UR Medicine Labs
Hematology and Chemistry Laboratory
Strong Memorial Hospital
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Closure Time
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□ Non-Technical

Procedure Name: Closure Time Standard Operating Procedure
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Closure Time Standard Operating Procedure

I. Purpose

A. The purpose of this document is to provide instructions on how to perform a closure time assay on the Siemens PFA-100.

II. Principle

A. The PFA-100® is an instrument and test cartridge system in which the process of platelet adhesion and aggregation following a vascular injury is simulated in vitro. Platelet dysfunction detected by the PFA-100® system may be acquired, inherited, or induced by platelet inhibiting agents. The most common causes of platelet dysfunction are related to uremia, von Willebrand disease (vWD), and exposure to agents, such as acetyl salicylic acid (ASA, for example Aspirin®). The PFA-100® system allows for rapid evaluation of platelet function on small samples of anticoagulated whole blood based on work described by Kratzer and Born. The single use PFA-100® test cartridge consists of a number of integrated parts including a capillary, a sample reservoir and a biochemically active membrane with a central aperture. Anticoagulated whole blood is aspirated from the sample reservoir through the capillary and the aperture, which exposes platelets to high shear flow conditions. The membrane is coated with collagen, a subendothelial protein generally believed to be the initial matrix for platelet attachment. The attachment of platelets to collagen is thought to trigger the initial physiologic stimulus for platelet activation. In addition, the membrane is coated with either epinephrine or ADP, which are other physiologic agonists that, along with collage, are widely used to activate platelets in aggregometry testing. At the beginning of a PFA-100® test, Trigger Solution is dispensed to wet the membrane. During the test, platelets adhere to the collagen-coated membrane. Then, similar to aggregometry, platelets become activated and release their granule contents upon contacting agonists such as ADP or epinephrine. The release of granule contents is followed by adherence of platelets to each other to form aggregates. As a measure of platelet function in the PFA-100® system, the process of platelet aggregation builds a platelet thrombus at the aperture thereby gradually diminishing and finally arresting the blood flow. The PFA-100® instrument determines the time from the start of the test until the platelet plug occludes the aperture, and reports that time interval at the Closure Time (CT). The CT is an indicator of platelet function in the analyzed whole blood sample. As expected, platelet plug formation in the PFA-100® system is affected by low platelet counts and/or activity, inadequate plasma von Willebrand factor status, and additionally by, inadequate hematocrit because of the flow process. The Collagen/Epinephrine (COL/EPI) Test Cartridge is the primary cartridge used to detect platelet dysfunction induced by intrinsic platelet defects, von Willebrand disease or exposure to platelet inhibiting agents. The Collagen/ADP (COL/ADP) Test

Cartridge is used to indicate if an abnormal result obtained with the COL/EPI Test Cartridge may have been caused by the effect of ASA or medications containing ASA.

III. Scope

A. This procedure will be used by the University of Rochester Medical Center, Hematology and Chemistry Laboratory.

IV. Responsibilities

Person/Group	Responsibility
Quality Assurance	• Supports the development of this document. • Review and approval of this document.
Medical Director	• Ensures that the procedure is followed. • Review and approval of this document.
Supervisor	• Ensures that the procedure is followed. • Review and approval of this document.
End User	• Follows the procedure.

V. Specimens

A. Acceptable Sample Types:

Citrated whole blood – 9:1 blood to anticoagulant ratio of 3.2% buffered sodium citrate. Follow NCCLS guidelines H3-A3 and H21-A2. No other anticoagulant is acceptable. Do not use hemolyzed blood samples. Do not refrigerate or use centrifuged samples.

B. Collection Criteria:

Venipuncture should be performed using a 21G or larger needle. Blood should be drawn directly into an evacuated plastic or siliconized glass tube containing 3.2% buffered sodium citrate. Sample should be properly mixed after collection, by inverting by hand 3-4 times. Samples must be stored undisturbed at room temperature and are stable for up to four hours. For the COL/Epi test cartridge, it is recommended that testing not be performed until 10 minutes after blood collection.

VI. Quality Control

A. The PFA-100® Self Test from the Maintenance Menu should be performed at the start of each shift that the system is in use. Refer to the PFA-100® Operating Manual QC procedures section for instructions.

B. To perform Self Test:

2. From the display **System Ready**, press the softkey located next to [**Menus**].
3. From the display **Menu**, press the numeric key [**2**] to select the option [**Maintenance**].
4. Press the numeric key [**2**] to select the option **Self Test**.
5. Press the softkey located next to [**Yes**] to continue the self test.
6. The system will instruct the user to load a vacuum test cartridge (create the vacuum test cartridge by inserting a grayish blue vacuum test cup into the blue priming cartridge) in position A. Place a priming cartridge (blue cartridge with no vacuum cup) in position B. The system will then rotate the carousel and instruct the user to load the O-ring cleaning pad (circular foam sponge) in the well. Once the cleaning pad is in position in the carousel well, apply 4-5 drops of Isopropanol to the center of the pad. Press gently on the pad with gloved index finger 2-3 times to help distribute the Isopropanol. Press the softkey located next to [**Continue**].
7. The system will perform the O-ring cleaning procedure and a vacuum test in addition to the power on diagnostics test. The memory test is performed only during power on and not performed during the self test. The system will print the pass/fail results as each test is completed.
8. After the self tests are completed, the system will print the high/low flag ranges for each test type.
9. At the end of the self test, the system will prompt the user to remove the O-ring cleaning pad. Remove the pad and dispose of in a suitable biohazard waste container. Remove the vacuum test cartridge from position A. Remove the vacuum test cup from the vacuum test cartridge with gloved index finger and discard in a suitable biohazard waste container. Remove the priming cartridge from position B, rinse with purified water and save for further use.
10. Press the key [Previous Screen] twice to return to the display System Ready.

C. As part of the instrument quality control: test a normal donor in duplicate with each new shipment of cartridges received or every 31 days. The QC testing is performed using the RUN CONTROL mode of the PFA-100.

D. The system will be considered under control if the mean CT falls within the established reference range.

1. If the mean CT is outside the reference range, repeat this procedure with a second individual from the laboratory's established control donor group.
2. If the mean CT's from both individuals are outside the reference range, contact Technical Assistance Center.
3. If the mean CT from the second individual is within the reference range, the platelet function status and medication history of the first individual should be suspected.

VII. Safety Precautions

All patient specimens and reagents should be considered potentially infectious. Precautions should be taken in accordance with laboratory safety policy (SH.CP.AU.gen.0005.0001), CDC recommendations and Federal OSHA Blood Borne Pathogen Standard, 29 CFR part 1910.1030.

VIII. Materials

Item	Supplier	Description	Storage
800 μL Pipette	Fisher	Ensures accuracy in measured sample volume	In Lab
Collagen/Epinephrine Cartridge	Siemens	A test cartridge unit containing a membrane coated with 2μg of equine Type I collagen and 10μg epinephrine bitartate.	Test cartridges in an unopened pouch are stable 2°-8° C until the expiration date printed on the label. Test cartridges are stable up to 3 months after opening the pouch when stored at 2°-8° C. Test cartridges stored at room temperature (16°-26° C) in a sealed or unsealed pouch are stable for up to 4 hours.
Collagen/ADP Cartridge	Siemens	A test cartridge unit containing a membrane coated with 2μg of equine Type I collagen and 50μg adenosine-5'-diphosphate (ADP).	

Trigger Solution	Siemens	A trigger solution vial containing 11mL isotonic saline (0.9% aqueous sodium chloride).	Trigger solution in an unopened vial is stable at room temperature (16°-26° C) until the expiration date printed on the label. Trigger solution is stable up to 60 days after the vial is placed on the instrument. Discard if turbid or particulate matter is visible.

IX. Procedure

A. Make sure the test cartridges have reached room temperature before utilization (approximately 15 minutes). Peel off and discard the top foil seal of the test cartridge.

B. Snap the test cartridge into position "A" of the PFA-100® cassette. When running two tests, place a second test cartridge into position "B". Two different types of test cartridges may be placed in the cassette (eg. Collagen/EPI and Collagen/ADP). The analyzer will automatically determine the test cartridge type before testing the sample.

C. Check the sample for a clot using two applicator sticks. Re-suspend the whole blood sample by gently inverting the collection tube 3-4 times by hand.

D. Holding the cassette with test cartridge(s) on a flat surface, pipette 800μL of sample into the smaller opening (sample reservoir opening) of the test cartridge.

E. Place the cassette with the test cartridge(s) into the incubation well(s) of the instrument so that the cassette is flush to the carousel surface. Do not apply pressure to the sample reservoir opening. The test can now be started.

F. Press the softkey located next to [RUN] on the System Ready display.

G. Use the numeric keypad to enter the patient order ID.

H. The instrument will start testing without action from the operator. When testing is complete, the system will print and display the results.

I. Remove the cassette carefully from the carousel. Holding the cassette in one hand, remove the test cartridge by gently pushing the bottom of the cartridge toward you until it unsnaps. Dispose of the test cartridge in a suitable biohazard waste container.

X. Interpretation

A. Results of the PFA-100® test are reported by the instrument as Closure Time (CT) in seconds. The PFA-100® test provides an indication of platelet function.
B. Closure Time above laboratory established cut-off may indicate the need for further diagnostic testing.
C. Results should always be evaluated in conjunction with clinical history and other laboratory findings (such as bleeding time and platelet aggregometry).

D. In cases where PFA-100® results do not agree with the clinical assessment, additional tests should be performed

	Normal (n=176)	**ASA (n=120)**	**vWD (n=28)**	**Glanzmann's thrombasthenia**
COL/EPI	Normal	Abnormal	Abnormal	Abnormal
COL/ADP	Normal	Normal	Abnormal	Abnormal

XI. Results Reporting

Results of the PFA-100® test are reported by the instrument as Closure Time (CT) in seconds. Results >300 sec for either cartridge should be repeated. If the test remains >300 sec after repetition or if there is an error message, have a slide made to check for platelet clumps. Platelet clumps indicate that the platelets have been activated, and the sample is invalid for testing. If platelet clumps are present, the sample should be cancelled and a redraw requested (21g or larger needle should be used during the draw). Record the result on the worksheet and in the LIS. If the Collagen/EPI cartridge results are >181 sec the LIS will reflex order the test for the Collagen/ADP cartridge. The procedure should then be repeated using the Collagen/ADP test cartridge. The printout from the analyzer is retained adjacent to the corresponding page of the laboratory test log.

Reference Interval:

Reference interval values:

Cartridge Type	**Mean (sec)**	**Reference Interval (sec)**
Collagen/EPI	141	0 - 181
Collagen/ADP	88	0 - 112

These ranges should only be used as a guide for interpretation together with other clinical signs and symptoms.

XII. Interferences

A. Presence of hemolysis may interfere with test results. The presence of free hemoglobin from lysis of red cells could affect the PFA-100 closure time for two reasons:

1. Reduction in hematocrit, and
2. Release of ADP.

Therefore, use of hemolyzed blood for PFA-l00® testing is not recommended.

B. Certain fatty acids and lipids found in various human diets are widely known to inhibit platelet function, for which the PFA-l00®system was designed to detect. Neutral lipids, such as cholesterol, generally have no effect on platelet function.

C. Platelet inhibiting agents, such as aspirin and anti-glycoprotein IIb/IIIa antagonists, directly affect platelet function.

XIII. Limitations

A. Microthrombi in the sample or particulates introduced into the sample from the environment could adversely affect the test results and/or cause a cancellation of the test by the instrument due to the detection of a flow obstruction.

B. Blood samples with high sedimentation properties may experience some settling in position B while waiting to be tested in sequence with position A. Should settling occur, the hemodynamic properties of the sample may be altered, potentially affecting the result. Thus, it is recommended that samples exhibiting high sedimentation properties be run as single tests. In order to obtain duplicate measurements, two separate runs should be performed.

C. Many medications are known to affect platelet function. Therefore, the medication history of the patient should be reviewed.

D. Closure time above laboratory established cut-off could reflect reduced platelet function caused by hematocrit levels <35% or platelet counts <150,000/mL. Specimens with hematocrit levels >50% or platelet counts >500,000/mL have not been evaluated.

E. Certain fatty acids and lipids found in various human diets are known to inhibit platelet function; physicians may wish to advise patients to refrain from fatty foods prior to testing.

XIV. Training

Role	Training Needed
Management	Read
Employees	Read

XV. References

A. PFA-100®, Platelet Function Analyzer, Dade Behring/Siemens, 2003.

B. PFA-100® System Risk Assessment to Help in Risk Mitigation, Siemens Healthcare Diagnostics Inc., 2015