PFA-100®
Getting Started

Table of Contents

PFA-100® Installation Checklist

Instrument Startup

	Yes	No
Instrument received with accessory box?	☐	☐ *
All components received in accessory box?	☐	☐ *
After fuse installation, instrument powered on successfully?	☐	☐ *
Paper installed	☐	☐
Language, Date, Time and Patient ID formats selected?	☐	☐
Reviewed and selected other options (Ops Manual, section 7)?	☐	☐
(Low/High Flag ranges, Audible keys, Laboratory Information System)		
Trigger prime performed?	☐	☐
Self Test performed?	☐	☐

*****If no answered, please contact Dade Behring Technical Assistance Center at (800) 242-DADE, option 1.

Patient Testing

Sample requirements reviewed	☐	☐
Common status messages reviewed	☐	☐
Limitations and interfering substances reviewed	☐	☐
Reference range protocol reviewed	☐	☐
QC donor pool protocol reviewed	☐	☐
Ready to perform testing?	☐	☐

Training Checklist for the Platelet Function Analyzer (PFA)

Name _____ **Title** _____

The operator and instructor should initial each item. A signoff designated by the operator and instructor indicates that the material has been reviewed and that the operator demonstrates knowledge or understanding of the requirements for performing testing on the PFA.

Operator	Instructor	Sample Requirements
		1. Veinipuncture should be performed using a 21G or larger needle.
		2. Blood should be collected directly into a buffered vacutainer tube, which should NOT be the first tube collected. Discard the sample if there is a venous collapse or stoppage of blood flow collection. Do not use hemolyzed blood samples.
		3. Samples are stable for up to 4 hours at room temp, DO NOT centrifuge or refrigerate.
		4. Samples should NOT be placed on a rocker or rotator while awaiting testing.
		5. The PFA-100 requires 800uL of whole blood per cartridge.
Operator	**Instructor**	Limitations and Interfering Substances
		1. Microthrombi can affect results and/or cause flow obstructions.
		2. Samples with high sed rates may not duplicate well between positions A & B.
		3. Reviewed list of medications that can affect platelet function.
		4. Closure time above lab cut-off could reflect reduced platelet function caused by hematocrit levels <35% or platelet counts <150,000/uL.
		5. Certain fatty acids and lipids found in various human diets are known to inhibit platelet function and physicians may with to advise patients to refrain from fatty foods prior to testing.
Operator	**Instructor**	PFA Instrument
		1. Operator understands the principle of operation of the PFA analyzer.
		2. Operator can identify the components of the analyzer (carousel, trigger solution compartment, LCD display, keyboard, and printer).
		3. Operator can identify the items needed to perform daily preventative maintenance, and can perform daily PM procedures, and change printer ribbon and paper.
		4. Operator understands the consumables required for sample testing, as well as their storage requirements, and can load and prime a new bottle of trigger solution.
Operator	**Instructor**	Sample Testing
		1. Operator understands the laboratory's protocol for QC on the PFA.
		2. Make sure test cartridges have reached room temp before testing (approx. 15 minutes).
		3. Load cartridges on analyzer and snap into position.
		4. Resuspend the whole blood sample by gently inverting the tube 3-4 times by hand.
		5. Pipette at least 800uL of sample into the cartridge without trapping air at the bottom.
		6. Enter patient ID and request test.
		7. Operator understands how to request another printout of patient results.
Operator	**Instructor**	Troubleshooting
		1. The operator is aware of the most common status messages and what they mean, is familiar with the troubleshooting section of the procedure manual, and can locate the Dade Behring technical assistance hotline number.
Operator	**Instructor**	Result Interpretation and Reporting
		1. The operator is familiar with and can locate the laboratory's defined reference ranges, and understands that the reference range is specific for the concentration of sodium citrate in the collection tube.
		2. The operator can explain the three types of results that can be interpreted from the closure times using the two types of cartridges on the PFA.
		3. The operator understands how to correctly enter and release a PFA result into the computer.

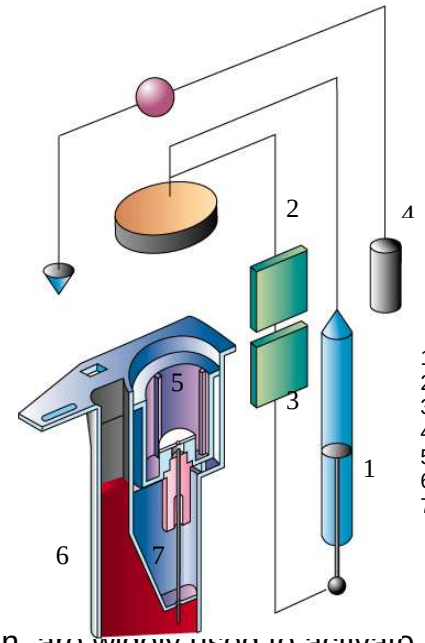
Instructor Name _____ **Title** _____ **Date** _____

PRINCIPLE OF PLATELET FUNCTION ANALYSIS

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 ®).[1]

SYSTEM FUNCTIONAL OPERATION

The PFA-100 ® system allows for rapid evaluation of platelet fuction on small samples of anticoagulated whole blood based on work described by Kratzer and Born.[2,3] 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 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 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, [4] 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. [5] In optical aggregometry, platelet function is assessed by aggregate formation detected by changes in light transmittance.

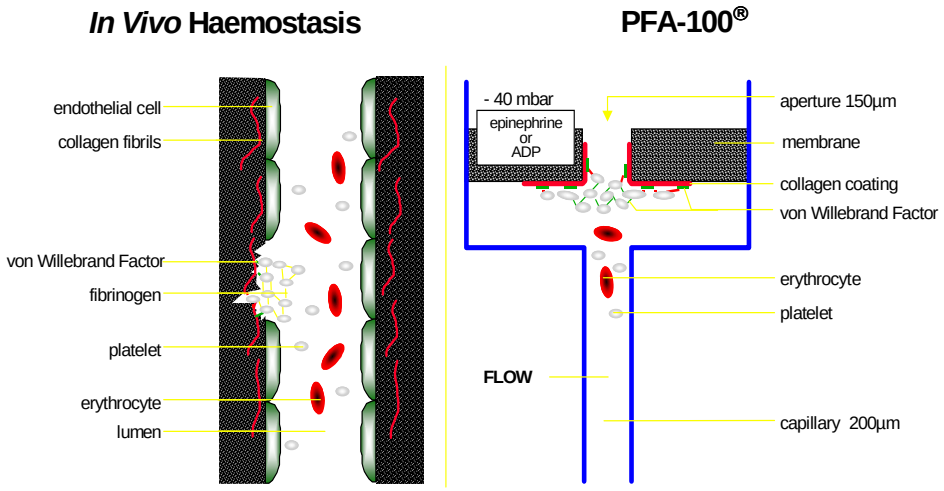


1. Syringe
2. Microprocessor
3. Pressure Transducer
4. Trigger Solution
5. Membrane
6. Sample Reservoir
7. Capillary

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 as 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. [5]

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.

The diagram below illustrates how the PFA-100 ® system simulates the process of platelet adhesion and aggregation following a vascular injury in comparison to in vivo hemostasis.



REFERENCES

1. Bick, R: Platelet function defects: a clinical review. Semin. Thromb. Hemost. (1992) 18,167-185.
2. Kratzer, MAA; Born, GVR: Simulation of Primary Hemostasis in Vitro. Haemostasis (1985) 5, 357-362.
3. Kratzer, MAA; Bellucci, S; Caen, JP: Detection of Abnormal Platelet Function with an in vitro Model of Primary Hemostasis. Haemostasis (1985) 15, 363-370.
4. Born, GVR: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 194:927, 1962.
5. Kundu, SK; Heilmann, EJ; Sio, R; Garcia, C; Ostgaard, RA: Characterization of an in vitro platelet function analyzer, PFA-100. Clinical Applications in Thrombosis/Hemostasis 2:241-249, 1996.

Sample Requirements

The PFA-100® requires 800 µL of citrated whole blood to perform one test. The maximum amount of sample that can be added to the test cartridge is 900 µL (Refer to Common Status Messages).

Sample Collection

Venipuncture should be performed using a 21G or larger needle. Blood should be drawn directly into an evacuated plastic or siliconized glass tube or syringe containing 3.8% (0.129M) or 3.2% (0.105M) buffered sodium citrate (1 part anticoagulant to 9 parts blood). After sample collection, ensure proper mixing of anticoagulant by gently inverting the tube by hand 3 to 4 times. Discard the sample if there is a venous collapse or stoppage of blood flow collection. Do not use hemolyzed blood samples.

Samples must be stored undisturbed at room temperature and are stable for up to 4 hours. For the Col/Epi test cartridge, it is recommended that testing not be performed until 10 minutes after blood collection. The collection method (both citrate concentration and venipuncture method) should be kept consistent. Do not refrigerate or centrifuge samples.

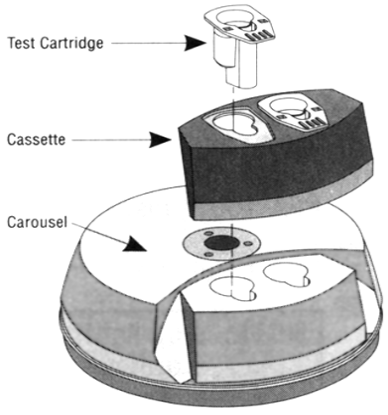
Note: Use of unbuffered sodium citrate anticoagulant is not recommended.

Performing Tests

1. Preparation of the test cartridge(s)
   (a) Allow the pouch containing the test cartridges to warm-up to room temperature prior to opening (apx. 15 min.) and remove the desired number of cartridges for testing. Close the pouch by using the reclosable seal and return the pouch to 2-8°C storage.

   (b) Remove and discard the top foil seal from the test cartridge(s).

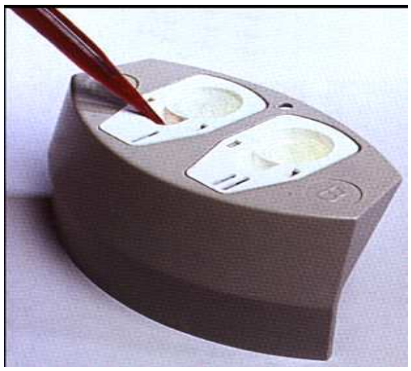
   (c) Place the test cartridge(s) in the cassette and push until the test cartridge(s) securely snaps in place.



2. Sample loading

Note: The following steps must be performed in sequence without interruption

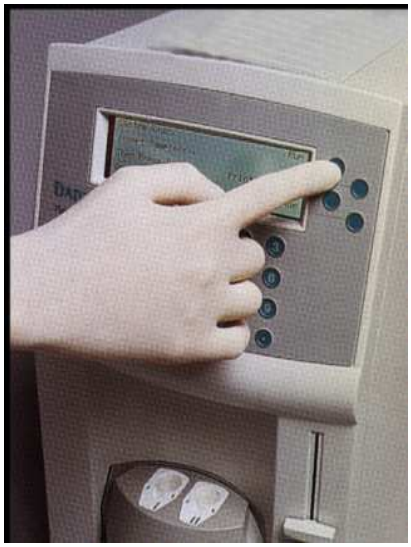
(a) Mix the blood sample by inverting the collection tube gently 3-4 times by hand. Holding the cassette with test cartridge(s) on a flat surface, pipette 800 µL of blood into the smaller opening (sample reservoir opening) of the test cartridge by dispensing slowly along one of the inside corners. This will reduce the possibility of air entrapment in the sample reservoir.



(b) 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 small reservoir opening. The test can now be started.

3. Starting the test(s)

(a) Select run from the system ready display.



4. Disposal of used test cartridges

Remove the cassette carefully from the carousel. Holding the cassette in one hand, remove these cartridge(s) by gently pulling the bottom of the cartridge(s) sideways until it unsnaps. Dispose of test cartridge(s) in a suitable biohazard waste container.

Expected Values

Specimens collected in 3.8% (0.129 M) buffered sodium citrate, from 176 ostensibly healthy individuals were evaluated to establish a reference range. These individuals had no previous history or laboratory results indicative of platelet dysfunction induced by intrinsic platelet defects, von Willebrand disease or exposure to platelet inhibiting agents. The following reference ranges were determined based upon a 90% central interval of results from duplicate determinations on these 176 subjects.

Cartridge Type	Mean (sec)	Reference Range
Collagen/Epinephrine	132	94-193
Collagen/ADP	92	71-118

In a limited study, on 36 apparently normal subjects, up to 12% shorter closure times were observed for samples collected in 3.2% (0.105 M) buffered sodium citrate.9 Differences in technique, equipment, reagents, donor population, etc. may produce results other than those listed. **Each laboratory should establish its own reference ranges** (non-parametric procedures from NCCLS document C28-A may be used as a guideline).10 The provided ranges should only be used as a guide for interpretation together with other clinical signs and symptoms.

Interpretation of Results

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. 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 (such as bleeding time and platelet aggregometry). In cases where PFA-100 ® results do not agree with the clinical assessment, additional tests should be performed.

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

	Normal (n=176)	ASA* (n=120)	vWD (n=28)	Glanzmann's thrombasthenia (n=4)
COL/EPI	normal	abnormal	abnormal	abnormal
COL/ADP	normal	normal	abnormal	abnormal

ASA-induced Platelet Dysfunction

Specimens drawn in 3.8 % (0.129 M) buffered sodium citrate from normal patients following ASA ingestion were tested using COL/EPI test cartridges in conjunction with COL/ADP test cartridges on the PFA-100 ® to evaluate platelet dysfunction due to ASA ingestion. A total of 120 specimens were tested in duplicate between 2 and 30 hours after ASA ingestion (325 mg). The results were as follows:

	COL/ADP Normal PFA CT ≤114 sec.	COL/ADP Abnormal PFA CT >114 sec.
COL/EPI Normal PFA CT ≤ 170 sec.	5	1
COL/EPI Abnormal PFA CT >170 sec.	87	27

The PFA-100 ® system detected platelet dysfunction in 95% of 120 ASA ingestion cases as indicated by the abnormal result obtained with the COL/EPI test cartridge. The pattern of abnormal COL/EPI and normal COL/ADP was observed in 72.5% of these 120 cases.

Differences in subject population, aspirin dosage, the time of testing after aspirin ingestion, and the anticoagulant used during blood sample collection, may produce results other than those listed.

Limitations of Procedure

1. 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.
2. 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, thehemodynamic 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.
3. Many medications are known to affect platelet function. Therefore, the medication history of the patient should be reviewed.
4. Closure Time above the 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.
5. Certain fatty acids and lipids found in various human diets are known to inhibit platelet function and physicians may wish to advise patients to refrain from fatty foods prior to testing.

Interfering Substances

1. 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-100 ® testing is not recommended.
2. Certain fatty acids and lipids found in various human diets are widely known to inhibit platelet function,[6,7] for which the PFA-100 ® system was designed to detect. Neutral lipids, such as cholesterol, generally have no effect on platelet function.[8]
3. Platelet inhibiting agents, such as aspirin ® and anti-glycoprotein IIb/IIIa antagonists, directly affect platelet function.

Influence of Hematocrit and Platelet Count on PFA-100® Measurements

Hematocrit

Hemorheology (fluidic properties of blood) plays an important role in the proper distribution of blood cells within a blood vessel during flow. Factors that affect normal hemorheology, such as viscosity, hematocrit etc. have been linked to abnormal hemostasis and in some cases to thrombosis. Several clinical studies have indicated a definitive association between significant bleeding and anemia. It is possible that this association is due to an abnormal distribution of blood cells (inability of red blood cells to push platelets toward the periphery of the vessel) during flow. In the PFA-100® system, platelet plug formation occurs under flow conditions similar to those encountered in a small or stenotic blood vessel. It is; therefore, not surprising that platelet function measured by PFA-100® is also affected by hematocrit below the normal range. Our studies in artificially-created low hematocrit samples (with normal levels of functional platelets) have indicated generally longer closure time with hematocrit of approximately 30% compared to closure times at normal hematocrit, and a statistically significant prolongation at hematocrit of 20%. The closure times do not appear to have any dependence on the variance of hematocrit within the normal range in normal platelet function subjects. It is important to understand that prolongation of closure time in low hematocrit samples is not an artifact of the PFA-100® system, although, the link between low hematocrit and lack of platelet function may not always be apparent to a user unfamiliar with the system. The prolongation of closure time is very likely a true reflection of the platelet function in a patient with abnormal hematocrit (e.g., anemia), and consequently, this information may be beneficial to a clinician involved in the hemostatic management of this patient.

Platelet Count

The clinical association of platelet count and bleeding is well documented. Thrombocytopenia (lower than normal platelet count) and thrombocytosis (higher than normal platelet count) have been definitively linked to bleeding diatheses. Since platelet plug formation in the PFA-100® system requires normal functionality as well as adequate number of platelets, abnormal PFA-100® measurements are often observed in the setting of thrombocytopenia. Our internal studies have indicated significant increase in closure times when the count of functionally normal platelets is reduced from 200,000/mL to 50,000/mL with a constant hematocrit. Several clinical studies are now underway to critically evaluate the PFA-100® system in thrombocytopenic patient populations. As with hematocrit, the closure times do not appear to be dependent on the variance of platelet counts within the normal range in normal platelet function subjects.

The Effect of Sedimentation of Blood Samples on PFA Measurements

The clinical studies conducted with the PFA-100® System have indicated that the closure time results may be influenced by the sedimentation properties of a sample. The following discussion provides insights to the effect of sample sedimentation on closure time and guidance for the PFA users concerning this issue:

Why is the PFA-100® System sensitive to sedimentation of the blood sample?

The PFA-100® system does not have an on-board sample mixing mechanism. The user is instructed to mix the sample tube or syringe by gentle inversion immediately prior to sample loading. Once the blood sample is pipetted into the test cartridge, there is no further mixing of the sample prior to or during the test. During a PFA test, the capillary aspirates the blood sample from the bottom of the sample reservoir. If the sample has a tendency to settle, a more pronounced gradient in cell density forms over time within the blood sample in comparison to what would be expected in a sample with normal sedimentation properties. Thus, within the blood sample in the test cartridge, the blood cells tend to accumulate in the bottom and the plasma accumulates in the top. When testing a sample with high sedimentation properties, the segment of the sample (accumulated in the bottom) with higher cell density is aspirated first. On occasion, these samples show aberrant closure time results (typically shorter than what would be expected with a well-mixed sample). In samples with severely impaired platelet function, occlusion of the aperture is usually not achieved and during the testing of these samples, a sudden increase in the flow rate is observed once the cellular layer passes through and plasma enters the capillary. The test usually becomes non-measurable at this point as plasma alone does not support platelet plug formation, and a status message typically follows the closure time result. The time elapsed prior to the start of a test in the PFA-100® system is typically about 3 minutes. Therefore, the potential exists for some blood samples with high sedimentation properties (e.g., severe sepsis, chemotherapy or highly hemodiluted samples such as those obtained during cardiopulmonary bypass) to sediment during the sample incubation period and influence the closure time.

What is Dade Behring's recommendation on the issue of blood sample sedimentation?

Product literature for the PFA-100® system provides proper instructions and guidance to the user regarding mixing and loading of blood samples. These instructions should be followed as closely as possible during subsequent usage of the system. The product literature also recommends measurement of samples with high sedimentation properties in singlicate and not in duplicate using both positions A and B. In a clinical setting, it may not always be possible to judge the sedimentation properties of an unknown sample. However, the origin of the sample (which department or floor it came from), diagnosis (e.g., disease state, presence of substantial hemodilution due to cardiopulmonary bypass or ECMO, etc.), and other available information (e.g., hematocrit values) may assist the user in determining whether sedimentation of the sample should be considered when testing with the PFA-100® system. If abnormally high sedimentation properties are suspected (due to any of the pertinent factors described above), PFA-100® tests should be run in singlicate (one test per run). When two patient samples are being tested in the same run, the sample from the subject with higher sedimentation properties should be tested first (in position A). The mixing of the sample tube, pipetting of blood into the cartridge and initiation of the test should be done without any intermediate delays. Shaking, tilting or inversion of the test cartridge after loading the blood sample is not recommended due to the risk of sample spillage and foaming. The user should always be conscious of the potential for sample sedimentation during the test procedure and take appropriate precautions. It is extremely important that the PFA-100® results are utilized in conjunction with the history and other clinical indications of the patient when making a clinical decision.

Commonly Used Medications that Induce Temporary Platelet Dysfunction

Antibiotics

Ampicillin
Chlortetracycline (Areomycin)
Carbenicillin
Nitrofurantoin (Furadantin)
Gentamicin
Cephalothin (Keflin)
Moxalactam
Nafcillin
Piperacillin
Quinacrine

Cardiovascular/Respiratory

Aminophylline
Clofibrate
Phenoxybenzamine (Dibenzyline)
Dicumarol
Dihydroergotamine
Dipyridamone (Persantine)
Heparin
Hydralazine
Isoproterenol (Isuprel)
Nitroglycerin
Nitroprusside
Papaverine
Propranolol
Phentolamine (Regitine)
Reserpine
Theophylline
Verapamil

Miscellaneous Drugs

Alcohol
Aminocaproic acid
Diphenhydramine (Benadryl)
Caffeine
Cyclosporine
Dextran
Glycerol guaiacolate
Hydroxyethyl starch
Hydrocortisone
Methylprednisolone
Cyproheptadine
Promethazine (Phenergan)
Methysergide maleate
Tocopherol
Tranexamic acid
Vinblastine
Vincristine

Anti-Inflammatory Drugs

Sulfinpyrazone
Aspirin
Colchicine
Ibuprofen (Motrin)
Indomethacin
Fenoprofen
Naproxen (Naprosyn)
Phenylbutazone
Mefenamic acid (Ponstel)

Psychiatric Drugs

Nortriptyline (Aventyl)
Amytriptyline (Elavil)
Desipramine (Norpramine)
Doxepin (Sinequan)
Tryfluoroperazine (Stelazine)
Chlorpromazine (Thorazine)
Imipramine (Tofranil)

Anesthetics

Cocaine
Dibucaine (Nupercaine)
Procaine
Lidocaine (Xylocaine)

Diuretics

Acetozolamide
Ethacrynic acid
Furosemide

Antiplatelet Drugs

ReoPro
Integrelin
Aggrastat
Clopidogrel
Ticlid

List of Commonly Used Medications Known to Induce Temporary Platelet Dysfunction

Common Medications Containing Nonsteroidal Anti-Inflammatory Agents

Advil
Alka-Seltzer
Anacin
Anahist
Anaprox
APC
APC w/codeine
APC w/demerol
A.S.A.
A.S.A. compound
A.S.A. compound w/codeine
Ascriptin A/D
Aspergum
Aspirin (USP)
Aspirin-childrens
Bayer
Bayer-childrens
Bayer timed release
Bufferin
Calurin
Cama inlay
Cope
Coricidin
Coricidin “D”
Coricidin Demilets
Coricidin Medilets
Darvon w/A.S.A.
Darvon-N w/A.S.A.
Darvon Compound
Dolene Compound
Dristan
Easprin
Ecotrin
Ecotrin
Empiral
Empirin
Empirin w/codeine
Emprazil
Emprazil-C
Equagesic
Excedrin
Excedrin PM
Fiorinal
Fiorinal w/codeine
Fizrin
4-way cold tablets
IBU (Ibuprofen Tablets)
Liquiprin
Lortab A.S.A.
Lodine Capsules
Measurin
Midol
Meclomen Capsules
Motrin
Nalfon
Naprosyn
Norgesic
Nuprin
PAC compound
PAC compound w/codeine
Pedia-Profen
Percodan
Ponstel
Relafen
Robaxisal-PH
Sine-Off
St. Joseph’s
St. Joseph’s for children
Super-Anahist
Synalogs
Synalogs-DC
Triaminicin
Toradol Vanquish

Medications Containing Aspirin

PRESCRIPTION	NON-PRESCRIPTION
Aggrenox	Alka-Seltzer Effervescent Tablets
Ascriptin with Codeine Tablets	Alka-Seltzer Plus Cold Medicine
A.S.A. and Codeine Compound	Anacin Tabs & Caps., Max strength
Axotal Tablets	Arthritis Str. Bufferin Tablet
Bufferin with Codeine #3 Tablets	A.S.A. Tablets
Darvon with A.S.A. Pulvules	Ascriptin Tablets
Darvon Compound-65	Ascriptin A/D Tablets
Disalcid Capsules	Aspergum
Easprin	Aspirin Tablets 5 grain
Empirin with Codeine Tablets	BC Tablets and Powder
Equagesic Tablets	Buffering Tablets
Fiorinal Tablets	Cama Arthritis Pain Reliever
Fiorinal with Codeine	Congesprin Chewable Tablets
Magan Tablets	Cope Tablets
Micrainin Tablets	Coricidin “D” Decongestant Tablets
Norgesic & Norgesic Forte Tablets	Coricidin Tablets
Pabalate-SF Tablets	Doan’s Pills
Percodan & Percodan-Demi Tablets	Ecotrin Tablets
Robaxisal Tablets	Empirim Tablets
Synalgos-DC Capsules	Excedrin Tablets & Capsules
Trilisate Tablets & Liquid	4-Way Cold Tablets
Talwin Compound	Measurin Tablets
Zorprin Tablets	Midol Caplets

IBUPROFEN

The ibuprofen medications (such as Advil, Nuprin, Motrin, etc.) also cause a tendency towards bleeding. For this reason avoid all ibuprofen medications beginning 2 days before testing.

PFA-100® Evaluation Protocol

This protocol is designed to assist the laboratory in its evaluation of the PFA-100® system. The protocol is only a recommendation, the laboratory may wish to design their own protocol.

1. Select 20 donors known to have normal platelet function. Verification of normal platelet function by aggregation and history would be ideal.

2. Test the 20 subjects in duplicate using collagen/epinephrine (col/epi) cartridges and collagen/ADP (col/ADP) cartridges.

3. Place 10 of the 20 donors on one dose of 325 mg non-enteric coated aspirin.

4. Test the subjects between 3 and 30 hours after Aspirin ingestion with both types of cartridges (col/epi and col/ADP).

5. The typical pattern seen in subjects with normal platelet function is a CT result within the reference range for both the col/epi and col/ADP cartridges (normal). In general, the pattern seen after aspirin ingestion is a CT result outside the reference range (abnormal) with col/epi and within the reference range for col/ADP (normal).

EVALUATION WORKSHEET

Instrument SN#: _____

Lot Numbers: _____

	3.2% Citrate*	3.8% Citrate
Collagen/Epinephrine reference range:	**80 - 184 sec**	**94 - 193 sec**
Collagen/ADP reference range:	**56 - 102 sec**	**71 - 118 sec**

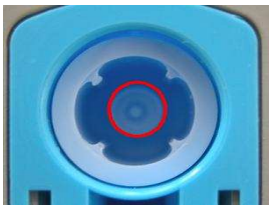
***Reference ranges in 3.2% only were determined based upon a 95% central interval of results from singlecate determinations (six US customer sites; n=120 for Col/Epi; n=118 for Col/ADP).**

DATE	SAMPLE ID	CLOSURE TIME Col/Epi (sec)	CLOSURE TIME Col/ADP (sec)	COMMENTS	TECH

The PFA-100® Self Test from the Maintenance Menu should be performed at the start of each shift the system is in use.

2.1 Self Test
(including Trigger Solution dispensing alignment check)

1. From the display **System Ready**, press the softkey located next to **[Menus].**
2. From the display **Menu**, press the numeric key **[2]** to select the option **Maintenance**.
3. Press the numeric key **[2]** to select the option **Self Test**.
4. Press the softkey located next to **[Yes]** to continue the self test.
5. 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 and B.
   Disregard the instructions displayed on the screen. According to the new recommended procedure two blue vacuum cups are required.
   Press the softkey located next to **[Continue]**.
   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]**.
6. 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.
7. After the self tests are completed, the system will print the high/low flag ranges for each test type.
8. 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.
   Press the softkey located next to **[Continue]**.
9. Remove the vacuum test cartridge from position B and inspect the trigger solution dispensing. Check that the trigger solution is dispensed on the raised platform of the vacuum cup (red circled in Figure below).
   Please note that the size and exact centering of the drop is not critical. As long as there is a drop visible on the platform, trigger solution dispensing is adequate.
   In case there is no drop visible on the platform, please call your Dade Behring Service.



Target area for the trigger solution drop on the vacuum cup

10. Remove the vacuum test cartridge from position A. Remove the vacuum test cup from both cartridges with gloved index finger and discard in a suitable biohazard waste container.
    Rinse both priming cartridges with purified water and save for further use.
11. Press the key **[Previous Screen]** twice to return to the display **System Ready**.

PFA-100® Quality Control Control Donor

As part of the instrument quality control it is recommended to test in duplicate a control donor with each new shipment of cartridges received or whenever the institution wishes to verify the performance of the system. The system will be considered under control if the mean closure time (CT) falls within the established reference range. If the mean CT is outside the reference range, repeat this procedure with a second individual from the laboratory's established control donor group. If the mean CT's from both individuals are outside the reference range, contact Technical Services. 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.

For the purpose of QC testing a control donor group should be previously established. The qualified QC donors should have a closure time near the middle of the reference range and acceptable replicate results. The reference range provided in the package insert may be used as a guideline. Dade recommends that each laboratory establish its own reference range. The following procedure is an example of how to establish the control donor group:

1. Individuals who are potential donors must be free from any medication known to affect platelet function.
2. Test each potential donor by performing two replicates with col/epi cartridges only.
3. Qualify the donor if the printed duplicate mean is within 110-160 seconds and the duplicate CV is less than or equal to 15%. (Note: This range was determined from the mean CT +/- 25 seconds of blood samples collected in 3.8% buffered sodium citrate from adult normal subjects in a US-based multicenter study. The group of 176 normal subjects consisted of 61% females and 39% males with an age range between 18 and 57 years.)

Note: The acceptable range may need to be modified depending on the mean closure time established by individual laboratories for normal subjects.

It is recommended that the laboratory run the quality control procedure in a manner consistent with its established quality control program and in conformance with local, state, and/or federal regulations or accreditation requirements.

PFA -100® Establishment of Control Donor Group Data Form

Specification:

Collagen/Epinephrine (PCE) qualifying range: 110 - 160 seconds
Duplicate CV less than or equal to 15%

Date	Donor ID	PCE Lot #	PCE Result #1	PCE Result #2	Status Code	Duplicate CV%	PCE Mean	Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected
								Accepted Rejected

Comments:

_____

_____

Technologist: _____Date: _____

O-RING MAINTENANCE

NOTE: It is recommended to perform manual O-Ring cleaning on a weekly basis. The O-Ring should be replaced on a yearly basis. The manual O-Ring cleaning procedure should be performed whenever the status message " **VACUUM TEST FAI**L" is obtained after a "Self Test" is performed from the Maintenance menu or whenever the status message " **TESTTERMINATED DUE TO AIR LEA**K" is printed after a test.

MANUAL O-RING CLEANING AND O-RING REPLACEMENT PROCEDURE

Gloves must be worn when performing this cleaning procedure.

1. Remove the O-Ring by performing **Remove O-Ring** option from the Maintenance menu.
2. Rinse the O-Ring under running tap water. Place O-Ring between forefinger and thumb and remove any debris by using a rubbing motion while rinsing under tap water. Visually inspect the O-Ring for debris or unusual wear and tear such as cracks. If the O-Ring requires replacement, follow the **Install O-Ring** option from the Maintenance menu to install new O-Ring. Discard the old O-Ring in a suitable biohazard container.
3. Shake excess water off and soak in 70% Isopropanol for approximately 15 seconds.
4. Shake excess Isopropanol off and install the O-Ring by performing **Install O-Ring** option from the Maintenance menu.
5. Perform a "Self Test" from the Maintenance Menu to verify that the system has no vacuum leak.
6. If problems persist contact Technical Services.

NOTE: The " **Ru**n" and " **Run Contro**l" functions are disabled after completion of " **Remove O-Rin**g" and " **Install O-Rin**g" options. A "Self Test" must be performed from the Maintenance Menu.

REMOVE O-RING

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 so that the cassette is flush to the carousel surface. 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" of the O-Ring Service Tool. After approximately 30 seconds, the carousel will rotate back allowing the removal of the O-Ring Service Tool.

5. Remove the O-Ring Service Tool and press the softkey located next to [CONTINUE].
6. If the O-Ring Service Tool fails to remove the O-Ring, step 4 should be repeated once more. If the Service Tool fails to remove the O-Ring, contact Technical Services*****.
7. Invert the O-Ring Service Tool and tap against the palm of your gloved hand to remove the O-Ring. Follow the Manual O-Ring Cleaning or Replacement Procedure.
***** Before contacting Technical Services clean O-ring with cleaning pad and isopropyl alcohol as described below then repeat step 4. If the tool fails to remove the O-ring after this procedure contact Technical Services..

INSTALL O-RING

NOTE: To minimize dirt or debris accumulating on the O-Ring Service Tool, maintain tool stored in the packaging provided. Make sure the O-Ring placement surface in position "B" of the O-Ring Service Tool is free of debris before loading the O-Ring. If required, clean the O-Ring placement surface with Isopropanol.

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 [7] to select Install O-Ring option. The system will display the message "Load O-Ring, Service Tool, Then Press Continue".
4. Load O-Ring in Position "B" of the O-Ring Service Tool. Place the O-Ring Service Tool into the incubation wells of the instrument so that the cassette is flush to the carousel surface. 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 the O-Ring Service Tool. After approximately 30 seconds, the carousel will rotate back allowing the removal of the O-Ring Service Tool.
5. Remove the O-Ring Service Tool and press the softkey located next to [CONTINUE].
6. Perform a "Self Test" from the Maintenance Menu to verify that the system has no vacuum leak. When performing the "Self Test" to insure proper installation of O-Ring, it is not necessary to insert a cleaning pad
when prompted, press continue to proceed with the "Self Test".

PFA-100® QC AND MAINTENANCE FORM

MONTH/YEAR _____ **Col/Epi Ref. Range _____** **Col/ADP Ref. Range _____**

DAILY PM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30 31
CLEAN WORK SURFACE																														
CHECK PRINTER PAPER																														
CHECK TRIGGER SOLUTION																														
PERFORM SELF TEST																														
SELF TEST PASS/FAIL																														
WEEKLY PM																														
CLEAN AND INSPECT O-RING																														
YEARLY PM																														
REPLACE 0-RING																														
TECH INITIALS																														

QC	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30 31
COL/EPI CLOSURE TIME																														
LOT NUMBER																														
COL/ADP CLOSURE TIME																														
LOT NUMBER																														
TECH																														

PFA-100® QC AND MAINTENANCE FORM

MONTH/YEAR _____ Col/Epi Ref. Range _____ Col/ADP Ref. Range _____

CORRECTIVE ACTION

DATE	PROBLEM	ACTION TAKEN	TECH

SUPERVISOR REVIEW	DATE

Estimating a Normal Reference Interval for PFA-100®

The procedure described below is an adaptation from the NCCLS document C28-A (June 1995) on transferring a reference interval from manufacturer's claim to clinical laboratories. The procedure can be used for each test cartridge type col/epi and col/adp.

- Test a minimum of 40 ostensibly healthy adult individuals with no previous history or laboratory results indicative of platelet dysfunction. A confirmation of normal platelet function for these subjects at the time of blood sampling for PFA-100® is preferable. An even distribution by gender is recommended. As a general rule, test a minimum of 30% subjects from each gender.

- Testing should be performed over a period of several days and by different personnel, if possible, to minimize day to day variation.

- Donors must not be on any medications, especially aspirin or any medication containing aspirin. You may select donors using a sample collected in 3.8% sodium citrate anticoagulant. Accept donors whose closure time falls within the reference interval listed in the package insert. Test qualified donors with samples collected in the anticoagulant used in your laboratory.

- Rank the closure times in ascending order and remove the lowest and highest values. The range for the resulting data after removing the two extreme values constitutes a 95% reference interval. The larger the number of subjects the more reliable the reference interval.

- If the resulting reference interval does not compare well with the manufacturer's reference interval a more comprehensive study should be conducted (see above mentioned NCCLS guideline).

REFERENCE INTERVAL WORKSHEET

Instrument SN#: _____

Cartridge Type: _____

Lot Number: _____

Citrate Concentration: _____

	DATE	SAMPLE ID	CLOSURE TIME (SEC)	Male □ Female □	TECH.
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

REFERENCE INTERVAL WORKSHEET

Instrument SN#: _____

Cartridge Type: _____

Lot Number: _____

Citrate Concentration: _____

	DATE	SAMPLE ID	CLOSURE TIME (SEC)	Male □ Female □	TECH.
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
33					
34					
35					
36					
37					
38					
39					
40					

GUIDE TO COMMON STATUS MESSAGES

The following list of messages is uniquely associated with events or conditions detected during a test run. These messages report events that occur only when blood is flowing through the test cartridge. The instrument flags the event or condition with a “>” sign followed by the time of the event or condition, the status code letter and a description of the event. For example, > 300A SEC MAX TEST TIME EXCEEDED.

1. Maximum test time exceeded (A)
2. Air leak (B)
3. Flow obstruction (C)
4. Insufficient sample (D)
5. Maximum syringe travel reached (E)

MAXIMUM TEST TIME EXCEEDED (A)

Description	• The sample did not achieve closure of the aperture within the maximum time for a test (>300 seconds, not including the incubation period).
Causes	1. Sample with abnormal platelet function resulting in non-closure (NC) of the aperture. 2. A possible vacuum leak in the system causing a NC. 3. Defective test cartridge.
Solutions/Comments	1. If a NC is obtained with a normal donor control or the result does not agree with the patient's clinical history a possible vacuum leak may be suspected. Perform a Self Test via the Maintenance Menu without loading the O-ring cleaning pad. This will test if dirt or debris on the O-ring is the cause for the vacuum leak. If Self Test passes a defective cartridge may have caused the vacuum leak. If the vacuum leak test fails, perform the manual O-Ring maintenance procedure via the Maintenance Menu and rerun the Self Test. Cleaning or replacing the O-ring may correct the vacuum leak. If problem persists, contact Technical Assistance Center. 2. Rerun the sample with new cartridge to verify the test result. If daily Self Test OK then most likely this is an abnormal sample.
Result Interpretation	• Report as CT > 300 sec, abnormal platelet function as confirmed by retest.

Test Terminated Due to Air Leak (B)

Description	The instrument has detected an initial air leak in the vacuum system. This condition is detected only at the beginning of a test.
Causes	• Possible vacuum leak in the system or a malfunctioning trigger solenoid pump. • No sample in test cartridge. • Air entrapped in fluid lines. • Air entrapped in test cartridge when sample was loaded. • Defective test cartridge.
Solutions/Comments	• Verify that sample was added to test cartridge. Rerun the test with appropriate sample volume (800 µL). • Perform a Self-Test via the Maintenance Menu without the O-Ring cleaning pad to test the vacuum system. If the vacuum test passes, a malfunctioning solenoid pump may be the cause for the air leak. Contact Technical Assistance Center for solenoid pump troubleshooting procedure. If the vacuum test fails, perform manual O-Ring maintenance procedure via the Maintenance Menu. Clean or replace O-Ring as per maintenance instructions. Perform Self Test. If vacuum leak test passes rerun sample. If problem persists contact Technical Assistance Center. • Prime system from Maintenance menu. • Rerun the test with a new cartridge and sample. • Rerun the test with a new cartridge and sample.
Result Interpretation	• Non-reportable.

Test Terminated Due to Flow Obstruction (C)

	Test Terminated Due to Flow Obstruction
Description	• The instrument has detected a sudden stoppage of blood flow during the test. A flow obstruction can occur at the start of a test or during a test a run. 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 specifications subsequent to the first 30 seconds of testing. The instrument aborts the run if these specifications are not met. The result is reported as "Test Terminated Due to Flow Obstruction". This condition may be caused by microthrombi in the sample or particulates introduced into the sample or test cartridge from the environment. 2. Flow Obstruction During a Test - A flow obstruction that occurs after initial blood flow rate conditions have been established is reported by the instrument at the time the flow obstruction occurs; i.e. " > 80C sec Flow Obstruction". This type of flow obstruction occurs when the capillary or the test cartridge membrane aperture is suddenly plugged by microaggregates that may form during the test or by particulates introduced into the sample or test cartridge from the environment. 3. Defective test cartridge.
Solutions/Comments	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 have resulted from problems during blood collection. Recollect blood sample and repeat test. 3. If problem persists, contact Technical Assistance Center.
Result Interpretation	• Non-reportable.

Test Terminated Due to Insufficient Sample (D)

Description	• The system has detected air being drawn subsequent to the first 30 seconds of testing. This 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 in the test cartridge. 2. Sufficient sample was loaded in the test cartridge but closure of the aperture did not occur due to platelet dysfunction and/or low sample viscosity (↓ hematocrit, high sedimentation rate).
Solutions/Comments	1. Verify that 800µL was added to the cartridge. Rerun sample using 900µL. 2. Verify sample hematocrit is within normal range. Refer to the package insert (Limitations of Procedure section) for further information. An abnormal hematocrit may impair platelet function and result in prolongation of the closure time. Platelet dysfunction in combination with low hematocrit will often induce this type of status message. Samples from patients treated with platelet antagonist drugs may exhibit characteristics that cause this type of status message. Adding additional specimen (up to 900µL) could eliminate the insufficient sample message but a combination of low hematocrit and platelet dysfunction will most likely result in a closure time >300 sec. • The test cartridge cup serves as the receptacle where blood is collected after it passes through the membrane aperture. During a test, the instrument vacuum chuck interfaces with the cup and comes in close proximity to blood. Air that is drawn into the cup whenever an insufficient sample occurs can cause blood to foam and contaminate either the vacuum chuck or O-ring. It is recommended to perform manual cleaning of the O-ring with the cleaning pad to avoid potential contamination.
Result Interpretation	• An insufficient sample message that occurs at a time that is greater than the upper limit of the reference range may indicate abnormal platelet function due to the reasons stated above. In such cases this result may be reported as the time in which the test ended,"> xxx sec", with a statement qualifying the properties of the sample (i.e. abnormal hematocrit and/or low platelet count) and suspicion of platelet dysfunction.

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. Sufficient sample in the cartridge but closure did not occur due to platelet dysfunction and/or low viscosity (↓hematocrit, high sedimentation rate). 2. A defective test cartridge causing a small vacuum leak. 3. Dirt or debris is present on the vacuum seal between the instrument and the test cartridge, causing a small vacuum leak. 4. Instrument malfunction causing a small vacuum leak.
Solutions/Comments	1. Perform a Self Test via the Maintenance menu without the O-Ring cleaning pad to rule out vacuum leaks. If no errors are reported by the Self Test the system is in control. 2. Verify sample hematocrit before performing a second test. If hematocrit is abnormal the sample may have low viscosity which may induce platelet dysfunction. Refer to the package insert (Limitations of Procedure section) for further information. An abnormal hematocrit may impair platelet function and result in prolongation of the closure time. Platelet dysfunction in combination with low hematocrit will often induce this type of status message. Samples from patients treated with platelet GPIIb/IIIa antagonist drugs may exhibit characteristics that may induce this type of status message. Adding additional specimen (up to 900µL) could eliminate maximum syringe travel but will most likely result in a closure time >300 sec. If the repeat result confirms the abnormality, platelet dysfunction may be suspected possibly due to abnormal hemodynamic properties of the sample and/or anti-platelet agents. 3. If problem persists contact Technical Assistance Center.
Result Interpretation	• A maximum syringe travel message that occurs at a time that is greater than the upper limit of the reference range may indicate abnormal platelet function. The result may be reported as “> xxx E sec” only if the time lies above the reference range. The report should include a statement qualifying the abnormal qualities of the sample and suspicion of platelet dysfunction.

Communication to Medical Staff

One of the final steps after understanding the characteristics and performance of the PFA-100® system, is the communication of information to the laboratory customers. It is important to plan ahead and begin communication early when introducing a new test to the medical staff. Physician education is the key to successful implementation. Elicit support from the pathologists and laboratory director. Insure that all questions are answered prior to the start date.

Who should be informed?

- Laboratory staff
- Physicians: house staff: Hematologists, Anesthesiologists, Internists, Surgeons, Cardiologists. Include residents, interns and medical students
- Physicians who refer patients to the lab for testing
- Nursing staff
- Out patient clinics

How should they be informed?

- Memos Example included on following page
- Posters/Flyers
- Hospital Computers
- Grand Rounds
- Presentations at key department meetings (CV surgery, Medicine, Anesthesiology, Hematology)

What to supply when asked for additional information.

- Question and answer sheet See enclosed
- Recent publications See Bibliography enclosed
- PFA-100® information sheet Can be provided by Dade Behring

To: Medical Staff and Nursing Staff

From: Laboratory Manager

The laboratory is pleased to announce the introduction of a new technology for evaluating platelet function, PFA-100®, Platelet Function Analyzer. The PFA-100® is the first *in vitro* test system designed for clinical laboratory use that is capable of detecting platelet dysfunction under high shear flow conditions in a small citrated whole blood sample. Two types of test cartridges are used with the system, Collagen/Epinephrine or Collagen/ADP. 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, such as Aspirin® or Aspirin® containing medication. 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 Aspirin® or medications containing Aspirin®.

The instrument aspirates a blood sample under constant vacuum from the sample reservoir through a capillary and a microscopic aperture cut into the membrane of the test cartridge. The membrane is coated with collagen and epinephrine or ADP. The presence of these biochemical stimuli, and high shear rates generated under standardized flow conditions that simulate *in vivo* vascular injury, result in platelet attachment, activation and aggregation, slowly building a platelet plug at the aperture. The time required to obtain occlusion of the aperture is reported as the "closure time"(CT). The CT is indicative of the platelet function in the sample.

Beginning (Date) the Laboratory will offer both tests, Epinephrine and ADP on the PFA-100®. The patient results will be reported as closure time in seconds (CT). The reference range for both types of cartridges are as follows:

Specimens collected in 3.8% (0.129 M) buffered sodium citrate

Cartridge Type	**Mean (sec)**	**Reference Range (n=176)**
Collagen/Epinephrine	132	94-193
Collagen/ADP	92	71-118

Specimens collected in 3.2% (0.1 M) buffered sodium citrate

Cartridge Type	**Mean (sec)**	**Reference Range (n=309)**
Collagen/Epinephrine	110	82-150
Collagen/ADP	78	62-100

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

If you should have any questions or concerns, see the attached "Questions and Answers" or please feel free to contact me at extension _____.

PFA-100® Platelet Function Analyzer
Questions and Answers

What is the PFA-100® test?

The PFA-100® is a high shear flow system that measures platelet adhesion and aggregation in citrated whole blood. The PFA-100® test induces platelet activation as blood is made to flow through an aperture cut into a membrane that is coated with collagen fibrils and epinephrine (CEPI) or collagen fibrils and ADP (CADP). The time taken for blood to form a platelet plug that occludes the aperture is an indication of global platelet function and is referred to as the Closure Time (CT). The system is used as an aid to assess platelet dysfunction.

What is the mechanism of platelet plug formation measured by PFA-100®?

In vitro studies performed to characterize the mechanism of platelet plug formation revealed that von Willebrand Factor (vWF) is the key adhesive protein that mediates platelet adhesion and aggregation in the PFA-100® test cartridge. Other studies have confirmed that in a high shear environment it is vWF and not fibrinogen that mediates platelet aggregation. Consequently, clinical studies have confirmed that the CT is highly sensitive to von Willebrand disease.[1-4]

Likewise, the CT is highly sensitive to qualitative and quantitative defects to platelet receptors that mediate adhesion (glycoprotein Ib/IX complex) and aggregation (glycoprotein IIb/IIIa).[5-6]

Thus, the CT is sensitive to inherited or acquired defects in platelet function.

What other factors influence the closure time?

Closure Time above the 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. In simulated thrombocytopenic samples with normal platelet function a marked prolongation of the CT was observed at platelet counts between 50,000/mL to 100,000/mL.[5] Similarly, in artificially created erythropenic blood samples with normal platelet function the CT increased significantly below hematocrit values of 30%.[6] A prolonged closure time caused by low platelet counts and/or low hematocrit is not necessarily an artifact of the PFA-100 test. The test is effectively simulating reduced primary hemostasis as would occur in vivo when these blood parameters are affected by underlying disease states.[7]

Differences in closure time may be obtained depending on the blood collection system used. In a limited study, on 36 apparently normal subjects, up to 12% shorter closure times were observed for samples collected in 3.2% (0.105 M) vs. 3.8% (0.129M) buffered sodium citrate.[8]

Platelet inhibiting agents , such as aspirin, NSAID's, glycoprotein IIb/IIIa antagonists (ReoPro, Integrelin and Aggrastat) and Plavix directly inhibit platelet function. The list of medications that affect platelet function is extensive. Certain foods, vitamins and herbs have been reported to induce platelet dysfunction.[9]

What is the reproducibility of the test?

The simplicity of the PFA-100 test belies the complexity of the reaction occurring in the cartridge membrane. The test is measuring the biological function of blood cells in a whole blood environment at high shear rates. Specifically, the test monitors the hemostatic capacity of platelets to adhere, aggregate and occlude a microscopic aperture in an environment that mimics the in-vivo process. It is therefore not surprising to find that the CV of this biological reaction is typically around 10% to 12%. In addition, the greatest amount of variability can be attributed to the within donor component. Various studies have shown that platelet receptor phenotype polymorphisms, platelet vWF content, blood group and diurnal variations can be potential causes for inter-individual variability.[10-13]

An imprecision study was conducted to characterize the variability of the PFA-100® system. The samples used in this study were collected in evacuated tubes containing 3.8% (0.129M) buffered sodium citrate.

The study was performed on three cartridge lots for each type of cartridge. Blood samples were collected from four subjects with normal platelet function on three separate days over a period of eight days. Six replicates from each subject were tested on each day with both types of cartridges. Results are as follows:

	COL/EPI	COL/ADP
Within lot CV	12.4%	12.7%
	(8.8 - 14.0)	(10.9 - 15.9)
Between lot CV	0.6%	4.0%
	(0.0 -1.1)	(0.0 - 7.4)

In addition, data collected during a multicenter study was analyzed for variability due to test position. This data represented results from 176 subjects with normal platelet function tested in positions A and B. The duplicate CV was calculated at 13.5% for COL/EPI cartridges and 10.0% for COL/ADP cartridges. Results by site ranged from 10.4% to 17.4% with COL/EPI cartridges and from 7.7% to 11.2% with COL/ADP cartridges. For samples with closure time near the cutoff, the duplicate CV's are 13.7% for COL/EPI cartridges and 10.0% for COL/ADP cartridges.

Interpretation of Results

The CT is dependent on platelet function, von Willebrand Factor level, platelet count and hematocrit. It is important for each laboratory to establish its own reference ranges (non-parametric procedures from NCCLS document C28-A may be used as a guideline). If possible a CBC may be run to ensure that specimens used to establish the reference range have normal hematocrit and platelet count. The upper limit of the

reference range is used as the cut-off value to assess platelet function. Closure times above the cut-off indicate abnormal platelet function. On the other hand, the clinical significance of CT below the lower limit of the normal reference range (i.e. more rapid closure) has not been fully determined. Results should always be evaluated in conjunction with clinical history and other laboratory findings (i.e. platelet aggregometry). In cases where PFA-100® results do not agree with the clinical assessment, additional tests should be performed.

Expected Patterns

Col/Epi normal and Col/ADP normal – Platelet function is normal. If patient history/physical examination give strong indication of a bleeding disorder repeat testing for confirmation. If still normal, consider testing for factor deficiencies.

Col/Epi abnormal and Col/ADP normal – This pattern is indicative of drug induced platelet dysfunction. Most commonly seen after aspirin ingestion. Review patient chart information for prescription and non-prescription medication.

Col/Epi abnormal and Col/ADP abnormal – 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. If the patient is not anemic, thrombocytopenic or has cardiovascular or renal disease, the underlying reason for the abnormality should be determined. Most patients with prolonged Col/ADP closure times manifest abnormalities in primary hemostasis that could potentially place them at an increased risk of bleeding during a surgical procedure.[14]

NOTE

1. The Co/Epi abnormal and Col/ADP normal pattern has also been reported in patients with storage pool deficiency and platelet secretion defects.[11]

2. Although rare, some investigators have reported Col/Epi normal and Col/ADP abnormal pattern with Type 1 vWD patients.[1]

The performance of PFA-100 has not been defined in any of these two conditions.

PFA-100 test results should always be evaluated in conjunction with clinical history and other laboratory findings (i.e. platelet aggregometry). In cases where PFA-100® results do not agree with the clinical assessment, additional tests should be performed.

Reporting Closure Time Results

The following table provides an example of how PFA-100 results may be reported.

Test Cartridge	Patient Closure Time	Platelet Function Status	Normal Range
Col/EPI	199 seconds	Abnormal	94 – 193
Col/ADP	96 seconds	Normal	71 – 118
Platelet count	256,000/µL		
Hematocrit	37%		
Interpretation	Results suggest drug impaired platelet function, most commonly due to aspirin or NSAID's. Review patient medication history.		

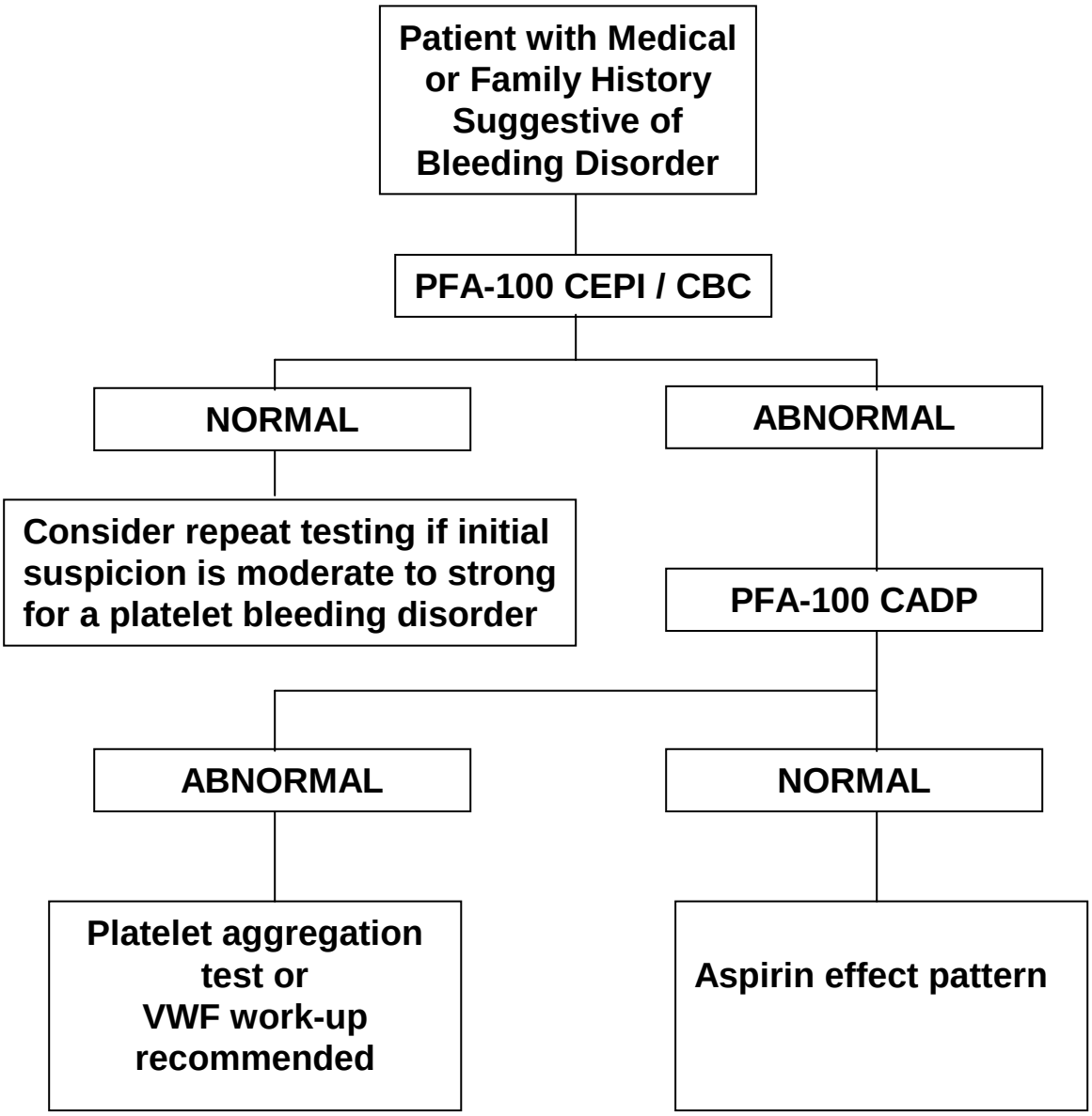
Patient platelet count and hematocrit may also be reported. These parameters aid in the interpretation of results. Normal closure times in samples with low hematocrit (< 35%) indicate that primary hemostasis under the conditions tested is not affected by the reduced red cell count. Likewise, normal closure times in samples with platelet counts below < 150,000/µL suggest normal platelet function in the platelet population tested. However, intrinsic platelet dysfunction cannot be ruled out when abnormal closure times are obtained with thrombocytopenic or anemic samples. The association between low hematocrit and impaired platelet function is well recognized.[15,16] The fact that closure times are prolonged when red blood cell mass is reduced suggests that PFA-100 is able to simulate in-vivo hemodynamic conditions. Thus PFA-100 may offer an assessment of platelet hemostatic capacity when platelet function is tested in samples with sub-optimal hemodynamic conditions.

Assessment of Platelet Function

The PFA-100® test is initially performed with the Col/Epi test cartridge. Col/Epi is sensitive to vWD, platelet disorders, the effects of aspirin and other platelet inhibiting drugs. A normal value is indicative of normal platelet function. A positive Col/Epi result may be indicative of drug-induced platelet dysfunction. A second measurement with the Col/ADP test cartridge is recommended to differentiate a drug-induced vs. a congenital platelet defect. A negative Col/ADP test indicates drug-induced platelet dysfunction caused by aspirin. A positive Col/ADP test warrants further investigation particularly when this test is significantly prolonged or reaches maximum test time (CT > 300 sec). Platelet aggregation tests and/or vWF work-up is recommended.

The following PFA-100® algorithm may be used as an aid in the assessment of platelet dysfunction when a congenital or acquired platelet defect is suspected in patients with a medical or family history suggestive of bleeding disorder.

PFA-100 Algorithm



Abnormal Platelet Function

Positive results with both test cartridges indicate abnormal platelet function. However, the effects of low platelet counts (< 150,000/mL) or hematocrit (<35%) should be first excluded. If both platelet count and hematocrit are normal the closure time result is indicative of platelet dysfunction. It is suggested that the patient be referred to a hematologist for further evaluation. Since the PFA-100® test has been shown to be very sensitive to von Willebrand disease as well as inherited/acquired platelet defects it is suggested that appropriate follow up should include: 1) a thorough medical history, 2) vWF:RCof and vWF:Ag determination and 3) platelet aggregation /release profile.

The Aspirin Response

Early in the development of PFA-100®, particular attention was paid to the system's sensitivity to the most widely used anti-platelet agent, aspirin (ASA). Homoncik et al. demonstrated the high sensitivity of PFA-100® to low dose ASA (<100 mg) on normal subjects but also highlighted the existence of a significant variability of response among individuals, due at least in part to plasma levels of von Willebrand factor (vWF).[17]

Aspirin-like Defect

An abnormal result with the Col/Epi followed by a normal Col/ADP is the classic "aspirin pattern" detected with the PFA-100® test. This pattern suggests that the most likely cause of the positive result with Col/Epi be due to aspirin or similar medication. In many cases, the results with the Col/Epi test cartridge will be reported as > 300 sec. In contrast, the Col/ADP result will fall within the normal range. In relatively few cases the Col/ADP result may be slightly prolonged (i.e. less than 10 sec below the cut-off). If the patient denies aspirin intake it is suggested to follow up with platelet aggregation/release studies to exclude congenital platelet defects. The aspirin pattern has also been observed in a limited number of patients with storage pool and release defects.[11] The prevalence of these defects is unknown but may be more common than previously recognized. Platelet aggregation/release studies are being used to diagnose storage pool and release defects.

Aspirin Resistance

Various studies have estimated that 8% to 45% of the population do not respond to aspirin. Poor response to aspirin has been documented in healthy controls, cerebral vascular disease and cardiovascular disease. Although the molecular mechanism of aspirin resistance have yet to be discovered there is increasing evidence that the Col/Epi test cartridge may play a role in the detection of aspirin non-responders.[18] In patients with acute coronary syndromes the incidence of aspirin non-responders has been reported to be as high as 50% and in one study was even shown to be associated with a higher risk of death at 6 months post-first ACS.[19] However, the clinical significance of aspirin resistance in patients with ACS remains to be determined in prospective trials.

Thienopyridines

Thienopyridines (Ticlopidine®, Clopidogrel®) have become common anti-platelet agents in cardiology. Although limited, information on PFA-100®'s sensitivity to thienopyridines has become available this past year. First, Raman reported that daily ingestion of 75 mg of Clopidogrel® was usually not accompanied by a significant prolongation of the PFA-100® CT's in stroke patients after 5 days.[20] But when followed for up to 4 weeks, some patients showed markedly prolonged CT's while others remained at the same baseline level. Second, Fischetti et al. compared patients on ASA and ASA+Ticlopidine® before and 24 hrs. after angiography and angioplasty, respectively.[21] They found that both procedures induced hyper-aggregability (i.e. shorter CT's) at 24 hrs. but that ASA+Ticlopidine® treatment failed to suppress this phenomenon. The results from these two investigations raise the legitimate question of whether or not PFA-100® has an adequate sensitivity to thienopyridines. In absence of standard platelet aggregometry results and more importantly because of the lack of clinical outcome data to explain these results, understanding their significance is pure speculation at this point. It is important to note that it is becoming more and more common to administer a high loading dose of Clopidogrel® prior to angioplasty. In a small study, van der Planken et al. reported that both PFA-100® and standard aggregometry detected very early the effect of a bolus of 375 mg or 450 mg Clopidogrel® on platelet function in patients already on ASA.[22]

GPIIb/IIIa Antagonism

In the past several years, the use of GPIIb/IIIa inhibitors (ReoPro®, Aggrastat®, and Integrilin®) has become fairly routine in certain angioplasty settings to further reduce the risk of thrombotic events. In a recent publication, Madan et al. evaluated the performance of PFA-100® during standard ReoPro®-assisted angioplasty.[23] Results obtained with PFA-100® were similar to those obtained by aggregometry and a receptor occupancy assay. PFA-100® detected maximal platelet inhibition (CT > 300 sec.) in 24 of 25 patients post-bolus. Maximal platelet inhibition was maintained throughout the ReoPro® infusion and variable degrees of recovery from platelet inhibition were observed at 24 hrs. Interestingly, a post-procedural myocardial infarction occurred in one patient. Despite having received the standard ReoPro® bolus and infusion, this subject failed to achieve significant levels of platelet inhibition (as measured by PFA-100®) immediately after the ReoPro® bolus. Platelet aggregometry and receptor occupancy studies confirmed the apparent lack of initial platelet inhibition in this individual. Similar results were found by Hezard et al. suggesting the potential of PFA-100® in verifying the inhibition of platelet function during this type of procedure.[24]

CLINICAL UTILITY OF PFA-100

Can the PFA-100® test replace the Bleeding Time to predict surgical bleeding?

The bleeding time (BT) test was originally designed as an aid in the diagnosis of platelet dysfunction. The use of the BT as a preoperative screening to predict bleeding during surgical procedures was implemented as routine clinical practice without supporting evidence of its clinical utility. Several studies have shown that the preoperative BT lacks clinical benefit.[25,26] Recently, a position article by the College of American Pathologists and the American Society of Clinical Pathologists concluded that:

(1) In the absence of a history of a bleeding disorder, the bleeding time test is not a useful predictor of the risk of hemorrhage associated with surgical procedures. (2) A normal bleeding time does not exclude the possibility of excessive hemorrhage associated with invasive procedures. (3) The bleeding time cannot be used to reliably identify patients who may have recently ingested aspirin or non-steroidal anti-inflammatory agents or those who have a platelet defect attributable to these drugs. In the absence of a history of excessive bleeding, the bleeding time fails as a screening test and is, therefore, not indicated as a routine preoperative test.[27]

Furthermore, Lehman et. al., recently reported no adverse clinical impact upon discontinuation of the BT test at a busy tertiary-care, university medical care center.[28]

PFA-100 evaluation studies were not designed to predict bleeding during surgical procedures. To date, there is no evidence that the PFA-100 test can predict perioperative bleeding. In fact, the best pre-operative screening test available remains a detailed clinical history that includes family, surgical and drug histories. In the absence of well documented clinical history or in cases were there is clinical history suggestive of a bleeding disorder, the PFA-100 test can be used to rule out platelet dysfunction or von Willebrand Factor defects.

Can the PFA-100 be used as a pre-operative screening test?

The PFA-100 test should be used to decide whether patients with suspected bleeding disorders have von Willebrand disease or a significant platelet adhesion or aggregation disorder prior to surgical procedures known to have higher risk for hemorrhagic complications. Patients with underlying medical conditions often have an acquired platelet defect that may lead to excessive bleeding during a surgical procedure. In this context, the PFA-100 test can only be used to assess the platelet related bleeding risk in order to manage the overall risk of bleeding.

If the intent of the test is to detect the presence of aspirin or other anti-platelet drugs prior to surgery, the PFA-100 test is well suited for this purpose. If the “aspirin pattern” (abnormal Col/Epi but normal Col/ADP) is observed and aspirin ingestion is confirmed by history the surgeon should determine how to manage the bleeding risk in conjunction with the patients clinical history and other laboratory tests. On the other hand, a normal Col/Epi result with confirmed aspirin ingestion might indicate sub-optimal platelet inhibition (i.e. “aspirin resistance”). Normal closure times with Col/Epi have been reported in patients on low dose (< 100 mg ASA) as well as high dose (> 325 mg ASA) aspirin therapy. In both normal subjects and patients the individual response to aspirin has been shown to be highly variable. The aspirin response can be modified by other blood cells, aspirin dose, duration of therapy and the time of testing after aspirin ingestion. All of these factors will impact the closure time. Recovery of platelet function

as assessed by PFA-100 test has been documented in normal subjects within 24 hours post aspirin ingestion. However, there have not been any PFA-100 studies that address whether patients not responding to aspirin therapy would bleed or not bleed during surgery. Likewise, there are no PFA-100 studies showing that patients responding to aspirin have excessive bleeding during surgery. It is doubtful whether any such studies have been performed with platelet aggregometry or the bleeding time. What is important is that the PFA-100 can assess the patient's response to aspirin and thereby provide the clinician with useful information that can lead to better management of platelet related bleeding risk.

Can the PFA-100 be used to assess post-operative bleeding?

The intended use of PFA-100 is to aid in the detection of platelet dysfunction. If the intent of the test is to assess platelet function in the bleeding patient the closure time is a good indicator of global platelet function. In a recent study closure times with the Col/ADP cartridge were shown to increase during CABG. Interestingly, after protamine neutralization closure times returned to baseline values in approximately 90% of patients. The specificity to identify patients with low bleeding risk was 80% and negative predictive value was 96%. The authors suggest that post-op prolongation of the Col/ADP closure time may warrant use of platelet concentrates in a bleeding CABG patient.[29] The utility of the closure time to identify CABG patients who may not benefit from platelet transfusions was further investigated in a retrospective analysis of Col/ADP closure time measured after the administration of protamine.[30] In this study, normal closure times identified a subgroup of patients whose bleeding could not be controlled by platelet transfusions alone and required other means. This was in sharp contrast to a second group of patients with prolonged closure time whose bleeding was controlled by platelet transfusion only. These studies suggest that closure time may be able to discriminate platelet related bleeding from other causes. Nevertheless, post-op bleeding is multi-factorial in nature and these findings warrant further studies to determine the clinical utility of the closure time in cardiothoracic surgical procedures involving extracorporeal circulation.

Can the PFA-100 be used to assess therapeutic monitoring of vWD?

Desmopressin (DDAVP) is the treatment of choice for Type 1 vWD and has been used to treat patients with congenital defects of platelet secretion. This analog of vasopressin has antidiuretic properties and releases von Willebrand factor from storage sites in endothelial cells. A rise in von Willebrand factor has been demonstrated to occur in normal subjects and in certain subtypes of vWD after infusion of DDAVP. Fressinaud reported a normalization of the closure time with both types of test cartridges in all Type 1 vWD patients following infusion of DDAVP.[31] Similarly, Cattaneo observed a shortening of the closure time in patients with platelet secretion defects or Type 1 vWD post DDAVP therapy.[2,11] Cattaneo et. al., found that the quantity and quality of platelet vWF in Type 1 vWD patients has a significant effect in the response to therapy as measured by the PFA-100. The closure time in these patients post treatment were unchanged from baseline values. These results suggest that the PFA-100 may be a useful tool to aid in the therapeutic monitoring of patients with vWD.

The following tables list the overall sensitivity of the Col/Epi and Col/ADP test cartridge in various bleeding disorders.[32]

Table 1. PFA-100 in Various Bleeding Disorders Col/Epinephrine Test Cartridge

	Hemophilia			Platelet Dysfunction					Von Willebrand disease subtype							
	FVIII	FIX	FXI	GT	BSS	SPD	PSD	HPS	1	2A	2B	2N	2M	3	Acquired.	Pseudo/ platelet
Positive cases	0/26	0/5	0/1	14/14	2/2	12/16	4/9	10/11	154/174	33/33	33/36	0/3	12/12	31/31	8/8	4/4
Sensitivity (%)	0	0	0	100	100	75	44	91	89	100	92	0	100	100	100	100
				Overall Sensitivity 81% n = 52					Overall Sensitivity 92% n = 301							

Notes: Values given for positive cases are total number of samples with prolonged CT/number of samples tested per group.

Abbreviations: GT, Glanzmann thrombasthenia; BSS, Bernard Soulier Syndrome; SPD, storage pool disorder; HPS, Hermansky-Pudlak Syndrome; PSD, primary secretion disorder.

Table 2. PFA-100 in Various Bleeding Disorders Col/ADP Test Cartridge

	Hemophilia			Platelet Dysfunction					Von Willebrand disease subtype							
	FVIII	FIX	FXI	GT	BSS	SPD	PSD	HPS	1	2A	2B	2N	2M	3	Acquired.	Pseudo/ platelet
Positive cases	0/26	0/5	0/1	14/14	2/2	6/16	0/9	4/11	142/174	33/33	33/36	0/3	12/12	31/31	8/8	4/4
Sensitivity (%)	0	0	0	100	100	38	0	36	82	100	92	0	100	100	100	100
				Overall Sensitivity 50% n = 52					Overall Sensitivity 87% n = 301							

Notes: Values given for patient positive cases are total number of samples with prolonged CT/number of samples tested per group.

Abbreviations: GT, Glanzmann thrombasthenia; BSS, Bernard Soulier Syndrome; SPD, storage pool disorder; HPS, Hermansky-Pudlak Syndrome; PSD, primary secretion disorder.

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NCCLS

Procedure Guidelines for the PFA-100®

This publication is intended only as a reference and formatting tool and is provided solely for your convenience. Reference should be made to the current package inserts, manufacturer's operating manuals and instructions and your standard operating procedures in preparing procedure manuals for particular applications. The information provided in this guide is based on current product information and equipment specifications available at the time of publication. The user is solely responsible for the updating and review/approval of the contents of the final manual. This review/approval is to be performed prior to adoption of the manual by the laboratory.

Procedure: PLATELET FUNCTION ANALYZER (PFA-100®)

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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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*. The PFA-100® can be used as an aid in the detection of platelet dysfunction in citrated human whole blood.

. The single use PFA-100® test cartridge consists of a capillary, a sample reservoir and a biochemically active membrane with a central aperture. The membrane is coated with collagen, 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.

Anticoagulated whole blood is aspirated from the sample reservoir through the capillary and the aperture, which expose platelets to high shear flow conditions. During the test, platelets adhere to the collagen-coated membrane, platelets become activated and release their granule contents upon contacting the agonists. The release 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 and formation of a platelet thrombus at the aperture thereby gradually diminishes and arrests 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 as the Closure Time (CT). The CT is an indicator of platelet function in the analyzed whole blood sample

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 acetyl salicylic acid (ASA) or medications containing ASA.

SPECIMEN:

All investigations of platelet function are strongly dependent on the correct method of blood collection. The collection method (both citrate concentration and venipuncture method) should be kept consistent.

No special patient preparation is required.

Type:

Using a 21G or larger needle, blood should be drawn directly into an evacuated plastic or siliconized glass tube or syringe containing 3.8% (0.129M) or 3.2% (0.105M) buffered sodium citrate (1 part anticoagulant to 9 parts blood).

Use of unbuffered sodium citrate anticoagulant is NOT recommended.

After sample collection, ensure proper mixing of anticoagulant by gently inverting the tube by hand 3 to 4 times. Discard the sample if there is a venous collapse or stoppage of blood flow during collection.

Do not use hemolyzed samples.

Specimen Identification:

Label each specimen tube with the patient's name.

Record the patient name, date and time of collection, and test name on the Laboratory Test Log along with the initials of the person performing the test.

Handling Conditions:

Patient samples are stable for up to 4 hours and must be stored at room temperature.

EQUIPMENT AND MATERIALS:

Equipment:

Dade PFA®-100 Instrument

Materials:

Dade PFA® Collagen/Epinephrine (COL/EPI) Test Cartridge
Dade PFA® Collagen/ADP (COL/ADP) Test Cartridge
Dade PFA® Trigger Solution

Preparation:

(1) Dade PFA® Collagen/Epinephrine (COL/EPI) Test Cartridge-- A test cartridge unit containing a membrane coated with 2 μg of equine Type I collagen and 10 μg epinephrine bitartrate.

(2) Dade PFA Collagen/ADP (COL/ADP) Test Cartridge--A test cartridge unit containing a membrane coated with 2 μg of equine Type I collagen and 50 μg adenosine-5'-disphosphate (ADP).

a. Test cartridges in unopened pouch are stable at 2-8ºC until the expiration date printed on the label.
b. Test cartridges are stable up to 30 days after opening the pouch when stored at 2-8ºC
c. Test cartridges stored at room temperature (16 to 26ºC) in a sealed or unsealed pouch are stable for up to 4 hours.

(3) Dade PFA® Trigger Solution: A trigger solution vial containing 11 mL isotonic saline (0.9% aqueous sodium chloride)
a. Trigger Solution in unopened vial is stable at room temperature (16 to 26ºC) until the expiration date printed on the label.
b. Trigger Solution is stable up to 60 days after the vial is placed on the instrument.

Discard if turbid or if particulate matter is visible.

These products are for in vitro diagnostic use.

QUALITY CONTROL:

1. The PFA-100® Self Test from the Maintenance Menu should be performed at least once per shift at the start of each shift the system is in use. Refer to the PFA-100® Operating Manual QC Procedures section for instructions. Instructions on how to establish the control donor group are given in the PFA-100 package insert.
2. As part of the instrument quality control, it is recommended to test in duplicate a control donor with each new shipment of cartridges received or whenever the institution wishes to verify the performance of the system.
3. The system will be considered under control if the mean closure time (CT) falls within the established reference range.
4. If the mean CT is outside the established reference range, repeat this procedure with a second individual from the laboratory's established control donor group.
5. If the mean CT's from both individuals are outside the reference range, contact Technical Services.
6. 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.
7. These donor controls are stored at room temperature for up to 4 hours prior to testing.

PROCEDURE - STEPWISE:

1. Refer to the PFA-100® Operating Manual for instrument operation instructions.
2. Singlicate testing can be performed.. Refer to package insert for a discussion of duplicate testing.

Preparation of the test cartridges

1. Allow the pouch containing the test cartridges to warm-up to room temperature prior to opening and removing cartridges for testing. This takes approximately 15 minutes. After removal of the cartridges, close the pouch by using the re-closable seal.

2. Remove and discard the top foil seal from the test cartridge
3. Place the test cartridge(s) in the cassette and push until the test cartridge(s) securely snaps in place. (Refer to picture in the Introduction section of the Operating Manual)

Sample loading

Note: The following steps must be performed in sequence without interruption.

1. Mix the blood sample by inverting the collection tube gently by hand 3-4 times.
2. Holding the cassette with test cartridge(s) on a flat surface, pipette 800 µL of blood into the smaller opening (sample reservoir opening) of the test cartridge by dispensing slowly along one of the inside corners. This will reduce the possibility of air entrapment in the sample reservoir.
3. 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.

4. The test can now be started.
5. Dispose of used test cartridge(s) after completion of test

CALCULATIONS:

All calculations and comparison to reference ranges are performed by the Dade Behring PFA-100. Results are printed on the report.

REPORTING RESULTS:

Results of the PFA-100® test are reported by the instrument as Closure Time (CT) in seconds.
These results should be related to the reference interval for each cartridge type. Record results on the worksheet, patient requisition (if applicable) and in the computer system. The printout from the analyzer is retained adjacent to the corresponding page of the LABORATORY TEST LOG.
Record quality control values.

Reference Interval:

Reference interval values:

Cartridge Type	Mean (sec)	Reference Interval (sec)
Collagen/Epinephrine	xxx	xxx
Collagen/ADP	xxx	xxx

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

The requesting physician or physician on call should be notified immediately when test results exceed the following limits:

HIGH LEVEL CRITICAL VALUE: xxxxx

INTERPRETATION OF RESULTS:

1. 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.
2. Closure Time above the laboratory established cut-off may indicate the need for further diagnostic testing.
3. 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®).

4. 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.
5. Results should always be evaluated in conjunction with clinical history and other laboratory findings (such as bleeding time and platelet aggregometry). In cases where PFA-100® results do not agree with the clinical assessment, additional testing should be performed.
6. 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

PROCEDURE NOTES:

Handling Precautions:

All blood samples and blood components should be treated as potentially infectious.
All samples should be handled in accordance with good laboratory practices using appropriate precautions.
Personal protective equipment should be worn when inserting or removing cartridges from the carousel.
To avoid injury, do not disassemble the test cartridge.
The PFA-100® is incapable of detecting bubbles in the test cartridge.
There is a risk of exposure to aerosolized blood droplets when removing the test cartridges.

LIMITATIONS OF THE PROCEDURE:

1. 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:
   a.reduction in hematocrit
   b.release of ADP

   Therefore, use of hemolyzed blood for PFA-100 testing is not recommended.

2. Certain fatty acids and lipids found in various human diets are widely known to inhibit platelet function, for which the PFA-100 system was designed to detect. Physicians may wish to advise patients to refrain from fatty foods prior to testing. Neutral lipids, such as cholesterol, generally have no effect on platelet function.
3. Platelet inhibiting agents, such as aspirin and anti-glycoprotein IIb/ IIIa antagonists, directly affect platelet function.
4. 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.
5. 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. Thus, it is recommended that samples exhibiting high sedimentation properties be run as a single test. In order to obtain duplicate measurements, two separate runs should be performed.
6. Many medications are known to affect platelet function. Therefore, the medication history of the patient should be reviewed.
7. Closure Time above the laboratory cut-off could reflect reduced platelet function caused by hematocrit < 35% or platelet counts < 150,000/μL. Specimens with hematocrit levels >50% or platelets counts >500,000/μL have not been evaluated.
8. If the PFA-100® system cannot be made to operate properly, send whole blood sample to _____ _____(Phone_____) for

assay. Store samples at room temperature (16 to 26°C) prior to courier pick-up. Samples are stable for up to four hours.

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