SIEMENS Healthineers

Dade® PFA Collagen/EPI Test Cartridge

COLL | EPI | CARTRIDGE

Dade® PFA Collagen/ADP Test Cartridge

COLL | ADP | CARTRIDGE

Revision bar indicates update to previous version.

Intended Use

To aid in the detection of platelet dysfunction in citrated human whole blood.

Summary and Principle

The PFA-100® System consists of an instrument and test cartridges 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].

The PFA-100® System allows for rapid evaluation of platelet function on small samples of citrated 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. Citrated whole blood is aspirated from the sample reservoir through the capillary and the aperture, which expose platelets to high shear flow conditions. The membrane is coated with collagen, a subendothelial protein generally believed to be the initial matrix for platelet attachment. The attachment of platelets to collagen is thought to trigger the initial physiologic stimulus for platelet activation. In addition, the membrane is coated with either epinephrine or ADP, which are other physiologic agonists that, along with collagen, are widely used to activate platelets in aggregometry testing. At the beginning of a 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.

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, VWD 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.

Reagents

Materials provided

COLL EPI CARTRIDGE, REF B4170-20A

20 x COLL EPI CARTRIDGE, Dade® PFA Collagen/EPI Test Cartridge

COLL ADP CARTRIDGE, REF B4170-21A

20 x COLL ADP CARTRIDGE, Dade® PFA Collagen/ADP Test Cartridge

Note: All Reagents are sold separately.

Composition

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

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

Stability and Storage

Test cartridges in a sealed pouch are stable at 2 to 25 °C until the expiry date printed on the label. The test cartridges are stable 3 months after opening and reclosing the pouch when stored at 2 to 25 °C. Test cartridges stored at 15 to 25 °C in an open pouch or outside the pouch are stable for 4 hours.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

Safety data sheets (MSDS/SDS) available on siemens-healthineers.com/sds.

CAUTION!
Federal (USA) law restricts this device to sale by or on the order of licensed healthcare professionals.

Caution
COLL EPI CARTRIDGE, COLL ADP CARTRIDGE
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

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.

Protective equipment should be worn when inserting or removing with whole blood loaded cartridges from the carousel.

Do not disassemble the test cartridge. There is a risk of exposure to blood droplets when removing the test cartridge from the carousel.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Materials required but not provided

1. PFA-100® System, REF B4170
2. Dade® PFA Trigger Solution, REF B4170–50
3. Evacuated blood collection tubes or syringes containing 3.8 % (0.129 M) or 3.2 % (0.105 M) buffered sodium citrate (1 part anticoagulant to 9 parts blood).
4. Pipetting devices capable of measuring up to 800 µL.

Specimen Collection and Preparation

All investigations of platelet function are strongly dependent on the correct method of blood collection. Venipuncture should be performed using a 21G or larger needle (20G or 19G). Blood should be drawn directly into an evacuated plastic or siliconized glass tube or syringe containing 3.8 % (0.129 M) or 3.2 % (0.105 M) **buffered** sodium citrate (1 part anticoagulant to 9 parts blood). **Use of unbuffered sodium citrate anticoagulant is not recommended**.

Important! 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 blood samples. Samples must be stored undisturbed at room temperature (16 to 26 °C) 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.**

Procedure

Refer to the PFA-100® Operating Manual for instrument operation instructions.

Notes

1. 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.
2. Personal protective equipment should be worn when inserting or removing cartridges from the carousel.
3. To avoid injury, do not disassemble the test cartridge.
4. The PFA-100® System is incapable of detecting bubbles in the test cartridge.
5. There is a risk of exposure to aerosolized blood droplets when removing the test cartridge.

No relevant differences were observed between singlicate and duplicate testing with respect to the reference intervals, clinical sensitivity and specificity, as well as clinical accuracy of the results. Therefore singlicate as well as duplicate testing can be performed with the PFA-100® instrument. For additional information about singlicate testing, refer to the "Clinical Performance" section of these Instructions for Use.

When performing duplicate testing, the mean of the test results (including discrepant results, one above and one below the laboratory established cut-off) should be reported unless:

1. a non-closure was obtained at one position and a measurable CT of less than, or equal to, the established cut-off was obtained at the other position; or
2. CT of one test position was greater than, or equal to, two times (2x) the value of the other replicate.

Samples with replicate test results meeting one of these criteria should be repeated with the same sample, if it is no older than four hours calculated from venipuncture. If the sample is older, the test must be repeated with a freshly drawn sample. Individual laboratories may wish to establish their own criteria for repeat testing. If repeat testing does not resolve the discrepancy, contact Siemens Healthineers for assistance.

1. Preparation of the test cartridges

a. Allow the pouch containing the test cartridges to warm-up to room temperature (16 to 26 °C) prior to opening. This takes approximately 15 minutes. After removal of the cartridges, close the pouch by using the reclosable seal.

b. Remove and discard the top foil from the test cartridge. This top foil only protects against dust and other particles. Performance and stability of the test cartridges is not affected in case of incomplete sealing by the top foil.

Note: If the top foil has clearly been damaged or is missing, do not use the test cartridges. Discard it in appropriate waste container.

c. Place the test cartridge(s) in the cassette of the PFA-100® instrument and push until the test cartridge(s) securely snaps in place. (Refer to picture in the Introduction section of the Operating Manual).

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 by hand 3 - 4 times. 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 sample reservoir opening**.

c. The test can now be started.

3. Disposal of used test cartridge(s)

Remove the cassette carefully from the carousel. Holding the cassette in one hand, remove test 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.

Quality Control (QC)

At least once per shift at the start of each shift the system is in use, perform the PFA-100® "Self Test" from the "Maintenance Menu". Refer to the PFA-100® Operating Manual "QC Procedures" section for instructions.

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 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 CTs from both individuals are outside the reference range, contact Siemens Healthineers. 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 CT near the middle of the normal range and acceptable replicate results.

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 Test Cartridges only.
3. Qualify the donor if the printed duplicate mean is within 110 to 160 seconds and the duplicate CV (coefficient of variation) 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 CT established by individual laboratories for normal adults.

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.

Interpretation of Results

Results of the PFA-100® test are reported by the instrument as Closure Time (CT) in seconds (s). The PFA-100® test provides an indication of platelet function. A CT above the laboratory established cut-off may indicate the need for further diagnostic testing. Results should always be evaluated in conjunction with clinical history, clinical presentation and other laboratory findings [such as bleeding time, complete blood count (CBC) 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-100® test on normal subjects and subjects with various disorders:

	Normal (n = 176)	ASA** (n = 120)	VWD (n = 28)	Glanzmann thrombasthenia (n = 4)
Col/EPI	normal	abnormal	abnormal	abnormal
Col/ADP	normal	normal	abnormal	abnormal

** See table under "ASA-Induced Platelet Dysfunction".

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, the hemodynamic properties of the sample may be altered, potentially affecting the result. Thus, it is recommended that samples exhibiting high sedimentation properties be run as single tests. In order to obtain duplicate measurements, two separate runs should be performed.
3. Many medications are known to affect platelet function. Therefore, the medication history of the patient should be reviewed.
4. A CT above the laboratory established cut-off could reflect reduced platelet function caused by hematocrit levels (< 35 %) or platelet counts (< 150,000/µL). Specimens with hematocrit levels > 50 % or platelet counts > 500,000/µL 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.
5. The PFA-100® System performance has not been established for platelet inhibiting agents other than acetyl salicylic acid.
6. Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

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® CT 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. Commonly used drugs or certain substances in food may influence the closure time of the Col/EPI and/or Col/ADP cartridges.

   The table below summarizes substances which affect the closure time of the Col/EPI and/or Col/ADP cartridges at the given and higher concentrations (according to an internal study). The results of the spiked sample and the native sample (same donation and position in the system) were evaluated using a paired t-test. A p-value ≤ 0.05 was defined as significant interference.

Drug Category	Substance	Concentration	Concentration (S.I. units)	Influence on closure time	
				COL/EPI	COL/ADP
Antibiotic	Penicillin G	10 000 IU/mL	10^7 IU/L	prolongation	no influence
Analgesic	Ibuprofen	5 µg/mL	24.2 µmol/L	prolongation	no influence
Thrombolytic agent	Streptokinase	100 IU/mL	100 000 IU/L	prolongation	prolongation
Anti-platelet drug	Cilostazol	5 µg/mL	13.5 µmol/L	prolongation	no influence
	Tirofiban	0.1 µg/mL	0.2 µmol/L	prolongation	prolongation

Additional drugs that have influence on the closure time of the Col/EPI and/or Col/ADP cardridges as described in the literature are listed in the following table:

Drug category	Substance	Influence on closure time		Reference
		COL/EPI	COL/ADP	
Anesthetic	Propofol[a]	prolongation	none	[9]
	Ropivacaine hydrochloride[b]	prolongation	prolongation	[10]
Hemostatic	Desmopressin (DDAVP)[c]	shortening	shortening	[11]
Analgesic	Diclofenac[c]	prolongation	n.d.[d]	[12]
	Ketorolac[c]	prolongation	n.d.[d]	[12]
	Indometacin[c]	prolongation	n.d.[d]	[13]
	Meloxicam[c]	prolongation	n.d.[d]	[13]
	Nabumeton[c]	prolongation	n.d.[d]	[13]
Plasma expander	Hydroxyethyl starch	prolongation	prolongation	[14]
Anti-platelet drugs	Abciximab[c]	prolongation	prolongation	[15]
	Eptifibatid[c]	prolongation	prolongation	[15]
Vasodilator	Prostacyclin[c]	prolongation	prolongation	[16]
	Iloprost[c]	prolongation	prolongation	[17]

[a] Plasma concentration: 20 µg/mL
[b] Plasma concentration: 1.88 mg/mL
[c] *Ex vivo* measurement following administration of therapeutic doses
[d] not determined

Non-interfering Substances

The following substances and drugs do not affect the closure time of the Col/EPI and Col/ADP cartridges when present in plasma at the concentrations indicated according to an internal study. When evaluating samples spiked with the respective substance and the native samples (same donation and position in the system) using a paired t-test, the p-value was > 0.05.

Drug Category	Substance	Concentration	Concentration (S.I. units)
ACE inhibitor	Captopril	25 µg/mL	115.1 µmol/L
Alcohol	Ethanol	5 µL/mL	85.7 mmol/L
Analgesic	Acetaminophen	25 µg/mL	165.4 µmol/L
Antiarrhythmic agent	Lidocaine	25 µg/mL	106.7 µmol/L
Anticoagulant	Low molecular weight Heparin	1.5 IU/mL	1 500 IU/L
Antidepressant	Fluoxetine	25 µg/mL	80.8 µmol/L
Antioxidant	Catechin	25 µg/mL	86.2 µmol/L
	α-Tocopherol	25 µg/mL	58.0 µmol/L
Beta-Blocker	Propranolol	25 µg/mL	96.4 µmol/L
Bronchodilator	Theophyllin	25 µg/mL	138.8 µmol/L
Diuretic	Hydrochlorothiazid	25 µg/mL	84.0 µmol/L

Drug Category	Substance	Concentration	Concentration (S.I. units)
Anti-inflammatory drug	5-Aminosalicylic acid	50 µmol/L	50.0 µmol/L
Glucocorticoid	Betamethason	25 µg/mL	63.7 µmol/L
Calcium channel blocker	Diltiazem	25 µg/mL	60.3 µmol/L
Coronar vasodilator	Nitroglycerin	0.1 µg/mL	0.4 µmol/L
Phosphodiesterase inhibitor	Caffeine	20 µg/mL	103.0 µmol/L
	Dipyridamol	10 µg/mL	19.8 µmol/L
Phosphodiesterase V inhibitor	Sildenafil	5 µg/mL	10.5 µmol/L
Statin	Pravastatin	25 µg/mL	58.9 µmol/L
Thyroid hormone	L-Thyroxin	0.4 µg/mL	0.5 µmol/L

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, VWD 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 [s]	Reference Range [s]
Col/EPI	132	94–193
Col/ADP	92	71–118

In a limited study, on 36 apparently normal subjects, up to **12 % shorter CTs** were observed for samples collected in **3.2 % (0.105 M)** buffered sodium citrate[18].

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 CLSI document C28-A2 may be used as a guideline)[19]. The provided ranges should only be used as a guide for interpretation together with other clinical signs and symptoms.

System Performance Characteristics

Imprecision

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 % (0.129 M) 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 (8.8–14.0)	12.7 (10.9–15.9)
Between lot CV	0.6 (0.0–1.1)	4.0 (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 CT near the cut-off, the duplicate CV are 13.7 % for Col/EPI cartridges and 10.0 % for Col/ADP cartridges.

Clinical Performance

A total of 328 specimens were tested in duplicate with the PFA-100® System in a multicenter study. This population included samples from 176 subjects with normal platelet function and samples from 152 subjects with platelet dysfunction. A total of 115 subjects who were tested and enrolled in the normal platelet function group were tested post ASPIRIN ingestion and also enrolled in the platelet dysfunction group. The group of 176 normal subjects consisted of 61 % females and 39 % males with an age range between 18 and 57 years. The group of 152 platelet dysfunction subjects consisted of 66 % females and 34 % males with an age range between 16 and 66 years.

The Platelet Function Status (PFS) of each specimen was determined based upon results from a platelet function test panel (PT, APTT, platelet aggregometry, bleeding time, CBC [including platelet count], fibrinogen, factor VIII:C, von Willebrand factor activity, von Willebrand factor antigen and multimer determination) and clinical history. Additional specimens for which platelet function test panel results could be interpreted differently by two individuals as being either nor-mal or abnormal were classified as inconclusive for PFS. Samples with inconclusive PFS (n = 26) were excluded from the study.

Note: The only categories of platelet disorders studied with the PFA-100® System included disorders that were ASA-induced, and disorders in patients with previous history or laboratory results indicative of platelet dysfunction induced by Glanzmann thrombasthenia or VWD. Patients on oral contraceptives, patients with coagulation factor abnormalities and patients with thrombocytopenia were not studied.

The abnormal group included specimens from subjects with (a) VWD (n = 28), (b) ASPIRIN-induced dysfunction (n = 120), and (c) Glanzmann thrombasthenia (n = 4). The PFA-100® clinical categorization for this study was based on a designated cut-off of 170 seconds. This cut-off was established with data from a previous study taking into account the overlap of CTs between the normal and abnormal populations. A concordance table for the PFA-100® System categorization against platelet function status for the 328 specimens is presented below:

PFA-100® System vs. Platelet Function Status Comparison

	PFS Normal [n]	PFS Abnormal [n]
Col/EPI PFA Normal	156	6
Col/EPI PFA Abnormal	20	146

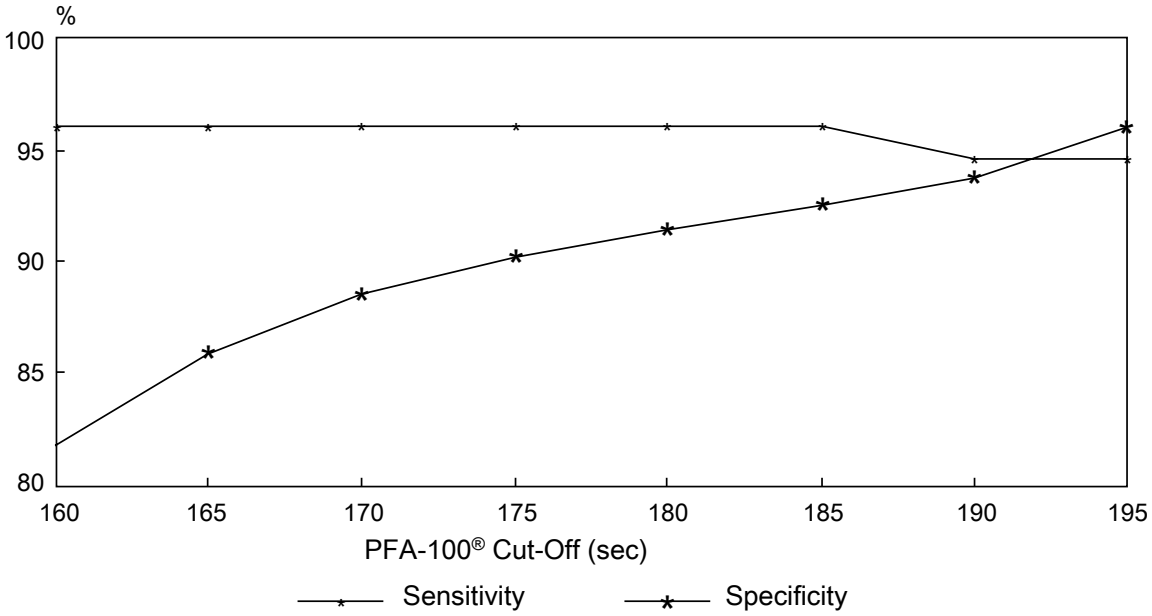
Overall clinical sensitivity and specificity were calculated from the table above at 96.1 % and 88.6 %, respectively. Also, results by clinical sites were computed and ranged from 86.7 % to 100.0 % for clinical sensitivity and from 79.3 % to 100.0 % for clinical specificity.

In addition to the calculation from the mean of duplicate determinations, clinical sensitivity, specificity and accuracy for the PFA-100® System were computed from single determinations. The table below presents the results for these calculations. These results demonstrate that the PFA-100® System clinical performance is not compromised by singlicate testing.

	Clinical Sensitivity [%]	Clinical Specificity [%]	Clinical Accuracy
Singlicate Determinations Position A	96.1	86.4	0.969
Position B	96.7	86.9	0.980
Duplicate Determinations	96.1	88.6	0.979
Confidence Limits	(91.6–98.5)	(83.0–92.9)	(0.961–0.997)

The data presented above reflect the clinical performance of the PFA-100® System with Col/EPI Test Cartridges using a cut-off at 170 seconds. The figure below depicts the observed clinical sensitivity and specificity of the PFA-100® System when a range of cut-off values was evaluated for this same study population. It is recommended that each laboratory establish its own cut-off based upon site population and internal procedure.

PFA-100® System Sensitivity and Specificity at Different Clinical Cut-Offs



The clinical concordance between the PFA-100® System and aggregometry is shown in the table below. This table excluded five (5) cases where aggregometry provided an inconclusive platelet function result. The overall proportion of agreement, expressed as a percentage, between the PFA-100® System and aggregometry was calculated at 90.1 % from this table:

	Aggregometry	
	Normal [n]	**Abnormal [n]**
Col/EPI PFA Normal	145	12
Col/EPI PFA Abnormal	20	146

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® analyzer 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 s [n]	**Col/ADP Abnormal PFA CT > 114 s [n]**
Col/EPI normal CT ≤ 170 s	5	1
Col/EPI Abnormal CT > 170 s	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.

Pediatric Testing

From one study[20], performance data are presented on the PFA-100® System in pediatric populations as a screening assay for VWD and for intrinsic and acquired disorders of platelet function is available. Fifty-two (52) children (ages 1-17) were evaluated for potential hemorrhagic disorders by standard coagulation testing, platelet aggregometry, bleeding time and von Willebrand factor (VWF) assays. Nineteen (19) subjects were diagnosed with platelet dysfunction (5 due to medication and 2 with Glanzmann thrombasthenia) or VWD (n = 12). Results of testing in parallel with the PFA-100® System on these patients are shown below.

Platelet dysfunction and VWD combined				**VWD only**	
Reagent	**Sensitivity**	**Specificity**	**Efficiency**	**Sensitivity**	**Efficiency**
Col/EPI	100 % (19/19)	97 % (32/33)	98 % (51/52)	100 % (12/12)	98 % (44/45)
(95 % CI)	(85–100 %)	(84–100 %)	(90–100 %)	(78–100 %)	(88–100 %)
Col/ADP°	87 % (13/15)	80 % (12/15)	83 % (25/30)	80 % (8/10)	80 % (20/25)
(95 % CI)	(60–98 %)	(52–96 %)	(65–94 %)	(44–97 %)	(59–93 %)

° Due to insufficient amount of specimens, only 32 patients were used to establish the values for Col/ADP testing.

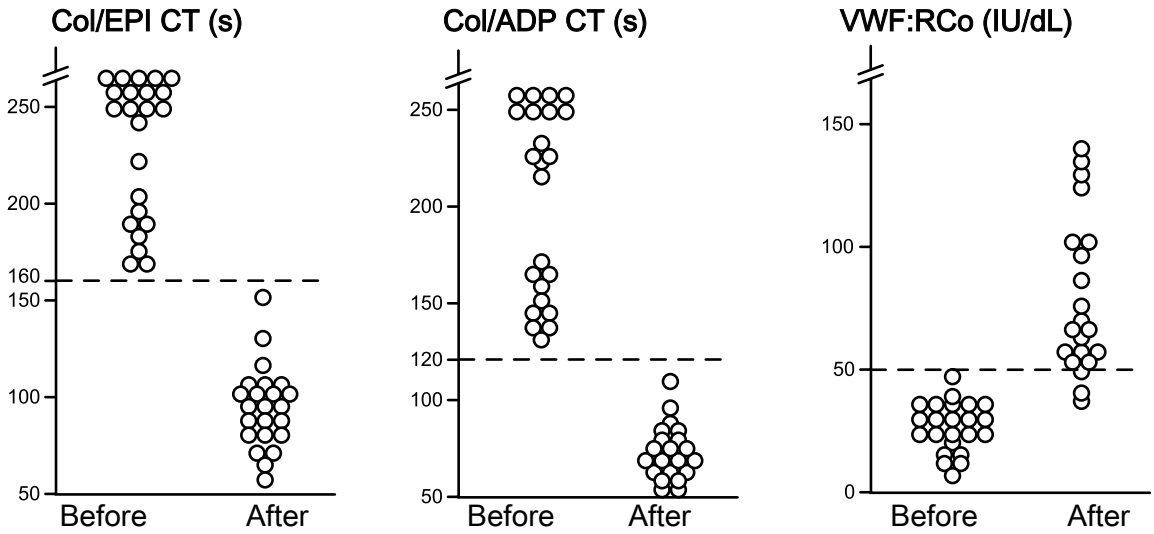
Data are also available from a separate study[21] in which children with previously diagnosed VWD were tested. The study results were obtained from 33 children (age range of 2 to 18 years) with well-characterized VWD (21 with definite type 1, 9 with type 2 and 3 with type 3). Of these children, 28 had abnormal CTs with Col/EPI and 29 had abnormal CTs with Col/ADP. Four children with definite type 1 VWD had normal CTs with both cartridge types and also showed normal levels of VWF antigen (VWF:Ag) and VWF ristocetin cofactor activity (VWF:RCo) on the same day of PFA-100® testing. Children receiving platelet inhibiting medications or having intrinsic defects of platelet function were not enrolled in this study.

In an additional study, the PFA-100® CTs of 52 children aged 1 to 15 years who were referred for allergy testing but were otherwise healthy and not taking any medications known to inhibit platelet function were compared to CTs of 20 healthy adults[22]. The mean CTs did not show any significant differences among the pediatric and adult groups. Neonates as well as children under 1 year of age were not included in this study.

Bleeding Management Testing

Although the performance of the PFA-100® System assessing therapeutic effects of desmopressin acetate (1-deamino-8-D-arginine vasopressin; DDAVP) has not been fully established, evaluations have been conducted in various patient settings. Data from several studies suggest that the PFA-100® System CT with both Col/EPI and Col/ADP Test Cartridges becomes measurably shorter following infusion of DDAVP. Shorter CTs indicate improved platelet function and may indicate improvement in bleeding symptoms and transfusion needs.

Data are presented from one study[23] of 23 patients with type 1 VWD. At baseline, all patients demonstrated prolonged CTs. Thirty minutes after infusion of DDAVP, CTs were within the laboratory reference range. A summary of results in these patients is shown in the figure below. These findings correlate inversely with the results of the VWF:RCo assay. When the VWF:RCo value is below the laboratory cut-off, CTs are prolonged, while both normalize following infusion of DDAVP. DDAVP triggers the release of high molecular weight multimers of VWF from the endothelium which augment platelet function under high shear conditions similar to the testing environment of the PFA-100® System.



Response to DDAVP in 23 patients with type 1 VWD. CTs in seconds (s) were measured with Col/EPI and Col/ADP Test Cartridges before and 30 min after infusion of DDAVP. VWF:RCo was also measured.

Data are presented from one prospective study[23] that evaluated impairment of primary hemostasis in 5649 unselected adult patients undergoing elective surgery. Procedures included vascular, thoracic, orthopedic, gynecologic, urologic, rhinolaryngologic and cranio-maxillofacial surgeries. Prior to surgery, patients completed a questionnaire to establish a bleeding history. Patients also underwent pre-operative hemostatic testing that included a platelet count, prothrombin time, activated partial thromboplastin time, bleeding time, VWF:Ag assay and PFA-100® Col/EPI and Col/ADP CT. Of 628 patients with a positive bleeding history, 256 had at least one abnormal hemostatic test. Of these 256 patients, 252 had Col/EPI or Col/ADP CTs above the normal range with blood collected in 3.2 % buffered sodium citrate.

Patients with CTs above the reference range had the following disorders: 54 VWD, 87 associated with ASPIRIN use and 113 due to other acquired or inherited problems of primary hemostasis. Prevalence of patients responding to DDAVP as measured by the PFA-100® System is shown in the table below[24].

Measure of improved platelet function following DDAVP therapy

Platelet Dysfunction	Total° [n]	Patients Responding † [n]	
		Col/EPI (CT<150 s)	Col/ADP (CT<100 s)
ASPIRIN	87	79	41
VWD‡	54	50	41
Other	113	100	88

° Patients receiving DDAVP.

† Patients with initially prolonged PFA-100® CT that corrected to normal following infusion of DDAVP. Reference ranges were 82 - 150 s (Col/EPI) and 62 -100 s (Col/ADP).

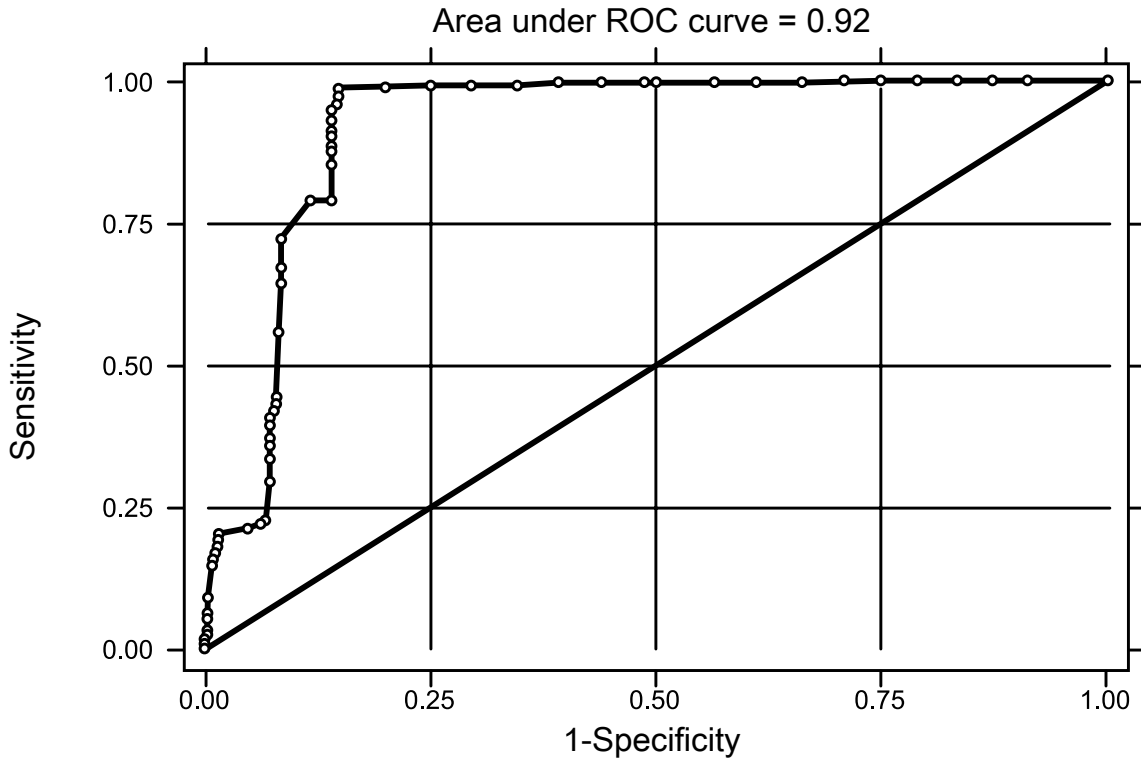
‡ VWD included 40 type 1, 12 possible type 1 and two type 2a. Two of the possible type 1 patients were not detected by either Col/EPI or Col/ADP Cartridges.

Clinical performance in 628 pre-operative patients having a positive bleeding history as defined in this study is shown in the table below.

Clinical performance characteristics for PFA-100® Col/EPI and Col/ADP CT in detection of primary hemostasis defects

	Sensitivity[oo] [%]	Specificity [%]	PPV [%]	NPV [%]
Col/EPI	90.8	86.6	81.8	93.4
Col/ADP	47.7	93.5	77.3	79.4

[oo] Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) were determined from ROC analysis as shown in figure below. Values were obtained using reference intervals established by the researchers which are different from those reported under Expected Values.



Receiver operator characteristic (ROC) curve for PFA-100® Col/EPI CTs in patients with a positive bleeding history and impaired platelet function based on all laboratory data (except PFA-100® System results), and patient medication history.

The 250 patients identified with prolonged Col/EPI CTs were administered DDAVP prior to surgery and then tested again with Col/EPI. In 229 patients (91.6 %), the Col/EPI CTs were within the laboratory reference range. After the DDAVP treatment blood transfusion needs among these patients were not significantly different than those of patients (n = 5393) with a negative bleeding history and normal primary hemostasis. In a related study[24] of 5102 patients, 317 were identified pre-operatively with having abnormal hemostasis, of which 311 demonstrated prolonged Col/EPI CTs. None of these patients were treated with DDAVP prior to surgery. The frequency of blood transfusion associated with surgery was significantly higher ($p<0.001$) in these patients than in those with Col/EPI CTs within the normal range (n = 4785).

Clinical performance characteristics for PFA-100® Col/EPI and Col/ADP CT in detection of transfusion needs is shown in the table below[24].

	Sensitivity [%]	Specificity [%]	PPV [%]	NPV [%]
Col/EPI	33.9	99.3	91.7	89.6
Col/ADP	19.9	99.4	86.5	86.6

Values were obtained using reference intervals established by the researchers which are different from those reported under Expected Values.

Pre-surgical correction of a prolonged PFA-100® CT as a result of DDAVP treatment, especially with the Col/EPI Cartridge, may provide useful information for blood transfusion management in patients undergoing different kinds of elective surgeries.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

siemens-healthineers.com

Applicable Version of electronic Instructions for Use

As Siemens Healthineers continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Siemens Healthineers ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via siemens-healthineers.com/eIFU.

Bibliography

1. Bick R. Platelet function defects: a clinical review. Semin Thromb Hemost 1992;18:167-85.
2. Kratzer MAA, Born. GVR: Simulation of primary hemostasis in vitro. Haemostasis 1985;5:357-62.
3. Kratzer MAA, Bellucci S, Caen JP. Detection of abnormal platelet function with an in vitro model of primary hemostasis. Haemostasis 1985;15:363-70.
4. Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962;194:927-9.
5. Kundu SK, Heilmann EJ, Sio R, et al. Characterization of an in vitro platelet function analyzer, PFA-100. Clinical Applications in Thrombosis/Hemostasis 1996;2:241–9.
6. MacIntyre DE, Hoover RL, Smith M et al. Inhibition of platelet function by cis-unsaturated fatty acids. Blood 1984;63:848-57.
7. Driss F, Vericel E, Lagarde M, et al. Inhibition of platelet aggregation and thromboxane synthesis after intake of small amount of icosapentaenoic acid. Thromb Res 1984;36:389-96.
8. Schick PK. Megakaryoryte and platelet lipids. In: Colman RW, Hirsh J, Marden VJ, Salzman EW, eds. Hemostasis and Thrombosis; Basic Principles and Clinical Practice, 3rd ed., Philadelphia: JB Lippincott Co., 1994: 574-89.
9. Reinhart W, Felix C. Influence of propofol on erythrocyte morphology, blood viscosity and platelet function. Clin Hemorheol Microcirc. 2003;29:33-40.
10. Porter JM, Crowe B, Cahill M, Shorten GD. The effects of ropivacaine hydrochloride on platelet function: an assessment using the platelet function analyser (PFA-100). Anaesthesia 2001:56:15-8.
11. Fressinaud E, Veyradier A, Sigaud M, et al. Therapeutic monitoring of von Willebrand disease: interest and limits of a platelet function analyzer at high shear rates. Brit J Haematol 1999;106:777-83.
12. Bauer KA, Gerson W, Wright C, et al. Platelet function following administration of a novel formulation of intravenous diclofenac sodium versus active comparators: a randomized, singledose, crossover study in healthy male volunteers. J Clin Anesth. 2010;22:510-8.
13. Van Kraaij D, Hovestad-Witterland A, De Metz M, Vollaard E. A comparison of the effects of nabumetone vs meloxicam on serum thromboxane B2 and platelet function in healthy volunteers. Br J Clin Pharmacol. 2002;53:644-7.
14. Liu FC, Liao CH, Chang YW, et al. Hydroxyethyl Starch interferes with human blood ex vivo coagulation, Platelet Function and Sedimentation. Acta Anaesthesiol Taiwan 2009;47:71-8.
15. Madan M, Berkowitz SD, Chrisitie DJ, et al. Determination of platelet aggregation inhibition during percutaneous coronary intervention with the platelet function analyzer PFA-100. Am Heart J 2002;144:151-8.
16. Zwerina J, Landsteiner H, Leitgeb U, et al. The influence of VIP and epoprostenol on platelet CD62P expression and primary haemostasis in vitro. Platelets. 2004;15:55-60. Dade® PFA Collagen/EPI Test Cartridge and Dade® PFA Collagen/ADP Test Cartridge 14/16 2012-05.

17. Kobsar A, Koessler J, Rajkovic M, et al. Prostacyclin receptor stimulation facilitates detection of human platelet P2Y(12) receptor inhibition by the PFA-100(R) system. Platelets. 2010:19. 112-6.
18. Heilmann EJ, Kundu SK, Sio R, et al. Comparison of four commercial citrate blood collection systems for platelet function analysis by the PFA-100™ system. Thromb Res 1997;87:159-64.
19. CLSI Approved Guideline C28-2A. How to define and determine reference intervals in the clinical laboratory. Wayne PA: Clinical and Laboratory Standards Institute, 2000.
20. Cariappa R, Hilhite TR, Parvin CA, Luchtman-Jones L. Comparison of PFA-100 and bleeding time testing in pediatric patients with suspected hemorrhagic problems. J Ped Hematol/Oncol 2003;25:474-9.
21. Dean JA, Blanchette VS, Carcao MD, et al. von Willebrand disease in a pediatric-based population – Comparison of type 1 diagnostic criteria and use of the PFA-100 and von Willebrand factor/ collagen-binding assay. Throm Haemost 2000;84:401-9.
22. Lippi G, Manzato F, Franchini M, et al. Establishment of reference values for the PFA-100 platelet function analyzer in pediatrics. Clin Exp Med 2001; 1:69-70.
23. Koscielny J, Ziemer S, Radtke H, et al. A practical concept for preoperative identification of patients with impaired primary hemostasis. Clin Appl Thrombosis/Hemostasis 2004;10:195-204.
24. Koscielny J, von Tempelhoff G-F, Ziemer S, et al. A practical concept for preoperative management of patients with impaired primary hemostasis. Clin Appl Thrombosis/ Hemostasis 2004;10:155-66.

Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community	Σ	Contains sufficient for <n> tests
	Biological Risks	IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation		Consult instruction for Use
NON STERILE	Non-sterile	CE	CE marking of conformity
CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.	CONTENTS	Contents
	Reconstitution volume	LEVEL	Level
	Keep away from sunlight and heat	WARNING	Warning
DANGER	Danger	RxOnly	Prescription device (US only)
UDI	Device Identification (UDI) barcode	REACH xx/xx/xx	REACH Authorization Number

Legal Information

Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestraße 127
91052 Erlangen
Germany
Phone: +49 9131 84-0
siemens-healthineers.com

Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg
Germany
siemens-healthineers.com