected specimen were determined by Sysmex CA-50 at La Croix du Sud Hospital. An independent *t*-test was used for comparison of quantitative measurements between the two groups (normal and icteric, normal and hemolyzed, normal and lipemic), and the level of statistical significance was set as 0.05 (5%) in all tests. This study reports that while PT and fibrinogen are not statistically ( $P > 0.05$ ) significantly affected by hemolysis, icterus, and lipemia among the participants; aPTT was affected by hemolysis (results of non-hemolyzed decreased from  $32.5 \pm 1.89$  to  $31.5 \pm 2.87$  for hemolyzed sample,  $P$  value=0.002) but it remains almost constant for both icteric and lipemic samples. After a careful visual inspection of blood specimen for coagulation tests, only aPPT test should cause rejection patient's samples with hemolysis while icteric and lipemic should not be rejected at all when aPTT, PT, and fibrinogen tests are to be performed. There is a need for further study to be done on effects of icterus, hemolysis, and lipemia on other clotting factors. There is a need for further studies to be done on effects of icterus, hemolysis, and lipemia on other clotting factors.

**Key words:** Hemolysis, icterus, lipemia, partial thromboplastin (PT), activated partial thromboplastin time (aPTT), fibrinogen.

## **INTRODUCTION**

Laboratory diagnosis is more and more prominent in modern medicine; it is commonly accepted that

approximately 70% of all medical decisions are based on the laboratory results (Arora, 2014). Accurate results

are therefore keys for appropriate diagnosis. Limiting errors is hence one of the major objectives of clinical laboratories. Sources of errors can occur at any steps of a test, being pre-analytical, analytical and postanalytical. The importance of pre-analytics has been emphasized in past years with between one-third and three quarters of laboratory errors being attributable to this phase (Bonini, 2002). Haemolysis, icterus and lipaemia (HIL) in patients' specimen may interfere in the measurement of many analytes, including coagulation parameters. *In vitro* haemolysis which occurs during sample collection transport or processing is a particular problem as it is the most common source of interference in plasma samples (Lippi, 2013).

This possible interference can be influenced by several factors such as (i) the level of interfering substance in plasma, (ii) the assay principle and (iii) the end-point detection system, that is, optical versus viscosity-based detection system (mechanical detection). One of the requirements for a clinical laboratory is that common interferences related to sample integrity such as haemolysis, icterus and lipaemia be evaluated with each reagent system. Because of limited resources and budgetary constraints, the clinical laboratory relies on the manufacturer to document hemolysis icterus and lipemia estimates. The clinical relevance of the observed bias should be reviewed (CLSI, 2012). Plasma prothrombin time (PPT) and activated partial thromboplastin time (aPTT) are coagulation tests routinely performed in laboratories to evaluate the function of the coagulation system (Laffan, 2011). The PPT test measures the extrinsic pathway, while APTT measures the intrinsic pathway activities. Both coagulation function tests are affected by preanalytical factors such as the venipuncture process, the dose of citrate anticoagulant, sample transportation, processing, and storing (Laffan, 2011). Fibrinogen levels are useful as part of the investigation of a bleeding tendency or an unexplained prolongation of the aPTT or PT. Elevated levels may correlate with increased risk of thrombosis in epidemiological studies although the significance in individual patients is unclear.

Currently, La Croix du Sud Hospital uses Sysmex CA-50 which utilizes optical detection method to measure coagulation analytes and thus detects the change in turbidity of blood during the coagulation process as the change in scattered light intensity. Hemolysis, icteric or lipemic samples or samples that contain a high degree of coloured or particulate may generate erroneous results when using an optical end-point detection method (Tantanate, 2011). Hemolysis effect on coagulation tests are not only restricted to the change in the colour of

the plasma samples under analysis but also on the direct interference with hemostasis (Woolley et al., 2016). For instance, phospholipid membranes from haemolytic red cells may interfere with the coagulation reaction by providing a phospholipid-rich surface that accelerates coagulation reactions. Furthermore, platelet activation by haemolysed red cells can impact on coagulation tests results (Tantanate, 2011). Icteric, lipemic, and hemolyzed samples are being rejected at La Croix du Sud Hospital Laboratory, when they are intended to be used for coagulation tests, mostly aPTT, PT and fibrinogen. The rejection of inappropriate blood samples for tests has become a policy that is applied in most laboratories (Stela, 2017) including the Laboratory of La Croix Du Sud Hospital.

The consequence of blood sample rejection is to repeat the whole process of blood sample collection that causes discomfort to patients, delayed test results, and increased laboratory operating costs (Arora, 2014). In addition, studies on the effect of hemolysis on coagulation tests have been rare and the results are still controversial (Stela, 2017). The current study aimed at determining the effect of hemolysis icterus and lipemia on aPTT, PT and fibrinogen as performed by Sysmex CA-50, a hemostasis analyzer at La Croix Du Sud Hospital, Kigali, Rwanda. The main objective of this study was to determine the effect of haemolysis, icterus and lipaemia on PT, aPTT and fibrinogen test results using Sysmex CA-50, at La Croix du Sud Hospital, Kigali-Rwanda. The specific objectives were to determine the effect of haemolysis on PT, aPTT and fibrinogen test results, to determine the effect of icterus on PT, aPTT and fibrinogen test results, to determine the effect of lipemia on PT, aPTT and fibrinogen test results.

## MATERIALS AND METHODS

This study was conducted at la Croix du Sud Hospital located in Gasabo district, Kigali, Rwanda and it was a cross sectional study design. Target populations of this study include all people whose samples show one or more of the following characteristics: hemolysis, icterus, or lipemia and are sodium citrate anticoagulated samples (9:1 ratio) for coagulation analysis that was received at La Croix du Sud Hospital during the period of 1st of September, 2017 to 31st of March, 2018. Normal specimens were sodium citrate anticoagulated blood specimen (9:1 ratio) without apparent hemolysis, icterus or lipemia and they served as control in this study. On the other hand, abnormal specimens were sodium citrate anticoagulated blood specimen (9:1 ratio) with apparent hemolysis, icterus or lipemia and they served as the study cases in this study. Any history of abnormal coagulation tests results made the subject to be excluded in this study.

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**Table 1.** Characteristics of the study subjects.

Characteristics	Gender		Total (%)
	Females	Males	
Age (years)			
< 5	8	6	14 (21)
15-29	7	4	11 (23)
30-44	26	12	38 (18.1)
45-59	20	29	49 (19.6)
60+	3	5	8 (14.4)
Total (%)	64 (53%)	56 (46%)	120 (100)

The sample size for this study was calculated to be 120 study subjects using Dennis's formula (1979). Personal protective equipment, blood collection tube (with sodium citrate anticoagulant), needles and their holders and other blood collection equipment were used to collect blood specimen. Sysmex CA-50 machine was used to analyze PT, aPTT and fibrinogen of collected samples. Data collection sheets and pen were used to record required results for the study and computer was used to store and statistically analyze the collected data. Macroscopic examination was used to identify hemolyzed icteric and lipemic samples to be used for this study. Patients who attended La Croix du Sud Hospital during the data collection period were selected and consented to participate in this study. This study utilized two samples from each and every patient; the first sample to be considered was sample collected at the first visit (sample which was hemolysed, icteric, or lipemic) while the second was collected when patient comes back to give acceptable sample. The later sample was considered as control sample without hemolysis, icteric, or lipemia. Blood specimens for coagulation tests were collected by venipuncture in a sodium citrate anticoagulant containing tubes as recommended (CLSI, 2012).

The tubes were filled with the blood adequately so that the blood/anticoagulant ratio was maintained at 9:1 and gently mixed then labeled. The collected samples were immediately delivered in the laboratory and centrifuged for 15 min at 2000 rpm (round per minute). All blood samples were visually inspected for hemolysis (hemolysis from venipuncture), icterus and lipemia. APTT, PT and fibrinogen of the selected specimen were determined by Sysmex CA-50 at La Croix du Sud Hospital. All the collected data were filled on data collection form then entered into SPSS (SPSS version 21) for data analysis. Coagulation Reaction Detection Method (Scattered Light Detection Method) was done using Sysmex CA-50 which is based on the principle of irradiating red light (660 nm) onto a mixture of blood plasma and reagent and detects the change in turbidity (when the fibrin clots are formed) as the change in scattered and measures the coagulation time. Coagulation Point Detection Method (Percentage Detection Method) calculates the coagulation time as the time required to achieve the amount of scattered light that is set for the coagulation detection point, using the amount of scattered light that is present just after the start of detection as 0%, and the amount of scattered light that is present at the completion of coagulation as 100%.

Range of analysis is set to a concentration of fibrinogen concentration from 50 to 450 mg/dL. The quantitative variables were presented as mean  $\pm$  standard deviation. An independent *t*-test was used for comparison of quantitative measurements between the two groups (normal and icteric, normal and hemolyzed, normal and lipemic). The statistical analysis was performed by IBM SPSS version 21, a statistical software package. The level of statistical significance was set as 0.05 in

all tests. A written permission letter to conduct a study was requested from MKU administration, followed by an approval letter from La Croix du Sud Hospital research committee to collect data. To assure confidentiality, no patient name was used and results were kept confidential between researchers and hospital management.

## RESULTS AND DISCUSSION

In this part, the results from this study were presented interpreted according to demographic characteristics, actual laboratory findings from testing. Moreover, the results were compared based on non hemolysed against hemolysed, nonlipemic against lipemic, non icterus against non icterus using independent *t*-test.

### Demographic characteristics of the study subject

The age and sex of the study subjects are shown in Table 1. This study included a total of 120 study subjects including 64 (53%) females and 56 (46%) males. The age of the participants ranged between 2 and 68 years with the mean of  $46 \pm 12.8$  and most of the patients were aged between 45 and 59. The patient's diagnosis varied. However, diagnosis, age and gender do not affect the PPT and APTT value.

### The effect of hemolysis on PT, aPTT and fibrinogen test results

Mean PT, aPPT and fibrinogen of hemolyzed and normal samples are shown in Table 2. On one hand, there was no statistically significant difference between the means of normal ( $16.60 \pm 0.9$ ) and hemolyzed ( $16.61 \pm 1.03$ ) blood samples ( $P > 0.05$ ) for both PT and fibrinogen, while on the other hand, means of aPPT of normal samples ( $32.5 \pm 1.89$ ) and haemolyzed samples ( $31.5 \pm 2.87$ ) do have *P* value of 0.002 which is less than 0.05.

The current study reports similar results with the ones reported by wooley (Woolley et al., 2016; Hernaningsih and Akualing, 2017; Novelli et al., 2017) which demonstrated that spontaneous haemolysis during sample collection and processing have no effect

**Table 2.** Mean PT, aPTT and fibrinogen according to the hemolysis status.

Coagulation test	Normal	Hemolyzed	P value
PT(s)	16.60 ± 0.9	16.61 ± 1.03	0.834
aPTT(s)	32.5 ± 1.89	31.5 ± 2.87	0.002
Fibrinogen (g/L)	5.22 ± 2.81	5.38 ± 2.78	0.927

**Table 3.** Mean PT, aPTT and fibrinogen according to the icterus status.

Coagulation test	Normal	Icteric	P value
PT(s)	13.62 ± 0.09	13.54 ± 0.07	0.837
aPTT (s)	29.72 ± 0.21	30.38 ± 0.14	0.189
Fibrinogen (g/L)	4.34 ± 0.23	4.23 ± 0.19	0.897

**Table 4.** Mean PT, aPTT and fibrinogen according to the lipemia status

Coagulation test	Normal	Lipemic	P value
PT(s)	16.95 ± 0.24	16.49 ± 0.19	0.985
aPTT(s)	28.87 ± 0.16	28.75 ± 0.13	0.654
Fibrinogen(g/L)	4.71 ± 0.07	4.34 ± 0.06	0.136

on PT and fibrinogen on one hand and that APTTs results with a slight statistical significance in haemolysed samples compared with non haemolysed samples is sufficient to impact patient management decisions, on the other hand. Moreover, these studies recommended the rejection of haemolysed samples for APTT determination.

#### The effect of icterus on PT, aPTT and fibrinogen test results

Table 3, comparing means of PT, aPTT and fibrinogen of normal samples against samples with icterus, shows that there is no significant difference between the means of normal and icteric samples for PT results (13.62 ± 0.09 with  $P > 0.05$ ), aPTT (30.38 ± 0.14 with  $P > 0.05$ ), and for fibrinogen (4.34 ± 0.23 with  $P > 0.05$ ). This shows that icteric samples have no statistical significant effect on PT, aPTT and fibrinogen.

There were several other studies that were conducted to investigate the effect of icterus on PT, aPTT and fibrinogen. For instance, in the study conducted by Wooley (2016), it was reported that there was no statistical difference ( $P > 0.5$ ) between the PT, aPTT and fibrinogen of icteric and non-icteric samples. In that study, the author concluded that PT, aPTT and fibrinogen were not clinically significantly affected by icterus (Woolley et

al., 2016). Similar results were obtained by the studies of hernaningsih and Akualing (2017) and Laga (2006) where icterus had no significant effect on PT, aPTT, or fibrinogen. All of the results of these studies agree with the results of the present study and all concluded that icteric blood specimens should not be rejected when they are to be used for PT, aPTT, and fibrinogen testing. In contrast, there was a study that was conducted in India by Tiker et al. (2005) who reported that the higher the conjugated bilirubin level, the longer the aPTT.

#### The effect of lipemia on PT, aPTT and fibrinogen test results

The mean PT, aPTT and fibrinogen of lipemic and normal samples are shown in Table 4. Like icterus, these results show that lipemia have no effect on any of PT, aPTT and fibrinogen results. Means PT, aPTT, and fibrinogen show no statistical significant difference among non-lipemic and lipemic samples as shown by their P values of 0.985, 0.654, and 0.136, respectively which are greater than 0.05.

These results were similar with the results of the study conducted by Wooley (2016), to assess the influence of hemolysis, icterus and lipemia on PT, aPTT and fibrinogen assays using a viscosity-based detection analyzer. In that study, lipemia did not significantly impact the results of either PT, aPTT or fibrinogen (Woolley et

al., 2016). This result agreed with the findings of Laga (2006) and Arora (2014) which clarified that the difference in PPT values between lipemic and non lipemic samples from healthy volunteers was insignificant, even when there was a statistical difference found in patient-subjects, the absolute difference between samples is incredibly small and without clinically significant effect.

This study reports that only aPTT results are statistically affected by hemolysed samples and this can result in misleading the clinician to offer medical care, thus the hemolysed sample should be rejected for aPTT testing but not PT and fibrinogen. For the current study, P value is greater than 0.05 for both PT and fibrinogen tests results, meaning that these results are closer to the samples without hemolysis (with P value of 0.834 and 0.927, respectively). The mean PT of normal blood samples ( $13.62 \pm 0.09$ ) was not significantly different from the mean PT of icteric blood samples ( $13.54 \pm 0.07$ ). The mean aPTT of the icteric samples was  $30.38 \pm 0.14$  and those of normal samples were  $29.72 \pm 0.21$ . Insignificant difference ( $P > 0.05$ ) was again found between the means fibrinogen of normal ( $4.34 \pm 0.23$ ) and icteric ( $4.23 \pm 0.19$ ) blood samples. The mean PT of normal samples was  $16.95 \pm 0.24$  whereas those of lipemic samples were  $16.49 \pm 0.19$ , and there was no statistically significant difference between the two ( $P > 0.05$ ). There was insignificant difference between the mean aPTT of the normal ( $28.87 \pm 0.16$ ) and lipemic ( $28.75 \pm 0.13$ ) blood samples ( $P > 0.05$ ). There was also the difference which was not statistically significant between the mean fibrinogen of the normal ( $4.71 \pm 0.07$ ) and lipemic ( $4.34 \pm 0.06$ ) blood samples ( $P > 0.05$ ).

## Conclusions

The results of this study confirm that PT and fibrinogen do not have significant clinical effect due to hemolysis, icterus and lipemia. However, while aPTT results are not affected by icteric and lipemia ( $P > 0.05$ ), it is significantly shortened by hemolysis ( $P < 0.05$ ).

All blood samples received in medical laboratory should be adequately visually inspected for hemolysis, icterus and lipemia and accepting or rejecting them should be based on which tests are needed to be performed. Icteric and lipemic plasma samples for coagulation testing should not be rejected. Hemolysis should be the major rejection criteria of the blood samples for coagulation tests especially when aPTT or hemostatic intrinsic factors are requested. There is a need for further studies to be done on effects of icterus, hemolysis, and lipemia on other clotting factors.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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