Sutter Health
Sutter Roseville Medical Center

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Applicability:	*Sutter Roseville Medical Center*

PFA-100: Platelet Function Analysis

Policy

This document details procedures specific to patient testing using the Siemens PFA-100 platelet function analyzer.

Principle

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*. This system can be used as an aid in the detection of platelet dysfunction in citrated human whole blood. The system allows for rapid evaluation of platelet function on small samples of blood, replacing the Bleeding Time procedure as a screening test for platelet dysfunction.

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. Citrated whole blood is aspirated from the sample reservoir through the capillary and the aperture, which expose 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 collagen, are widely used to activate platelets in aggregometry testing. At the beginning of a test, Trigger Solution is dispensed to wet the membrane. During the test, platelets adhere to the collagen-coated membrane. Then 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, the process of platelet aggregation builds a platelet thrombus at the aperture thereby gradually diminishing and finally arresting the blood flow.

The instrument determines the time from the start of the test until the platelet plug occludes the aperture, and reports that time interval as the Closure Time (CT). The CT is an indicator of platelet function in the analyzed whole blood sample. The collagen/epinephrine cartridge (Col/Epi) is the primary cartridge used to detect platelet dysfunction induced by intrinsic platelet defects, von Willebrand disease or exposure to platelet inhibiting agents (medications). The collagen/ADP cartridge (Col/ADP) is used to indicate if an abnormal result obtained with the collagen/epinephrine cartridge may have been caused by the effect of aspirin (ASA) or medications containing aspirin.

Supplies & Equipment

- Siemens PFA-100 analyzer
- Dade PFA priming cartridges
- Dade PFA vacuum cups
- Dade PFA O-ring cleaning pads
- Dade PFA O-ring service tool and O-rings
- MLA pipet: 900 µl (can also use 800 µl or 1000 µl if necessary)
- 3.2% buffered sodium citrate specimen collection tubes

Reagents

- Dade PFA Collagen/Epinephrine (Col/Epi) test cartridge and Dade ADP Collagen/Epinephrine (Col/ADP) test cartridge
  - Cartridges in an unopened pouch are stable at 2-25°C until the expiration date on the label.
  - Cartridges are stable up to 3 months after opening the pouch when stored at 2-25°C.
  - Cartridges are stable for 4 hours when stored at room temperature, regardless if the pouch is unopened or open.
  - Cartridges must be used within 15 minutes of removing the foil covering.
- Dade PFA Trigger Solution
  - Unopened vial is stable at room temperature until the expiration date on the label.
  - Opened vial is stable up to 60 days after being placed on the instrument.
  - The vial should be discarded if the solution is turbid.

Specimen Requirements

- 2 ml whole blood collected by venipuncture using a 21g or larger needle in 3.2% buffered sodium citrate anticoagulant. Mix by gentle inversion 3-4 times.
- Keep specimen at room temperature; DO NOT ICE SPECIMEN.
- DO NOT CENTRIFUGE SPECIMEN; testing is performed on whole blood. Resuspension of a sample after centrifugation is not acceptable for testing on the PFA-100.
- Do not use hemolyzed blood.
- Do not use samples that are clotted, or that were obtained by a difficult stick, where there was venous collapse or stoppage of blood flow during collection.
- Do not use partially filled tubes; tube must be at least 90% filled (same as the blue tops for routine coagulation testing.)
- Do not use resuspended previously spun blue top tubes, as centrifuging activates platelets.
- Samples are stable for 4 hours at room temperature.
- Wait at least 10 minutes between specimen collection and testing with the Col/EPI cartridge.

Maintenance

Refer to the operating manual for maintenance procedure instructions.

- Clean O-ring manually once a week and additionally as needed for troubleshooting.
- Replace O-ring at least once a year.
- Replace trigger solution at least every 60 days. The system must be primed after replacing the trigger solution.
- Trigger priming is recommended any time the system has not been used for more than 2 days to clear

bubbles.

- Clean the exterior with a weak bleach solution. Use the same solution to keep the black pad clean (pad is on the surface of the carousel underneath the cassette.)
- Replace paper and printer ribbon as needed.
- Replace the fuse as needed.

Calibration

No calibration is necessary for this procedure.

Quality Control

System and cartridge performance are verified using a combination of the diagnostic self-test procedure and testing of normal donor samples. No commercial external controls are available for the test system.

Quality Control - Self Test

The PFA Self Test should be performed **every 8 hours** that the system is in use.

STEP	ACTION
1.	From the System Ready display, press the softkey located next to [MENUS].
2.	From the menu display, press the numeric key [2] to select Maintenance.
3.	Press the numeric key [2] to select the Self Test.
4.	Press the softkey located next to [YES] to continue the self test.
5.	Load a priming cartridge and vacuum test cup into both positions. Press the softkey next to [CONTINUE]. Two blue vacuum cups are required, one each in positions A and B. • Disregard the instructions displayed on the screen. • Use new vacuum test cups every time the self test is done. • The priming cartridge may be reused. Check that the bottom foil is intact and that debris and scratches are not present. Replace as needed.
6.	Thoroughly dampen a new cleaning pad with an alcohol wipe and insert into the well when prompted. Press the softkey next to [CONTINUE]. • Wear gloves when handling cleaning pads. Contamination from bare hands may transfer from cleaning pad to O-Ring.
7.	The system will perform the O-ring cleaning procedure and vacuum test in addition to all Power On Diagnostics tests except for Memory Test.
8.	Pass/Fail results will print for each diagnostic test. High/low flag ranges for each cartridge type will also print.
9.	Remove the cleaning pad when prompted. Discard in biohazard waste. Press the softkey next to [CONTINUE].
10.	Visually inspect that a drop of trigger solution is on the platform of the test cup in Position B. • If no drop is visible in Position B, contact Siemens Technical Assistance Center.
11.	Discard both vacuum test cups in biohazard waste. Rinse both priming cartridges with DI water and save for reuse.

12.	Press the [PREVIOUS SCREEN] key twice to return to the System Ready display.
13.	Record Self Test results on the result log.

IF	THEN
All tests pass	Proceed with patient testing.
One or more tests fail	Manual O-ring cleaning must be performed. Refer to operator manual for additional troubleshooting.
Unable to resolve failure	Call Siemens technical support.

Quality Control - Normal Donor Control

Samples from a volunteer healthy adult free from any medication known to affect platelet function will be tested in duplicate for each new lot and/or shipment of cartridges. The mean CT of the duplicate tests should be in the reference range for the test cartridge and have a CT ≤ 15%

STEP	ACTION
1.	Collect a specimen from a volunteer healthy adult free from any medication known to affect platelet function
2.	Warm 2 test cartridge(s) from each lot and/or shipment to be tested for 15 minutes at room temperature.
3.	Load cartridges and sample into the cassette according to the patient testing procedure.
4.	From the System Ready display, press the softkey located next to [MENUS].
5.	From the menu display, press the numeric key [1] to select Run Control option.
6.	Use the keypad to enter the numeric portion of the cartridge lot. Press the softkey next to [ENTER].
7.	Use the keypad to enter the sample ID (optional.)
8.	Press the soft key next to [RUN].
9.	The results will be displayed and printed, along with mean CT and %CV values.
10.	Evaluate and record results on QC log.

IF	THEN
Mean CT is within reference range (Col/EPI: 83 - 153 Col/ADP: 57 - 100)	QC is acceptable.
Mean CT is outside the reference range	Repeat testing on a second normal donor.
2nd donor sample is acceptable	QC is acceptable. Review first donor's platelet function status and medication history.
2nd donor sample is not acceptable	Cartridge and analyzer performance is not verified. Do not run patient samples. Contact Siemens technical support.

Patient Testing

Initial testing is performed using the Collagen/Epinephrine cartridge. If the result on that cartridge is abnormal, follow-up testing is done using the Collagen/ADP cartridge.

STEP	ACTION
1.	Perform Self Test if not already successfully completed – must be performed **every 8 hours** of patient testing.
2.	Prepare test cartridges.

STEP	ACTION
A.	Select cartridges to be used. • Two cartridges may be tested at the same time if needed. Different cartridge types and patients may be run at the same time if needed.
B.	Remove cartridge from refrigerated pouches and warm at room temperature for 15 minutes. • Immediately seal and return pouch to refrigeration.
C.	Remove foil seal from the test cartridge.
D.	Load cartridge into cassette. • Use position A for a single test. • Make sure cartridge is seated properly in cassette to avoid carousel jams.

3.	Load samples. These steps must be done in sequence without interruption.

STEP	ACTION
A.	Place the cassette with the cartridge into the incubation wells. • Make sure that cassette and cartridges are flush to carousel surface.
B.	Gently mix sample by inversion several times.
C.	Pipette 900 µL (minimum volume 800 µL) of blood into the **smaller opening** (sample reservoir opening) of the test cartridge. • Dispense slowly along one of the inside corners to reduce the possibility of air entrapment. • Do not apply pressure to sample reservoir opening.

4.	Press the softkey next to [RUN].
5.	When prompted, enter the CID using the barcode reader or numeric keypad and press the softkey next to [RUN]. Accession number or MR# may be used if CID is not available. • If the same patient sample is used for 2 cartridges tested at the same time, the [RUN DUPLICATE] softkey can be used after entering the sample ID. • If 2 cartridges are loaded from different patients at the same time, separate prompts will be given for samples in position A and B. Press the softkey next to [RUN] after entering each sample ID. • The system automatically detects the cartridge type in use.

6.	If needed, cancel testing by pressing the softkey next to [CANCEL]. • The system may prompt for confirmation. Press softkeys next to [YES] or [NO] as needed.
7.	Results, including sample ID and test type, will print when testing is complete. • Place Sunquest patient label on the PFA tape.
8.	Remove cassette and cartridge from the carousel. • Remove the cartridge by gently pulling the bottom of the cartridge until it unsnaps. • Dispose of cartridge in sharps biohazard waste.

Status Messages

Status messages associated with events or conditions detected during the test run are indicated next to the printed test result.

<table>
<tr><th>MESSAGE</th><th>ACTION</th></tr>
<tr><td>Maximum Test Time Exceed</td><td>Sample did not achieve closure within 300 seconds.
Possible causes are:
• Sample with abnormal platelet function
• Possible vacuum leak
• Defective test cartridge.
<table>
<tr><th>IF</th><th>THEN</th></tr>
<tr><td>On normal control donor or result does not correlate with patient's history</td><td>Suspect vacuum leak. Perform Self-Test without loading the cleaning pad.
• If self-test passes, the test cartridge may have been defective. Repeat patient testing.
• If self-test fails, perform O-Ring maintenance or replacement. Repeat patient testing.

If test results are verified on repeat testing and the self-test passes, the sample is likely abnormal. Report as >300.</td></tr>
<tr><td>Other samples</td><td>Report as >300 if result correlates to patient's history.
Otherwise, follow troubleshooting steps in box above.</td></tr>
</table></td></tr>
<tr><td>Insufficient Sample</td><td>Air was detected in the first 30 seconds of testing.
Possible causes are:
• Not enough sample was loaded into the test cartridge.
• Sufficient sample was loaded but aperture closing did not occur due to platelet dysfunction or low sample viscosity (low HCT, high ESR.)
<table>
<tr><th>IF</th><th>THEN</th></tr>
<tr><td>No sample or sample</td><td>Repeat testing with correct sample volume.</td></tr>
</table></td></tr>
</table>

	volume <800 µL was added.	
	Air bubbles in sample reservoir.	Clean O-ring. Repeat testing with correct sample volume.
	Sample added properly	Check PLT and HCT results. If PLT<50,000/µL and /or HCT <20%, add ETC "PFAH" to result. See resulting section.

Flow Obstruction

Sample flow stoppage detected. If stoppage occurs in the first 30 seconds, the test is aborted and "Test termination due to flow obstruction" is reported by the analyzer.
If the stoppage occurs later in the test cycle, ">XX sec. flow obstruction" is reported by the analyzer (XX=seconds at which the obstruction is detected.)
Possible causes are:

- Microthrombi present in sample or formed during testing procedure.
- Particulates from the environment in the sample or test cartridge.

IF	THEN
Clots in sample	Cancel specimen and recollect.
No clots in sample	Repeat testing. Cancel specimen and recollect if unable to obtain results.

Maximum Syringe Travel Reached

Test stopped because syringe reached end of its travel prior to maximum test time.
Possible causes are:

- Syringe piston moved too quickly due to low sample viscosity (low HCT, high ESR)
- Defective PFA test cartridge
- Debris or analyzer malfunction preventing complete vacuum seal
- Platelet dysfunction

IF	THEN
Sample added properly	Suspect vacuum leak. Perform Self-Test **without** loading the cleaning pad. • If self-test passes, the analyzer is in control. Check HCT and PLT count. Report result. • If self-test fails, perform O-Ring maintenance or replacement. Repeat patient testing.
If PLT<50,000/µL and/or HCT <20%	Add ETC "PFAH" to result. See resulting section.

Air Leak

The analyzer detected an initial air leak in the vacuum system at the beginning of a test.
Possible causes are:

- Vacuum leak or malfunctioning trigger solenoid pump
- No sample in test cartridge
- Air trapped in test cartridge during specimen loading
- Defective test cartridge

IF	THEN

	Sample added properly	Suspect vacuum leak. Perform Self-Test **without** loading the cleaning pad. • If self-test passes, the solenoid pump may be causing error. • If self-test fails, perform O-Ring maintenance or replacement. • Prime system • Repeat patient testing. Contact Siemens technical support if error persists.
	No sample or <800 µL was added.	Repeat testing with appropriate sample volume.
Other Messages	Refer the Diagnostics and Troubleshooting section of the operating manual for additional information.	

Result Reporting

Results of the PFA-100 test are reported by the instrument as Closure Time (CT) in seconds.

Worksheet	RVKM
Test Code	PFA
Field Name	CEPI = Col/EPI result CADP = Col/ADP result

STEP	ACTION
1.	Record results from PFA tape onto patient testing log.
2.	Enter results in Sunquest. • If Col/EPI result is normal, the CADP field is automatically hidden. • Add the ETC "PFAH" if PLT <50,000/µL or HCT<20% when indicated. "PFAH" = "The PFA cannot distinguish between possible platelet dysfunction and abnormal hematology results (platelet count <50,000/cumm and/or hematocrit below 20%."
3.	Sunquest will automatically attach interpretive comments to abnormal results. • Abnormal Col/Epi with Normal Col/ADP: CADP2 = "This finding is usually associated with drug interference. Rule out ASA or other platelet inhibiting drugs." • Both tests abnormal: CADP5 = "This screening test suggests an inherent platelet dysfunction. Further investigation for platelet abnormalities (platelet aggregation studies) or von Willebrand disease is suggested if clinically indicated. However, medication effect cannot be ruled out, since some medications may make both tests abnormal (i.e.: Plavix)."
3.	Notify ordering provider of critical results and document as needed.
4.	Verify accuracy of Sunquest entry and document check on patient testing log.

Reference Range

- Col/Epi Cartridge: 83 - 153 seconds (mean 118)
- Col/ADP Cartridge: 58 – 106 seconds (mean 82)

Critical Range

- Col/Epi Cartridge: > 153 seconds
- Col/ADP Cartridge: >106 seconds

Interfering Substances

Hemolysis may interfere with test results. Free hemoglobin from red cell lysis could affect results due to decreased hematocrit and release of ADP.

Neutral lipids, such as cholesterol, generally have no effect on platelet function. Certain fatty acids and lipids found in human diets are known to affect platelet function which may be detected by the PFA-100.

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

Limitations

- 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.
- Blood samples with high sedimentation properties may experience some settling in position B while waiting to be tested in sequence position A. Should settling occur, the hemodynamic properties of the sample may be altered, potentially affecting the result. It is recommended that samples exhibiting high sedimentation properties be run as a single test in position A only. In order to obtain duplicate measurements, two separate runs should be performed.
- Low platelet counts (<150,000 cumm) or low hematocrits (<35%) may result in higher closure times. The effect of platelet counts >500,000/cumm and hematocrits >50% have not been evaluated. Extremely high platelet counts may clog the aperture and make testing impossible.
- The PFA-100 system performance has not been established for platelet inhibiting agents other than acetyl salicylic acid.
- Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.
- A backup analyzer is available for use if the primary analyzer is out of service. The backup analyzer may be placed into service if the following are successfully performed:
    - Clean (or replace if necessary) O-ring
    - Load and prime trigger solution
    - Perform self-test
    - Quality Control testing with Normal Donor sample.
    - Alternatively, samples may be sent to other Sutter sites in the vicinity that perform PFA testing (SRMC recommended.) Store samples at room temperature prior to courier pickup. Samples are stable for 4 hours only.

Interpretation

Results of the PFA-100 test are reported by the instrument as Closure Time (CT) in seconds. Closure Time above the laboratory established cut-off may indicate the need for further diagnostic testing. Results should always be evaluated in conjunction with clinical history and other laboratory findings, if available (bleeding time and platelet aggregometry).

Platelet dysfunction detected by the PFA-100system may be acquired (as with uremia), inherited (von Willebrand disease and thrombasthenia), or induced by platelet inhibiting agents (aspirin, ReoPro). An abnormal Col/Epi result must always be followed by a Col/ADP test. If the Col/ADP result is normal, results are most likely due to drug effect. If both tests are abnormal, inherited platelet dysfunction should be considered. Medication effect cannot be ruled out, however, since some medications will make both tests abnormal (ie: Plavix).

The following are expected patterns observed with the PFA test on normal subjects and subjects with various disorders:

	Normal	ASA	vWD	Glanzmann's thrombasthenia
Col/Epi	normal	abnormal	abnormal	abnormal
Col/ADP	normal	normal	abnormal	abnormal

Supporting Documents

- Form A: PFA Self Test and Patient Result Log
- Form B: PFA Maintenance Log
- Form C: PFA Normal Donor QC Log

References

- PFA-100 System Instruction Manual (US) version 1.0.1 December 2017
- Dade PFA-100 Reagent Package Insert. Dade Behring, July 2014
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Attachments

Form A: PFA Self Test And Patient Result Log

Form B: PFA Maintenance Log
Form C: PFA Normal Donor QC Log

Approval Signatures

Step Description	Approver	Date
Medical Director	Lindsey Westerbeck: Dir, Lab	1/4/2021
Laboratory Director	Lindsey Westerbeck: Dir, Lab	12/30/2020