**TITLE: PFA-100® Platelet Function Analyzer**

**PRINCIPLE / PURPOSE:** The PFA-100 is an instrument test cartridge system in which

the process of platelet adhesion and aggregation following 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 acetylsalicylic acid (aspirin).

Anticoagulated whole blood is aspirated through the aperture, which exposes platelets

to high shear flow conditions. The membrane is coated with certain platelet activating

agents. Platelets adhere to the membrane and to each other. The time from the start of

the test until the platelet plug occludes the aperture is the closure time.

**COMPLEXITY LEVEL:** Moderate

**SAFETY:**

* The required personal protective equipment for this procedure

Gloves

Approved lab coats, worn closed

Shield

Approved protective eyewear

* Gloves and lab coats should be worn at all times during analysis of samples.
* Samples must be uncapped behind a safety shield.

**SPECIMEN:**

**Specimen Collection:**

**Venipuncture should be performed using a 21G or larger needle directly into an evacuated plastic tube. If it is not possible to collect the specimen with a 21G needle or if a traumatic venipuncture occurs, the procedure cannot be performed. Notify the ordering physician if this occurs.** Specimens are collected into two 3.2% buffered sodium citrate tubes with a collection ratio of 9:1 blood to anti-coagulant. No other anti-coagulant is acceptable. After sample collection, ensure proper mixing by gently inverting the tubes by hand 3 to 4 times. The specimens must be transported to the laboratory by hand.

**Do not** send the samples through the tube system. **Do not** centrifuge specimens.

**Stability and Storage:** Samples must be stored undisturbed at room temperature (16 to 26º C) and are stable for up to four hours.

**Unacceptable Specimens:** Samples that are short draws, clotted, over-filled,

hemolyzed or centrifuged may yield incorrect results and must be recollected.

**EQUIPMENT AND MATERIALS:**

1. Siemens PFA-100 Analyzer

2. Testing cassette

3. Pipette (up to 1000 μL)

4. Pipette tips

5. Priming cartridges

6. Vacuum test cups

7. O-ring cleaning pads

8. Isopropanol (70%)

**Reagents:**

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| **Product** | **Reconstitution** | **Stability Time Before Use** | **Open Expiration** |
| **T**rigger Solution | Ready to Use | Room Temp until expiry date on label | 60 days |
| Collagen/Epi  Test Cartridge | Ready to Use | 2-8 °C until expiry date on unopened pouch  **Or**  3 months at 2-8 °C after opening pouch | 4 hours at Room Temp |
| Collagen/ADP  Test Cartridge | Ready to Use | 2-8 °C until expiry date on unopened pouch  **Or**  3 months at 2-8 °C after opening pouch | 4 hours at Room Temp |

**Loading Trigger Solution**

1. Pull up the knob to remove the probe from the trigger solution bottle and unlatch the

access door. The door will automatically open when the knob is pulled up all the

way.

2. Remove the empty bottle.

3. Remove the flip-top cap and insert the new bottle in place. Do not use the trigger

solution if turbid or if particulate matter is present.

4. Push the access door closed.

5. Push the knob all the way down to insert the probe into the trigger solution bottle.

6. Snap a blue priming cartridge into a testing cassette in either position A or B.

7. From the *System Ready* display, press the soft key located next to [MENUS].

8. Press the numeric key [2] to select the *Maintenance* option.

9. Press the numeric key [4] to select the *Trigger Prime* option.

10.Press the soft key located next to [PRIME] to start priming the trigger system.

**QUALITY CONTROL:**

1. **Self-Test:** The Self-Test will be performed on 1st shift as part of daily maintenance.

a. From the *System Ready* display, press the soft key located next to [MENUS].

b. From the *Menu* display, press the numeric key [2] to select the *Maintenance*

option.

c. Press the numeric key [2] to select the *Self-Test* option.

d. Press the soft key located next to [YES] to continue.

e. Create a Vacuum Test Cartridge by inserting a blue Vacuum Test Cup into

the blue Priming Cartridge and placing one of these in to both positions A

and B. Load the Cartridge on to the analyzer. Press the soft key located

next to [CONTINUE].

f. As instructed, load the O-Ring Cleaning Pad (circular foam sponge) in the

exposed well. Apply 4-5 drops of Isopropanol to the center of the pad. Press

gently on the pad with a gloved index finger 2-3 times to help distribute the

Isopropanol. Press the softkey located next to [CONTINUE].

g. At completion, the system will prompt the user to remove the O-Ring

Cleaning Pad. Remove the pad and place in the container containing the extra O-Ring Cleaning pads. Press the soft key located next to [CONTINUE].

h. Press the [PREVIOUS SCREEN] key twice to return to the *System Ready*

display.

i. Review the instrument printout. The system will print the pass/fail results for

each test completed and the high/low flag ranges for each test type.

2. **Normal Donor Control:** With each new shipment or lot number, a control donor should be tested in duplicate. This will serve as a normal control. Blood drawn from a healthy adult individual will be used as a normal control for the PFA test. The mean of the closure time must fall within the established reference range.

a. From the *System Ready* display, press the soft key located next to [MENUS].

b. From the *Menu* display, press the numeric key [1] to select the *Run Control*

option.

c. Use the numeric keypad to enter the numeric portion of the lot number of the

test cartridge being used. This number is located on the test cartridge pouch

and on the test cartridge itself. Press the soft key located next to [ENTER].

d. Use the numeric keypad to enter the control ID. Press the softkey located

next to [RUN].

e. When testing is complete, the instrument will print and display the results

along with the test type, the control ID# and the lot number entered, a CV%

and a mean value for the two tests run. It is recommended that the

laboratory maintains a hard copy of the stored control results.

**PROCEDURE:**

**1. Collagen/Epinephrine Test (Phase 1)**

a. Allow the Collagen/Epinephrine test cartridge to warm to room temperature,

approximately 15 minutes.

b. Peel off and discard the top foil seal of the test cartridge.

c. Snap the test cartridge into position “A” (left position) of the testing cassette.

d. Re-suspend the whole blood sample by gently inverting the tube 3-4 times by

hand.

e. Pipette 800μL of sample into the smaller well of the cartridge. To minimize

the risk of air bubbles, place the tip of the pipette against the inside corner of

the sample well and slowly dispense the specimen.

f. Place the cassette into the incubation well of the instrument so that the

cassette is flush to the carousel surface.

g. Press the soft key located next to [RUN] on the *System Ready* display.

h. Use the numeric keypad to enter the patient ID #.

i. Press the soft key located next to [RUN]. The instrument will start testing.

j. When the testing is complete, the instrument will print and display the results

along with the patient ID # entered and the test type.

k. Remove the cassette from the carousel. Holding the cassette in one hand,

remove the cartridge by gently pulling the bottom of the cartridge sideways

until it unsnaps. Dispose of the test cartridge in a suitable biohazard waste

container.

l. Review the results. If the results for the Collagen/Epinephrine cartridge fall

below the normal Closure Time, **no** further testing is necessary. Proceed to

“Resulting”.

m. If the results for the Collagen/Epinephrine cartridge fall above the normal

Closure Time, proceed to Phase 2.

**2. Collagen/ADP Test (Phase 2)**

a. Allow the Collagen/ADP test cartridge to warm to temperature approximately 15 minutes.

b. Proceed with loading the cartridge, adding sample, and testing as stated in

Phase 1.

c. When testing is complete, the instrument will print and display the results along

with the patient ID # entered and the test type. Remove the cassette from the

carousel and dispose in a suitable biohazard waste container.

d. Review the results and proceed to “Resulting”.

**INTERPRETING AND REPORTING RESULTS:**

**Normal Values:**

Collagen/Epinephrine 0 -193 seconds Closure Time

Collagen/ADP 0 -118 seconds Closure Time

**Clinical Reportable Range:**

Collagen/Epinephrine cartridge: 0 – 300 seconds

Collagen /ADP cartridge: 0 – 300 seconds

Closure times greater than 300 seconds will be reported as >300

**Reporting format:**

An “Always Text” will be added to all patient reports. The text will state: “Results of the

test should always be interpreted in conjunction with the patient’s medical history,

clinical presentation and medication history. Patients with Hematocrit values <35.0% or

Platelet counts <150,000 / μL may result in values above the Laboratory established

reference range.”

**Instructions for Computer Data Entry:**

1. Log on to Sunquest
2. Select Result Entry and choose Manual
3. Enter Worksheet: **ARCOAG**
4. Select RESULT at the bottom of the screen
5. Choose the Binocular Icon at the bottom of the screen
6. Enter the container ID and press tab
7. At the prompt **COLEPI,** enter the Closure Time for the Collagen/Epinephrine cartridge in seconds.
   1. If the results are normal, type “HIDE” at the COLADP prompt
   2. If the results are abnormal, proceed to the next step
8. At prompt **COLADP**, enter the Closure Time for the Collagen/ADP cartridge.

* **PFAI** prompt: The PFA test interpretation will automatically result based on the

COLEPI and COLADP results entered. The comments will result as follows:

* **PFA1**: Platelet function is normal. If patient history/physical examination give

strong indication of a bleeding disorder repeat testing for confirmation.

* **PFA2**: This pattern is indicative of drug induced platelet dysfunction. Most

commonly seen after aspirin ingestion.

* **PFA3**: Platelet function is abnormal. This pattern is most commonly seen in

patients with von Willebrand disease or congenital platelet defects. Prolongation

of the Col/ADP closure time generally indicate a more extensive qualitative

platelet defect and/or an abnormality in von Willebrand factor.

**PROCEDURAL NOTES:**

* Results may be affected by a low hematocrit (<35.0%) and significant

thrombocytopenia. A sample size of 900 μl may be used for those samples with low hematocrits that produce QNS errors. See Troubleshooting charts.

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

**LIMITATIONS OF PROCEDURE:**

* The presence of hemolysis may interfere with testing. The presence of free

hemoglobin from lysis of red cell could affect the PFA-100 test closure time due to reduction in the hematocrit and the release of ADP.

* Certain fatty acids and lipids found in various diets are widely known to inhibit

platelet function. Neutral lipids, such as cholesterol, generally have no effect on

platelet function.

* Platelet inhibiting agents, such as aspirin and anti-glycoprotein llb/llla antagonists, directly affect platelet function.

**TROUBLESHOOTING:**

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| **MAXIMUM TEST TIME EXCEEDED (A)** | |
| Description | The sample did not achieve closure of the aperture within the maximum time for a test (>300 seconds). |
| Causes | 1. Abnormal sample  2. Possible vacuum leak in the system  3. Defective test cartridge |
| Solutions | 1. Perform a Self-Test to verify the system has no vacuum leak  2. Rerun the sample with a new cartridge to verify the test |
| Result Interpretation | Report the closure time as >300 seconds, abnormal plt function as confirmed by retest |

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| **TEST TERMINATED DUE TO AIR LEAK (B)** | |
| Description | The system has detected an initial air leak in the vacuum system. This condition is detected only at the beginning of a test. |
| Causes | 1. Possible vacuum leak in the system or a malfunctioning trigger solenoid pump  2. No sample in test cartridge  3. Air entrapped in fluid lines  4. Air entrapped in test cartridge when sample is loaded  5. Defective test cartridge |
| Solutions | 1. Verify that sample was added to test cartridge. If not, rerun the test.  2. Perform a Self-Test to verify the system has no vacuum leak. If the vacuum test fails, perform the manual O-ring maintenance procedure. If the vacuum test passes, a malfunctioning solenoid may be the cause for the air leak. Contact Technical Assistance for the solenoid pump troubleshooting procedure.  3. Prime the system from the Maintenance menu.  4. Rerun the test with a new cartridge and sample |
| Result Interpretation | Test is non-reportable |

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| **TEST TERMINATED DUE TO FLOW OBSTRUCTION (C)** | |
| Description | The system has detected a sudden stoppage of blood flow during the test. Refer to the package insert, “Limitations of Procedure” section, for further information. |
| Causes | 1. Initial Flow Obstruction – This type of flow obstruction occurs when the instrument detects a failure to establish initial blood flow rate. The system will abort the run and the test will be reported as “Test Terminated Due to Flow Obstruction”. This condition may be caused by microthrombi in the sample.  2. Flow Obstruction During a Test – A flow obstruction that occurs after initial blood flow rate conditions have been established is reported by the analyzer at the time the obstruction occurs, i.e. “>80C sec Flow Obstruction”. This occurs when the test cartridge membrane aperture is suddenly plugged by microaggregates.  3. Defective test cartridge |
| Solutions | 1. Verify that the sample does not contain clots or aggregates. If no aggregates are visible, repeat once to rule out a defective cartridge.  2. A sample that results in repeated flow obstruction may be a result of problems during collection. Recollect blood sample and repeat testing. |
| Result Interpretation | Test is non-reportable |

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| **TEST TERMINATED DUE TO INSUFFICIENT SAMPLE (D)** | |
| Description | The system has detected air being drawn subsequent to the first 30 seconds of testing. The condition is detected whenever the test runs out of sample and closure of the aperture has not occurred. |
| Causes | 1. Not enough sample was loaded to the cartridge.  2. Closure of aperture did not occur due to platelet dysfunction and/or low sample viscosity (hct <35.0). |
| Solutions | 1. Verify that 800 μL was added to the cartridge. Rerun sample using 1000 μL.  2. Verify the sample HCT is within normal range. An abnormal HCT may impair platelet function and result in prolongation of the closure time. Plt dysfunction in combination with a low HCT will often induce this type of message. |
| Result Interpretation | An insufficient sample message that occurs at a time that is greater than the upper limit of the normal range may indicate abnormal platelet function due to the reasons stated above. In this case, the result may be reported as the time in which the test ended (i.e. >216 sec would be reported as 216) with a statement  qualifying the properties of the sample such as an abnormal HCT and/or low platelet count and suspicion of platelet dysfunction. |

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| **TEST TERMINATED DUE TO MAXIMUM SYRINGE TRAVEL (E)** | |
| Description | The system has stopped the current test because the syringe has reached the end of its travel prior to maximum test time. |
| Causes | 1. Syringe piston moved too far too quickly as a result of low sample viscosity.  2. A defective test cartridge causing a small vacuum leak.  3. Dirt or debris are present on the vacuum seal between the instrument and the test cartridge.  4. Instrument malfunction causing a small vacuum leak. |
| Solutions | 1. Perform a Self-Test via Maintenance menu to rule out vacuum leaks.  2. Verify the sample HCT is within normal range. An abnormal HCT may impair platelet function and result in prolongation of the closure time. Plt dysfunction is combination with a low HCT will often induce this type of message. Adding additional specimen (1000 μL) could eliminate this message but will most likely result in a closure time >300 sec.  3. If problem persists, contact Technical Assistance Center. |
| Result Interpretation | A maximum syringe travel message that occurs at a time that is greater that the upper limit of the normal range may indicate abnormal platelet function. In this case, the result may be reported as the time in which the test ended (i.e. >216 E  sec would be reported as 216) with a statement qualifying the properties of the sample such as an abnormal hct and/or low plt count and suspicion of platelet dysfunction. |

**MAINTENANCE**

**Monthly: O-Ring cleaning**

1. From the *System Ready* display, press the softkey located next to [MENUS].

2. Press the numeric key [2] to select the *Maintenance* option.

3. Press the numeric key [6] to select the *Remove O-Ring* option. The system will

display the message “Load O-Ring Service Tool, Then Press Continue”.

4. Place the O-Ring Service Tool into the incubation wells of the instrument. Press

the softkey located next to [CONTINUE]. The system will rotate the carousel to

the O-Ring removal position and bring the O-Ring in contact with position A.

5. Remove the O-Ring Service Tool and press the softkey located next to

[CONTINUE].

6. Invert the O-Ring Service Tool and tap against the palm of your gloved hand to

remove the O-Ring. If the Service Tool fails to remove the O-Ring, repeat the

removal steps once more. If the Service Tool fails again, contact Technical

Support.

7. Rinse the O-Ring under running water. Remove any debris by using a rubbing

motion while rinsing under the tap water. Visually inspect the O-Ring for unusual

wear and tear.

8. Shake excess water off and soak in 70% Isopropanol for approximately 15

seconds. Shake excess Isopropanol off.

9. From the System Ready display, press the soft key located next to [MENUS].

10.Press the numeric key [2] to select the *Maintenance* option.

11.Press the numeric key [7] to select *Install O-Ring* option. The system will display

the message “Load O-Ring, Service Tool, Then Press Continue”.

12.Load the O-Ring in Position B of the O-Ring Service Tool. Place the O-Ring

Service Tool into the incubation wells of the carousel. Press the soft key located

next to [CONTINUE]. After approximately 30 seconds, the carousel will rotate

back allowing the removal of the O-Ring Service Tool.

13.Remove the O-Ring Service Tool and press the soft key located next to

[CONTINUE].

14.Perform a Self-Test to verify that the system has no vacuum leak.

**Yearly: O-Ring replacement**

* It is recommended the O-Ring should be replaced on a yearly basis.

**References:**

“Siemens PFA-100® Platelet Function Analyzer” Operating Manual, issued

09/2008.

“PFA-100® System, Getting Started/Training Guide”, by Dade Behring, January

2004.

Siemens PFA Package Insert 07/2014

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| Medical Director Approval **–**  **ARMC Cancer Center ARMC Main Lab** |  |  |
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SOP HISTORY PAGE

CLINICAL LABORATORY ALAMANCE REGIONAL MEDICAL CENTER

SOP Number: **COAG-0722-CH**

SOP Title: **Platelet Function**

Written By: **Quanisha Dyson, MT (ASCP) CM**

Manual in which Hard Copy of this SOP is located: **Coagulation Procedure Manual**

Distribution: **no other locations**

Supersedes Procedure:

SOP CHANGE CONTROL

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