Original Article

# Variables that Influence Platelet Function Analyzer-100™ Closure Times in Healthy Algerian Adults


## Abstract

**Context:** The Platelet Function Analyzer (PFA-100™) assesses primary hemostasis *in vitro* under high shear stress to simulate the conditions to which platelets are exposed at the site of an injured blood vessel wall. **Aims:** We investigated preanalytical variables in healthy Algerian adults, and we also assess the performance of the test. **Subjects and Methods:** Closure time (CT) was measured in 302 well-characterized healthy Algerian adults with the collagen/epinephrine (CEPI) and the collagen/adenosine diphosphate (CADP) cartridges. **Results:** Age and sex did not affect CT values. Blood group O was associated significantly with longer CEPI CT and CADP CT than non-O groups ($P \leq 0.0001$ for both). CTs were shorter in samples collected in the morning vs the afternoon ($P < 0.0001$ for both). We found a strong positive correlation between CT CEPI and CT CADP with $r = 0.72$ and $P < 0.001$ and an inverse mean correlation between von Willebrand factor level and CT with $r = -0.56$, $r = -0.45$ for CT CEPI and CT CADP, respectively $P < 0.001$. Duplicate analysis of PFA-100™ CT revealed a mean difference of 3.6% ±19.1% for the CEPI cartridge and 1.5% ±10% for the CADP cartridge. The mean coefficient of variation was 7.4% for the CADP CT and 7.6% for the CEPI CT. No marked difference between test positions. **Conclusions:** The PFA-100™ showed good reproducibility. The variables influencing the test in healthy Algerian adults are similar to Western and Asian populations. Standardization of preanalytical and analytical conditions is essential for obtaining reliable PFA-100™ results.


**Keywords:** *Healthy Algerian adults, platelet function analyzer-100™, platelet function test*

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## Introduction

Closure time (CT), measured by a platelet function analyzer device (PFA-100™), is now available to the clinical laboratory in place of the bleeding time test. The PFA-100™ assesses primary hemostasis *in vitro* under high shear stress by simulating the conditions to which platelets are exposed at the site of an injured blood vessel wall. We investigated preanalytical variables in healthy Algerian adults, and we also assess the performance of the test. The PFA-100™ uses two disposable cartridges that contain a membrane impregnated with platelet agonists, which simulated the vessel wall. A portion of citrated whole blood obtained by venipuncture is put into a test cartridge. It is aspirated into a capillary by a vacuum system that produces a shear force similar to that encountered in small vessels. The blood passes through an aperture in a membrane coated with collagen and adenosine diphosphate (CADP) or epinephrine (CEPI). These bioactive components are present in lyophilized form and are solubilized by the trigger solution (physiological water) automatically distributed at the beginning of the test. Mediated by the von Willebrand factor (vWF), platelets adhere to the collagen on the membrane, which then activates the platelets, causing them to aggregate in and around the aperture. The time needed to occlude the aperture by plug formation is the CT. The test is simple to perform, rapid (with maximal CT of 300 s), and can test relatively small volumes (0.8 ml/cartridge) of citrated blood up to 4 h from sampling. However, several variables affect the PFA-100™ test result. CT depends on citrate concentration, time of blood collection, plasma vWF level, ABO blood group, drugs, and some foods. Besides, a low platelet count or low hematocrit (Ht) may lead to prolongation of CT.

Recently, the PFA-100™ instrument has been updated to PFA-200™ with an

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additional cartridge: INNOVANCE PFA P2Y cartridge. The membrane is coated with adenosine diphosphate, Prostaglandin E 1, and Calcium chloride. The INNOVANCE® PFA P2Y cartridge detects congenital defects of the platelet P2Y12 receptor and platelet P2Y12-receptor blockades in patients undergoing therapy with P2Y12-receptor inhibitors. Most studies that use the PFA-100™ system have been performed in Western and Asian countries. So far, there is a lack of data on the use of PFA-100™ in the African population. Ethnic, genetic, dietary, and environmental factors influence the pathophysiology of hemostasis and thrombosis. In this study, we investigate in healthy adults from the West of Algeria the relationship between PFA-100™ CT and the following variables: Sex, age, ABO Rh blood group, the time of blood collection, the effect of cigarette smoking, hematological, and hemostatic parameters. We also assess the performance of the PFA-100™ test.

## Subjects and Methods

### Selection of healthy subjects and study design

We performed this prospective study at the blood transfusion department of our city. We invited regular blood donors to take part and to give their consent. The criteria for inclusion and noninclusion were those of blood donors as recommended by National guidelines of blood donor selection. We included in our study any Algerian adult male and female able to donate after the medical consultation. The exclusion criteria were as follows: Positive HIV serology, positive HCV serology, positive Hbs Ag serology, positive syphilis serology, taking aspirin or a nonsteroidal anti-inflammatory drug, antibiotic or other medications to affect platelet function within 10 days before collection, platelet count below 100 G/L or a Ht of <0.30. The local institutional ethics committee approved the study, and all the participants gave informed consent before enrolment. We recruited 303 healthy adult subjects (≥18-year-old) by random sampling method of 606 regular blood donors. We excluded one donor from this study because of an HCV-positive serology.

### Specimen collection and processing

Blood samples were collected from each subject's antecubital vein using the B. D. Vacutainer system. The samples were obtained in the morning (between 8:30 and 11:30 AM) and in the afternoon (between 2:00 and 5:00 PM). Whole blood was collected into EDTA collection tubes for complete blood counts (CBCs) determination. Blood for vWF Antigen (vWF: Ag) determination and routine hemostasis parameters was drawn into 0.129 M (3.8%) sodium citrate tube. Sample for PFA-100™ testing was also drawn into 0.129 M (3.8%) sodium citrate in another collection tube and stored at the room temperature before testing. The plasma was obtained by centrifugation at 3500 g for 10 min. Aliquots of 500 µl were snap-frozen and stored at −80°C. When required for further analysis vWF: Ag, samples were thawed at 37°C for 10 min. Hemolyzed or clotted samples were not used.

### Assay

#### *Closure time platelet function analyzer 100™*

CEPI CT and CADP-CT were determined between 30 min and 1 h after blood collection, using one single lot each of CEPI and CADP cartridges and a PFA100™ instrument from Siemens Healthcare Diagnostics (Marburg, Germany). Besides the single measurements, duplicate measurements of the CEPI and CADP were performed to assess the performance of the PFA-100™. For an optimal statistical interpretation of repeatability, the minimum number recommended is 30.

#### *CBC*

The CBC was performed on the Sysmex XT-2000i Automated Hematology Analyzer From Sysmex Corporation, Kobe, Japan.

#### *Routine hemostatic testing*

Was performed on the automated system STA Compact Stago from Diagnostica Stago, Asnieressur Seine, France.

#### *von Willebrand factor antigen*

von Willebrand factor antigen was determined by the immunological method (LiatestStago) on the automated system STA Compact StagoFrom DiagnosticaStago, Asnieressur Seine, France.

### Statistical analysis

We used the D'Agostino-Pearson omnibus test for normality to analyze the distribution of the values. As not all values were normally distributed, we gave data as either medians or ranges (minimum-maximum) or as mean ± standard deviation (S. D.). We compared unpaired data using the Mann–Whitney *U*-test. The Chi-square test checked the differences in the proportions of smokers, individuals with blood group O. We calculated Spearman rank correlation coefficients using the data from all subjects, and we verified it by multiple linear regression analysis. A correlation matrix was constructed to detect any multicollinearity between parameters. We calculated the percentages of differences between duplicate measurements of CEPI CT and CADP CT: ([CT1 − CT2]/average) ×100. We used to characterize imprecision the mean ± S. D. To check whether variability was linked to the magnitude of measures, we adopted Bland–Altman plots.

Data analyses were performed using the Statistical Package for the Social Sciences software SPSS version 25 (IBM SPSS Armonk NY, USA) and MedCalc Statistical Software version 15.0 (MedCalc Software, Ostend, Belgium). $P < 0.05$ was considered statistically significant.

## Results

Baseline data for all individuals, both men, and women, are shown in Table 1. We recruited 302 participants among them, 88% are males. Seventy-nine percent of the samples were collected in the morning. Females and males did not differ in age or in the proportion of blood Group O and collection time. Regarding smoking behavior, the proportion of female smokers was significantly deficient as compared to that of males.

According to the ABO Rh blood group, the distribution of participants was as follows: 44% are O, 33% are A, 18% are B, 5% are AB, 85.8% Rh positive, and 14.2% Rh negative. Knowing that the frequency of phenotype O, A, B, AB in the population of Western Algeria is, respectively, 44.04%, 39.28%, 12.84%, and 3.84%, there was no significant difference between our sample and the population from West Algeria. The median level of vWF Ag in the O group is 93% versus 100% in the non-O group, with a significant difference $P = 0.004$.

The CEPI and CADP CTs determined according to the various qualitative variables studied are shown in Table 2.

Calculation of the Spearman correlation coefficient confirmed by multiple linear regression analysis revealed a strong positive correlation between CEPI CT and CADP CT with $r = 0.72$ and $P < 0.001$ and an inverse mean correlation between vWF and CT. with $r = -0.56$, $r = -0.45$ for CEPI CT and CADP CT, respectively, $P < 0.001$. However, there was no correlation between C. T.(CEPI CT and CADP CT) and flowing parameters: White blood cell, red blood cell (RBC), Ht, hemoglobin (Hb), platelet count, platelet mean volume (PMV), fibrinogen level, prothrombin time (PT), and activated partial thromboplastin time (aPPT) [Table 3].

Duplicate analysis of PFA-100™ CT demonstrated a mean difference of 3.6% ±19.1% for the CEPI cartridge and 1.5% ±10% for the CADP cartridge. The mean coefficient of variation was 7.4% for the CADP CT and 7.6% for the CEPI CT. However, 9 of 30 CEPI CT differences exceeded 10% and 4 of 30 exceeded 15%. The corresponding data for CADP-CT were 9 of 30 higher than 10% and 5 of 30 higher than 15%. Bland–Altman plots revealed similar imprecision of duplicate measurements over the full measuring range [Figure 1]. CEPI-CT and CADP CT determined on channels A and B of the PFA-100™ equipment were not significantly different, with $P = 0.054$ and $P = 0.36$ [Figure 2].

## Discussion

We confirmed the previous finding that platelet CT in healthy subjects is not influenced by sex.[1] Furthermore, it is not correlated with age.[2] On the order hand, another study showed shorter CEPI-and CADP-CT in participants over 40 years compared with younger ones.[3] It has also been reported that older men appeared to have shorter CTs than younger men.[4] The increase vWF level in participants over 55-year-old may explain the shorter CT. We did not find a correlation between CT and age because 90% of the participants were younger than 47 years of age.

Our study showed the lack of correlation of PFA 100™ CT with leukocyte count, RBC count, Ht, Hb, platelet count, PT, aPPT, and fibrinogen level in healthy adult subjects. It has been published that some studies failed to observe, or only variably observed, a correlation between

**Table 1: Baseline data of 302 healthy individuals**

| | All | Male | Female | *P* (male versus female) |
|---|---|---|---|---|
| Age (years) | 32 (19-61) | 32 (19-61) | 31 (19-61) | NS† |
| Male/female ratio | 3.50 | 235 | 67 | |
| Smokers/nonsmokers | 77/225 | 75/160 | 2/65 | <0.0001 |
| Blood group O/non O | 133/169 | 101/134 | 32/35 | NS |
| RH positive/RH negative | 259/43 | 201/34 | 58/9 | NS |
| Time of blood collection AM/PM | 240/62 | 185/50 | 55/12 | NS |
| WBC (G/L), mean±SD | 6.78 (1.62) | 6.79 (1.63) | 6.71 (1.57) | NS |
| RBC (T/L), mean±SD | 4.79 (0.48) | 4.93 (0.41) | 4.32 (0.39) | <0.0001* |
| Hb (g/dl) | 14.5 (10.6-17.5) | 14.8 (11.0-17.5) | 10.60 (12.8-16.0) | <0.0001† |
| Ht | 42.8 (31.8-51.1) | 43.8 (31.8-51.1) | 37.5 (33.1-46.2) | <0.0001† |
| PLQ (G/L) | 209 (127-453) | 208 (127-453) | 146 (219-365) | 0.0294† |
| MPV fl, mean±SD | 10.44 (1.07) | 10.38 (1.04) | 10.68 (1.14) | NS* |
| PT (%) | 100 (70-100) | 100 (70-100) | 100 (70-100) | NS† |
| aPTT (s), mean±SD | 30.69 (4.41) | 31.07 (4.37) | 30.59 (4.55) | NS* |
| Fg (g/l), mean±SD | 2.98 (0.63) | 2.98 (0.63) | 2.98 (0.66) | NS* |
| vWF Ag (%)† | 97.5 (52-170) | 96 (52-153) | 98.75 (54-170) | NS† |

*Student test; †Mann-Whitney U test. Data are expressed as median value (minimum-maximum) and arithmetic mean±SD; Chi-square test. NS: Nonsignificant difference; SD: Standard deviation; WBC: White blood cell; RBC: Red blood cell; Ht: Hematocrit; Hb: Haemoglobin; PT: Prothrombin time; aPPT: Activated partial thromboplastin time; RH: Rhesus; AM: Antimeridian; PM: Postmeridian; PLQ; Platelet count, MPV: Mean Platelet volume, Fg: Fibrinogen level, vWFAg: von Willebrant factor Antigen

**Table 2: Comparison of platelet function analyzer-100™ closure times between different variables**

| Variables | CEPI CT Q1, M, Q3 (minimum-maximum) | CADP CT Q1, M, Q3 (minimum-maximum) |
|---|---|---|
| Sex | | |
| Male (*n*=235) | 122, 147, 174 (80-250) | 90, 102, 120 (63-150) |
| Female (*n*=67) | 122, 142, 179 (65-228) | 90, 108, 120 (67-152) |
| *P* | NS | NS |
| ABO blood group | | |
| O (*n*=133) | 129, 157, 180 (65-245) | 93, 108, 126 (67-152) |
| Non O (*n*=169) | 117, 139, 163 (80-250) | 80, 100, 113 (63-150) |
| *P* | 0.001 | >0.001 |
| RH blood group | | |
| RH positive (*n*=259) | 122, 144, 173 (80-245) | 90, 103, 118 (63-152) |
| RH negative (*n*=43) | 125, 159, 180 (65-250) | 90, 106, 126 (67-152) |
| *P* | NS | NS |
| Cigarette smoking | | |
| Smokers (*n*=77) | 116, 142, 172 (80-224) | 88, 100, 113 (63-150) |
| Nonsmokers (*n*=225) | 122, 146, 176 (65-250) | 90, 106, 122 (67-152) |
| *P* | NS | 0.038 |
| Time of blood collection | | |
| AM (*n*=240) | 119, 142, 171 (65-245) | 89, 102, 114 (64-152) |
| PM (*n*=62) | 131, 169, 190 (80-250) | 93, 115, 128 (63-148) |
| *P* | 0.001 | 0.006 |

Q1: 1st Quartile; Q2: 2nd Quartile; M: Median and range (maximum value-minimum value). RH: Rhesus; CEPI: Collagen/epinephrine; CT: Closure time; CADP: Collagen-adenosine diphosphate; AM: Antimeridian; PM: Postmeridian

**Table 3: Correlation between closure time and the different variables**

| | CEPI CT | CADP CT | vWF Ag | PT | aPTT | Fg | WBC | RBC | Hb | Ht | PLT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CEPI CT | | | | | | | | | | | |
| CADP CT | *r*=0.72 *P*<0.0001 | | | | | | | | | | |
| vWF Ag | *r*=−0.56* *P*<0.0001 | *r*=−0.46* *P*<0.0001 | | | | | | | | | |
| PT | NS* | NS* | NS | | | | | | | | |
| aPTT | NS* | NS* | NS | NS | | | | | | | |
| Fg | NS | NS | NS | NS | NS | | | | | | |
| WBC | NS | NS | NS | NS | NS | NS | | | | | |
| RBC | NS | NS | NS | NS | NS | NS | NS | | | | |
| Hb | NS | NS | NS | NS | NS | NS | NS | NS | | | |
| Ht | NS | NS | NS | NS | NS | NS | NS | NS | NS | | |
| PLT | NS | NS* | NS | NS | NS | NS | NS | NS | NS | NS | |
| MPV | NS* | NS* | NS | NS | NS | NS | NS | NS | NS | NS | NS |

*Confirmed by multiple regression analysis. Simple linear regression (spearman rank correlation coefficients, level of significance *P*<0.05). NS: Nonsignificant; CEPI: Collagen/epinephrine; CT: Closure time; CADP: Collagen-adenosine diphosphate; WBC: White blood cell; RBC: Red blood cell; Ht: Hematocrit; Hb: Hemoglobin; PT: Prothrombin time; aPPT: Activated partial thromboplastin time; MPV: Mean Platelet volume, Fg: Fibrinogen level, vWFAg: von Willebrant factor Antigen, PLQ; Platelet count

the PFA-100™ CT and hematocrit, leukocyte count, or platelet count.[5,6] These studies were mostly limited to healthy individuals. All these parameters may affect the PFA-100 CT, mainly when levels fall below the upper limit value. Thus, low hematocrits, low platelet counts, and low leukocyte counts can lead to prolonged PFA-100™ CT, and we observe correlation when we combined these data with the normal data set.[7,8] CT increases with decreases in hematocrit and conversely. Besides, CT increases as the platelet count decreases below 100 × 109/L.[9] PFA-100™ CT is usually prolonged when the platelet count is <50,000/µl or Ht is <25%.

In contrast, PFA-100™ CT appears mostly insensitive to the deficiency of coagulation factors (e.g., fibrinogen, factor V, factor VIII, and factor IX) because of insignificant thrombin generation and fibrin formation during the relatively short-time to platelet plug formation under the high shear conditions in the aperture of the cartridge

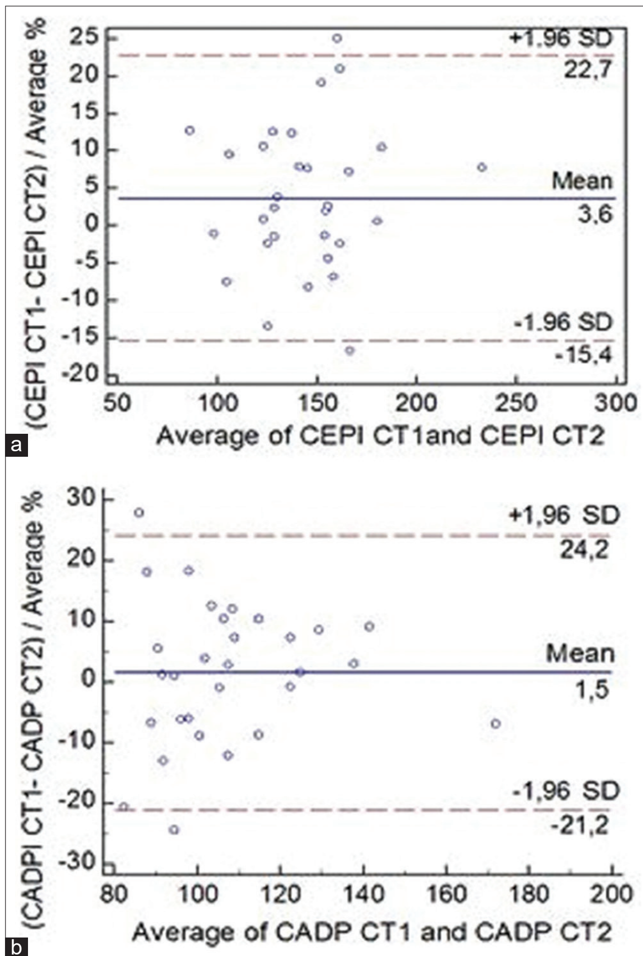


**Figure 1: Bland–Altman plots of duplicate measurements of collagen/epinephrine and collagen/adenosine diphosphate platelet function analyzer‑100™ closure times, respectively, a and b**

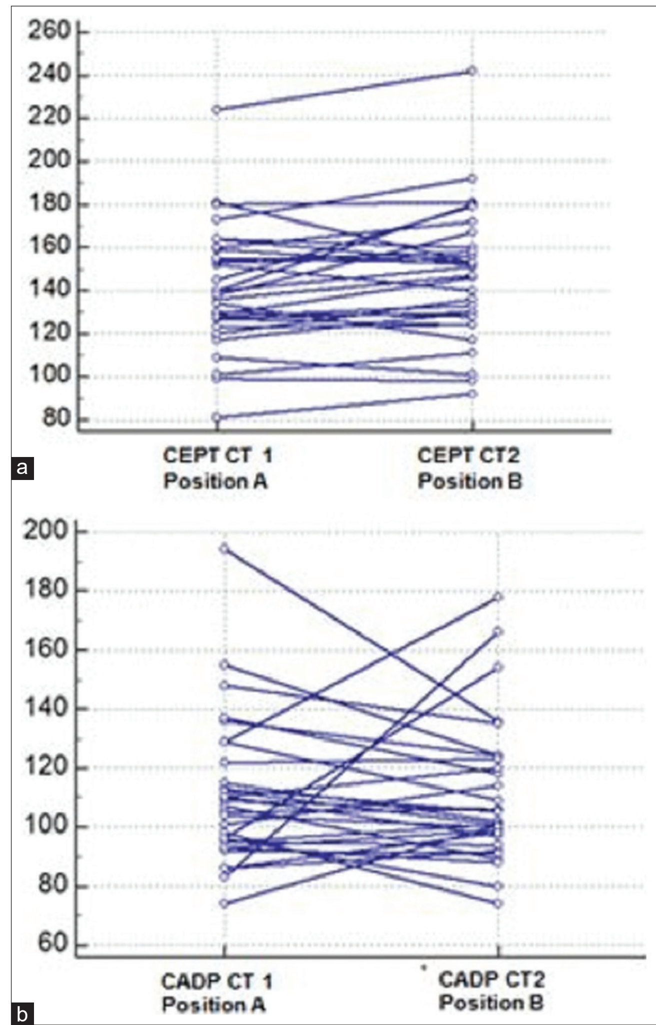


**Figure 2: Points and lines diagram of collagen/epinephrine and collagen/adenosine diphosphate platelet function analyzer-100™ closure times closure times determined, respectively, in position a and b**

membrane.[10] Thus, the PFA-100™ CT is not useful for assessing hypofibrinogenemia, dysfibrinogenemia, or hemophilia. Besides, it has been reported that PFA-100™ CT is abnormal in some congenital or acquired platelet function abnormalities but is no longer in cases of clotting factor deficiency. Hence, the interest in using the PFA-100™ CT is mainly for the assessment of primary hemostasis disorders.

In the present study, we observed no influence of cigarette smoking on CEPI CT among our blood donors. CADP CT was slightly shorter for smokers than for nonsmokers. The negligible difference did not support the establishment of specific reference ranges for smokers. Many studies confirmed the effect of cigarette smoke on platelet function.[11,12] Therefore, further assessments are needed to clarify whether the apparent effects of smoking on platelet function in some blood donors justify such donors stop smoking for some time before the donation.

Our findings show diurnal variations with CT PFA-100™. We confirmed that PFA-100™ CT increased significantly from morning to afternoon.[13] When using the PFA-100™ system as a routine clinical test, attention should be paid to the moment a blood collection. For example, a patient with discrete platelet dysfunction scheduled for surgery in the afternoon might go unnoticed if the test was performed in the early morning.

In agreement with previous work, PFA-100 CT strongly depends on the plasma vWF level.[14] During primary hemostasis and under the influence of high shear forces, the vWF mediates platelet adhesion and aggregation. Thus, lower levels of vWF affect the formation of a hemostatic plug and typically prolong PFA-100™ CT. Most studies showed that PFA-100™ CT has higher sensitivity for screening von Willebrand disease and monitoring DDAVP responses and FVIII/VWF substitution of patients.[15,16]

As expected, participants with blood group O had longer PFA-100™ CT than those with other blood groups, likely because plasma vWF levels are lower in blood group O than in other blood groups.[17,18] We know that vWF is one of the nonerythrocytic proteins that express ABH antigenic

determinants. The nature of carbohydrates in the antigens of the ABO blood group system determines the rate of proteolysis of vWF by ADAMTS13. Individuals who are not group O (AB, A, and B) have more carbohydrate antigen complex and thus have a slower rate of proteolysis. However, individuals of the Bombay phenotype who have low carbohydrate content have a faster rate of proteolysis. A recent study showed that the ABO locus exerts the main genetic influence on PFA-100™ CT, but it is not dependent on the vWF. However, while the effect of the ABO locus on CEPI CT PFA-100™ mediated through vWF, the effect of the ABO locus on the CADP phenotype is partly produced through vWF and partly through other mechanisms.[19]

Our duplicate analyses revealed acceptable mean values of the CV. This finding suggests that in routine usage, there is no need to perform duplicate testing. We found no marked differences between CT values determined in channels A and B of the PFA-100™ device, which agrees with previous observations.[20]

## Conclusions

The variables that affect CT PFA-100™ in healthy Algerian subjects were similar to those in Western and Asian populations, as reported in literature. Therefore, PFA-100™ CT should be interpreted according to the factors such as ABO blood group and diurnal variation. The small effect of smoking on CADP CT needs further assessments. The PFA-100™ showed good reproducibility. However, reliable PFA-100™ results depend on the standardization of preanalytical and analytical conditions. This report should help make this technique a more reliable and practical clinical tool, making it suitable for daily routine investigations.

### Ethical clearance

The ethic commitee of hospital university center Sidi Bel Abbes. Reference number :CHUSBA/CE/ 24/2013. Date: 11/August/2013. All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.


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Nil.

### Conflicts of interest

There are no conflicts of interest.


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