

### Aspirin and Plavix Sensitivity and Resistance: Are Different Tests of Platelet Function Comparable When Taking Aspirin and Plavix?

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This information is for education only and is not a product endorsement.

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### THE FOLLOWING MEDICATION(S) MAY AFFECT THE COAGULATION OF YOUR BLOOD!!

- Vitamin E-, Genseng, Garlic, Ginko Bilboa, Feverfew, Fish Oil, Glucosamine.
- Celebrex, Vioxx, Aspirin (ASA, Baby ASA, CAMA, Ecotrin, Fiorinal)
- Percondan, Dislacid, ECASA, Zorprin,
- Arthropan (Cholin salicylate); Voltaren (Diclofenac, Cataflam), Dolobid (Diflunisal); Lodine (Etodolac); Nalfon (Fenoprofen Calcium)
- Ibuprofen- Advin, Excedrin, Medipren, Menadol, Midol-200, Motrin, Motrin IB, Nuprin, Pamprin-IB, Rufen, Saleto (200,400, 600, 800), Trendar, Indocin, Orudis (ketoprofen); Toradol (Ketoralac), Doan's Pills, Magan, Modibin (Magnesium salicylate), Meclomen, Ponstel, Relafen, Aleve, Anaprox (Naproxen), Daypro, Feldene, Butazolidin, Amigenic,, Rexolate, Tusal, Tolectin.
- MAO Inhibitor: Nardil and Parnate
- Plavix, Prasugrel, Cangrelor, Ticagrelor, Ticlopidine, Coumadin, Pradaxa (Dabigatran), Heparinoids.
- Numerous antibiotics, Dilantin

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### Introduction

- Aspirin irreversibly acetylates platelet cyclooxygenase, preventing activation by blocking the prostaglandin pathway
- The platelet inhibiting effect of a single aspirin may be detectable by platelet function assays within 24 hours
- Failure to detect aspirin-induced platelet suppression may indicate physiological aspirin insensitivity, a phenomenon called "aspirin resistance"
- Aspirin resistance is a recognized cause of failed aspirin therapy and may imply increased risk arterial thrombosis.

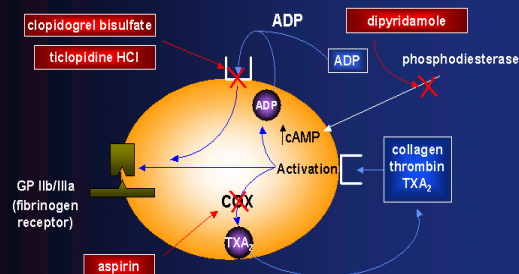
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### Why is it important ?

- Aspirin is used for prevention of complications of vascular diseases such as heart attack and strokes. Gender issues?
- Studies have shown using Aspirin alone reduced recurrent non-fatal stroke by 18%.
- However, studies have shown about 5-40% (about 1-2 million) of patients taking Aspirin may not be receiving full benefit because of resistance
- Several studies have suggested a significant increase of major vascular events associated with aspirin resistance. It may be reasonable to alter therapy in the aspirin resistant population rather than continue to take a drug that a test suggests is ineffective.

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### Mechanisms of Action of Oral Antiplatelet Therapies



ADP = adenosine diphosphate, TXA<sub>2</sub> = thromboxane A<sub>2</sub>, COX = cyclooxygenase.  
Schiefer AL. *Ann J Med*. 1996;101:193-209.

### ASPIRIN RESISTANCE

- **ASA resistance** refers to less than expected suppression of thromboxane A<sub>2</sub> production by ASA. Independently associated with an increased risk of adverse cardiovascular events.
- **Clinical resistance:** inability of ASA to protect subjects from cardiovascular events such as an acute MI.
- **Laboratory ASA resistance:** refers to the lack of anticipated effect of ASA on a laboratory assay of its antiplatelet effect.

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## POSSIBLE CAUSES OF ASPIRIN RESISTANCE

- Poor compliance by subjects.
- Drug interaction: ibuprofen, naproxen.
- Inadequate ASA dose.
- Increased turnover of platelets.
- Genetic polymorphisms of cyclo-oxygenase-1.
- Up regulation of alternate (non-platelet) pathways of thromboxane production.
- No standardized approach to the diagnosis and there are no proven effective treatments for aspirin resistance that improve outcome. Yet!

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## Research Background

- Eikelboom J et al: HOPE study: among patients with cardiovascular disease who take aspirin with persistent high 11-dehydro-thromboxane B2, had a 3.5 fold increase in the risk of death from heart attack
- Grottemeyer K, H et al: two year follow up of aspirin responders and non responders: Major end point (CVA, MI, Vascular death) seen in 4.4 % of aspirin responders but 40% in aspirin non-responders.
- Gum P., Topol E, et al: A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease among patient with aspirin resistance, 24% experienced death, MI, or CVA compared to 10% of patient who were not resistant
- Faraday et al: Relation Between Atherosclerosis Risk Factor and Aspirin Resistance in a Primary Prevention Population found that higher 11-DHT B2 levels is the only criteria associated with atherosclerosis risk factors.

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## Research Background

- Patrono et al: Low-Dose Aspirin for the Prevention of Atherothrombosis: Benefits fine for high risk subjects but may be marginal in low risk populations.
- Ridker PM et al: Women's Health study in healthy women ASA gave protection from stroke by 17% over men but no reduction in the risk of MI. Reverse effect for men in protection from MI but low protection from stroke.
- Becker DM et al: Women experienced the same or greater decrease in platelet reactivity after ASA therapy, retaining modestly more platelet reactivity compared with men.
- Bhatt DK et al: Overall clopidogrel + ASA was not significantly more effective than ASA alone in reducing MI, stroke and CVA.
- Lordkipanidze M et al: Aspirin resistance: Truth or dare. ASA resistance is poorly understood with testing not equivalent to each other. Like LA testing?

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## Research Background

- Goodman T, Sharma P, Ferro A. The genetics of aspirin resistance: ASA may not be effective in the prevention of thrombosis, depending on genetic makeup. Genetic testing is not currently used for predicting the effect of ASA clinically.
- Schwertner HA, McGlasson DL, Christopher M, Bush AC. Effects of different ASA formulations on platelet aggregation times and plasma salicylate concentrations.
- Feher G, Koltai K, Pappe E, et al: Aspirin resistance: possible roles of CV risk factors, previous disease history, concomitant medication and haemorrhological variables. Patients who demonstrated effective ASA inhibition had a significantly lower fibrinogen level (330 mg/dl vs 380 mg/dl).
- Cox D, Maree AO, Dooley M et al: Effect of enteric coating on antiplatelet activity of low-dose ASA in healthy volunteers.

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## Research Background

- Geske et al: Gender Variability of Urinary 11-DHT B2 levels in Diabetes Mellitus. Healthy females had higher levels than males. DM patients had higher levels than healthy controls. Female DM had higher levels than healthy females and DM males. No difference between DM males and healthy males. In response to ASA 325 healthy females levels were higher than healthy males.
- Garcia-Rodriguez 2011: Low dose ASA gave a 2.0 increase of UGIB over non-use; with clopidogrel added RR2.08; oral anticoagulants RR 2.0; NSAIDS 2.63; high dose steroids RR4.43; ASA with statins RR 0.99; ASA and low dose steroids RR 1.01.
- GRAVITAS Study with high dose clopidogrel (150 mg): no benefit over 75 mg dose based on platelet reactivity.

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## Introduction

- Assays that measure platelet response to aspirin may predict clinical outcomes
- We compared four methods for monitoring 24-hour platelet inhibition (single dose) and a 7 day dosing regimen in healthy subjects by 81 mg and 325 mg (standard child and adult) dosages
- We anticipated these assays will reveal a greater anti-platelet effect of 325 mg compared to 81 mg of aspirin
- We further anticipated the assays were comparable in their ability to detect aspirin effect
- We further anticipated that the 7-day dosing regimen would reveal a greater anti-platelet effect compared to the 24-hour regimen.

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## Research Objectives

- To measure platelet response to aspirin using four commercially available assays to determine:
  - Whether results of these assays compare and validate each other
  - Whether the degree of platelet inhibition under different single doses of Aspirin (81 and 325mg) are similar

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## Assays

Four commercially available assays were used in this study

- Whole blood aggregometry: examines platelet aggregation by using platelet agonists Collagen, ADP, Arachidonic Acid.
- PFA-100: tests platelet aggregation by measuring time to occlude an aperture. (Closure time)
- Verify/Now Accumetrics: studies platelet function by using arachidonic acid reagent. ASA inhibits platelet function and does not react to AA. Platelet aggregation is quantified as ARU (aspirin resistance units).
- Aspirin-works: Measure level of urine 11-Dehydrothromboxane (metabolite of Thromboxane A<sub>2</sub>) in µg/mg of creatinine.

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## Significance

- If these platelet function assays are found to be comparable, we may be able to choose the most time efficient, cost-effective approach to obtain this information.
- Data obtained can be used to distinguish aspirin resistant and aspirin sensitive individuals.
- The effectiveness of therapy for controlling glucose, cholesterol, and blood pressure is routinely monitored but the effectiveness of aspirin and Plavix therapy is not.
- If aspirin and plavix resistance is associated with increased risk of recurrent stroke, CVA, MI etc., then using platelet function assays could detect such individuals (who could then be offered other anti-thrombotic therapy?)

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## Plan for Data Analysis

- Whole blood platelet aggregation (WBPA) using Chronolog- 570Vs aggregometer was the gold standard test
- The other 3 assays results were compared to WBPA to validate equivalency

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## Chronolog WBA<sup>®</sup>

- Records whole blood platelet activation by platelet aggregation impedance
- Whole blood platelet aggregation is the reference method for aspirin detection
- 10 µL of aggregation agonists 1.0 µg/mL collagen (Coll) and 0.5 mM arachidonic acid (AA) were added to 1:1 saline/whole blood suspensions
- Aggregation impedance  $\leq$  8 ohms indicates aspirin effect

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Chrono-Log  
Model 700



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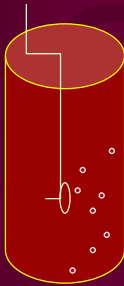
**Platelet rich plasma (light transmission aggregometry) LTA**

- Measures change in light transmission upon addition of agonist
- Considered by some the gold standard
- Labor intensive, not specific
- Sensitivity variable
- Correlates with clinical events

**WHOLE BLOOD AGGREGATION**

- Measures impedance: Superior to PRP?
- Evaluates platelets in a physiologic milieu in the presence of RBC and WBC which are known to modulate platelet function.
- Faster and uses less specimen making it better for children and hard to draw subjects.
- Higher sensitivity to medication responses.
- Does not require centrifugation thus avoiding injury to platelets and loss of giant platelets.

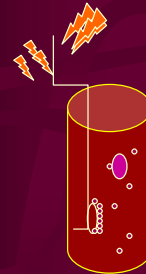
**Methods**



1. Whole blood sample is diluted with 0.9 % saline, 1:1 in cuvette

2. Electrode is placed in sample

**Methods**

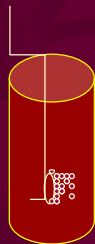


3. Platelets form a monolayer on the electrode

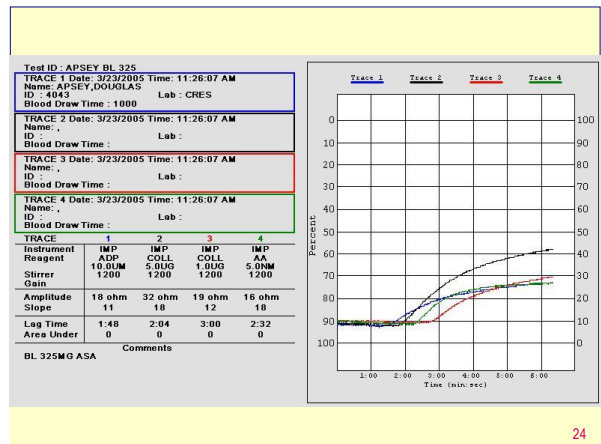
4. Voltage is run through the electrode and resistance baseline is assigned a value of zero ohms

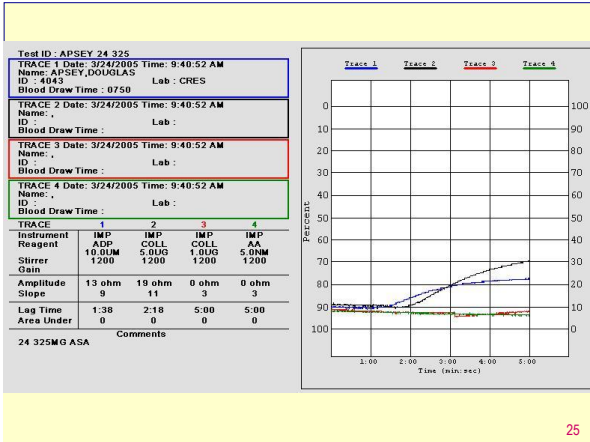
5. Agonist is added to stimulate aggregation

**Methods**

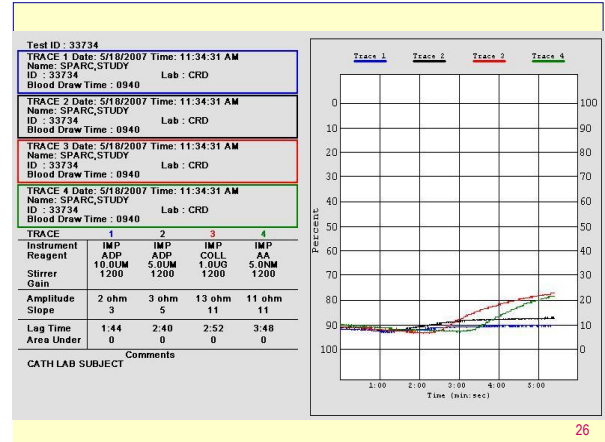


Amount of aggregation is directly proportional to the change in resistance in ohms

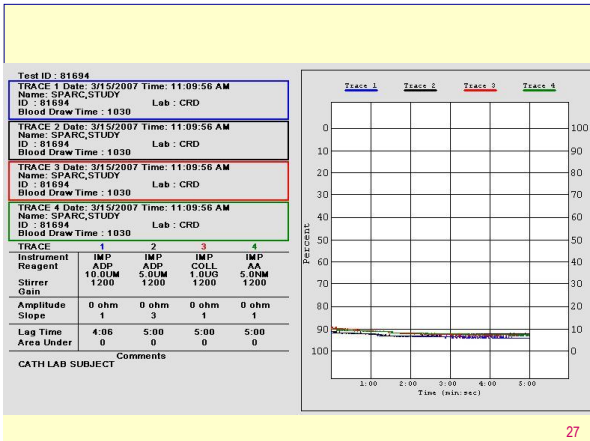




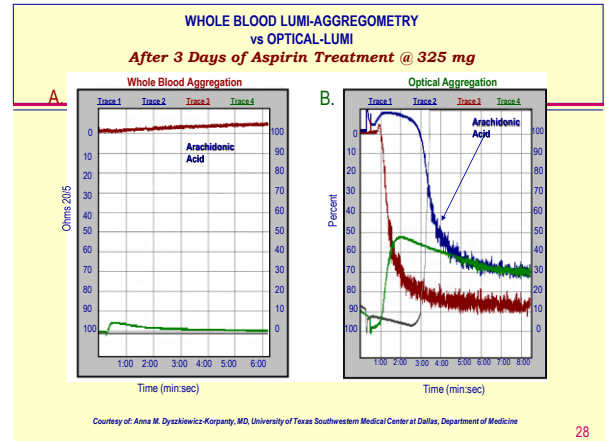
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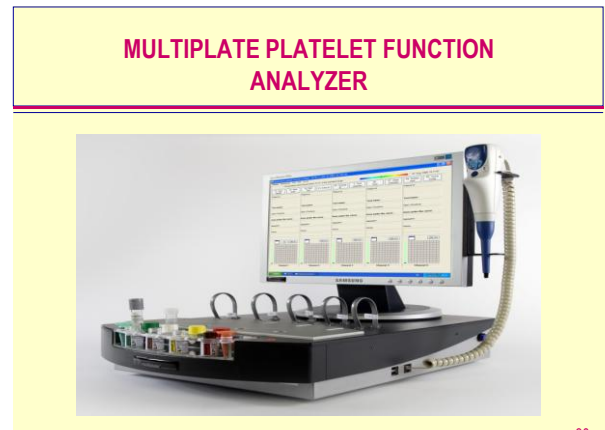
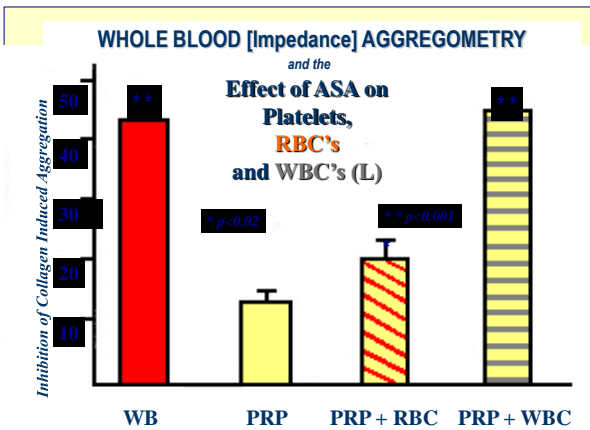
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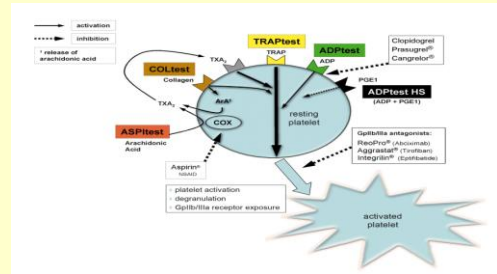
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## MULTIPLATE PLATELET FUNCTION ANALYZER: In use



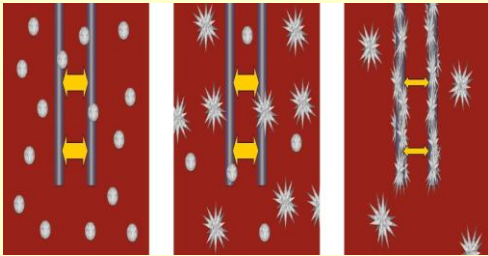
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## MULTIPLATE DIAGRAM OF TESTS



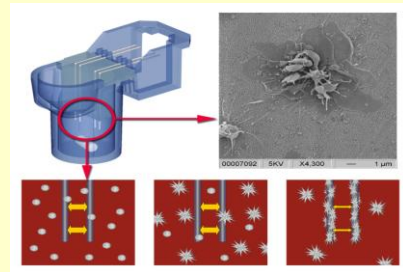
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## MULTIPLATE Detection Principle



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## MULTIPLATE TEST CELL MEASUREMENT



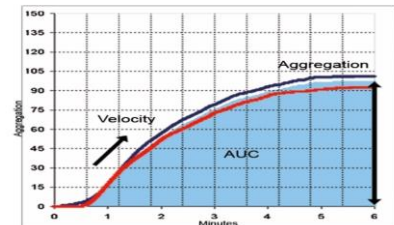
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## MULTIPLATE TEST CELL



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## MULTIPLATE AGGREGATION CURVE



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## Siemens Healthcare Diagnostics PFA-100®

- Simulates in vivo platelet plug formation by aspirating blood at a high shear rate through a small collagen-coated aperture.
- Records platelet-induced whole blood interval to occlusion of an aperture in a biochemically active membrane cartridge producing "closure time" (CT). Alternative to Ivy Bleeding Time.
- Specimens first assayed with ADP/collagen impregnated cartridges
- If ADP/collagen CT  $\leq 145$  s, aspirin effect was assessed by epinephrine/collagen (EPI/COLL) impregnated cartridges
- CT  $\geq 175$  seconds is anticipated aspirin response (time varies between different facilities)

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### PFA-100 CARTRIDGES

- Collagen/Epinephrine (CEPI) is the primary screening cartridge
- Collagen/ADP (CADD) indicates if a platelet dysfunction observed with CEPI is due to ASA or may reflect Plavix effect.
- INNOVANCE® P2Y12 cartridge specific for clopidogrel anti-platelet effect. Lower ADP concentration than CADD.

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### PFA - 100

- Requires PFA -100 instrument
- Rapid, easy to perform
- Whole blood – platelet count dependent. Hematocrit dependent. May be affected by high fibrinogen and vWF.
- Sensitivity variable
- Clinical outcomes studies limited
- Qualitative – results measured in closure time (sec)

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### PFA-100® Test Procedure



Pipette 800  $\mu$ L of citrated whole blood into the sample reservoir of the test cartridge(s).



Place the cassette onto the carousel of the analyzer.



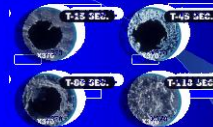
Using the integrated keypad, initiate the test run.

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### PFA-100® Test Principle

occlusion process

final result and print-out



PFA-100		
TEST: 2.00	3/M.	
00 070		
14/07/97	14:01	
ID#: 20456.17		
Test Type:	Collagen/EPI	
SAMPLE A:	110 SEC	

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The Solution: A diagnostic test that can help physicians determine if anti-platelet therapy is working for their patients.

## Verify Now by Accumetrics

- RAPID**

- Result available in less than 10 minutes

- EASY**

- Whole blood - no sample preparation
- Automatic sampling from closed tube
- Factory calibrated reagents
- CLIA - moderately complex; filed for waived status
- FDA Cleared
- Reimbursement/ CPT code



- ACCURATE**

- A quantitative reference point measured in Aspirin Reactive Units (ARU)

\*Correlates to optical platelet aggregometry

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Rapid

Easy

Accurate



Insert assay device



Add blood sample

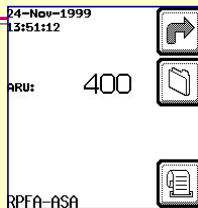


Result in minutes

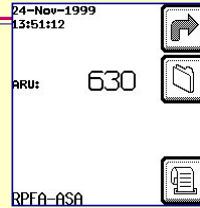
Clinical Lab  
Cardiac Cath Lab  
Point of Care  
Doctors' Office

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## VerifyNow® Aspirin Test Results



If a patient result is <550 ARU, then platelet dysfunction has been detected, indicating that Aspirin IS working.



If a patient result is  $\geq 550$  ARU, then no platelet dysfunction has been detected, indicating that the anti-platelet effect may not have been achieved or Aspirin IS NOT Working

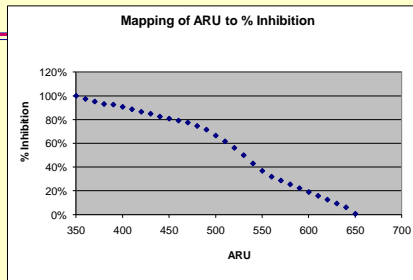
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## Verify Now

- Verify/Now Accumetrics instrument
- Cartridge containing fibrinogen-coated microparticles in a proprietary tube using Arachidonic Acid as agonist.
- Whole blood
- Rapid, easy to perform
- Sensitivity and specificity variable
- Clinical outcomes studies limited
- Qualitative – results measured as aspirin response units

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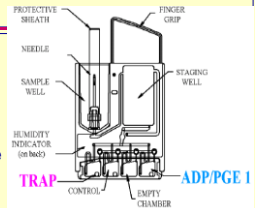
Mapping of ARU to % Inhibition



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## VerifyNow® P2Y12

- RAPID**
  - Result available in <3 minutes
- EASY**
  - Whole blood - no sample preparation
  - Automatic sampling from closed tube
  - Factory calibrated reagents
- ACCURATE**
  - More specific than optical aggregometry
  - Can measure % platelet inhibition without weaning patient off drug
- COST-EFFECTIVE**
  - Reimbursement
  - CPT code 85576 (2 times)
  - FDA cleared



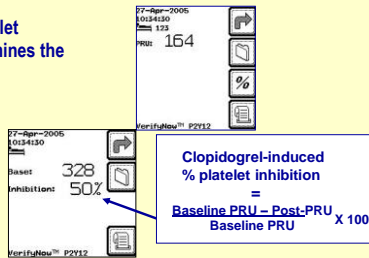
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## VerifyNow® P2Y12 Result Calculations

ADP-mediated platelet activation determines the PRU value

TRAP-mediated platelet activation approximates Baseline PRU



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## VerifyNow® P2Y12 Advantages

- Greater specificity for clopidogrel than test methods using ADP alone, e.g., optical aggregometry
- Ability to measure % platelet inhibition in patients on clopidogrel without first withdrawing clopidogrel
- Rapid - Time to result <3 minutes

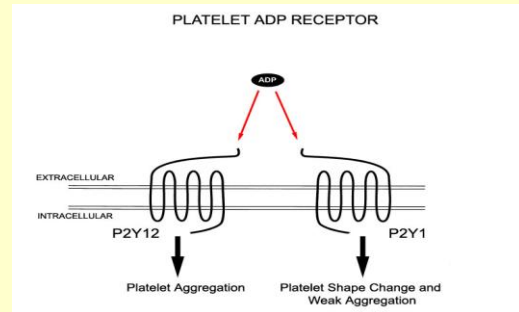
50

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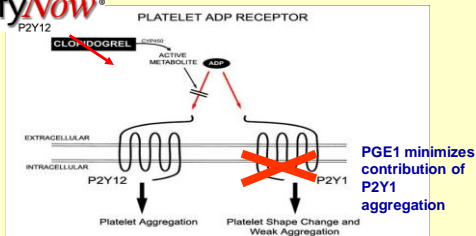
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## ADP activates platelets via two ADP receptors: P2Y12 and P2Y1...



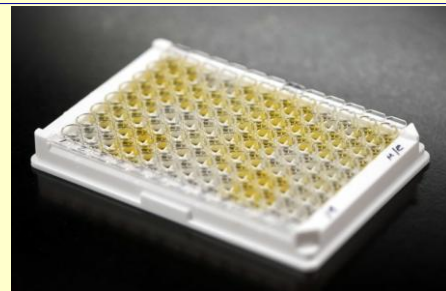
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VerifyNow®



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## Urinary 11-dehydrothromboxane B<sub>2</sub> AspirinWorks



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## Urinary 11-dehydrothromboxane B<sub>2</sub> AspirinWorks

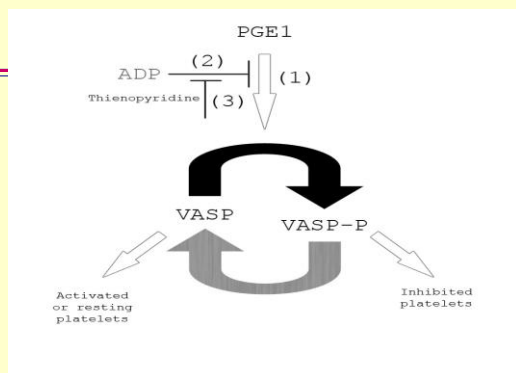
- Requires ELISA equipment and urinary creatinine result
- Random urine specimen that can be frozen until ready for testing.
- Sensitivity good
- Specificity uncertain
- Labor intense, not rapid. Two hour specimen incubation. Recently FDA approved. Established test in optimized format
- Quantitative - Results reported as pg 11-dehydrothromboxane B<sub>2</sub>/mg creatinine
- May be used to guide incremental aspirin therapy

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## VASP.P2Y12 Vasodilator Stimulated Phosphoprotein

- Dedicated to the monitoring of specific platelet ADP receptor (P2Y12) antagonists: Thienopyridines
- Regulated by cAMP cascade
- cAMP activated by PGE1 (1)
- Inhibited by ADP through P2Y12 receptors (2)
- VASP phosphorylation correlates with P2Y12 receptor inhibition. Non-phosphorylation state correlates with the active form of P2Y12 receptor.
- Thienopyridines can be demonstrated with PLT VASP/P2Y12 (3). Performed by flow cytometry on citrated blood.

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## VASP.P2Y12 Vasodilator Stimulated Phosphoprotein

- The aim of the assay is to evaluate the efficacy of Plavix therapy.
- Uses the PRI or platelet reactivity index expressed as a percentage to measure the difference in VASP fluorescence intensity between resting +PGE1 and activated +ADP platelets.
- Aleil B et al: J Thromb Haemost, 2005; 1:85-92 measuring VASP for clopidogrel resistance in patients with ischemic cardiovascular diseases found the following:
  - 85.8 to 6.6% PRI with the 85.8 being poor responders and 6.6% good responders. 30% of treated subjects were in range with bad responders. (PLAVIX RESISTANCE?)

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## Tests Requiring Blood Specimen

- Advantages
  - Point of Care
  - Rapid results
- Disadvantages
  - Preanalytical variables
  - Lack of standardization
  - Test must be run within 3-4 hours
  - Limited clinical outcomes data (except platelet aggregation)

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## Tests Requiring Blood Specimen Additional considerations

- Platelet function tests requiring whole blood may be impacted by:
  - Platelet count
  - Hematocrit
  - Fibrinogen - elevated levels (Lower fibrinogen levels have shown greater ASA response). Values above 380 mg/dl have been shown to affect assay.
  - Factor VIII – elevated levels
  - vWF – elevated levels
  - BMI and diabetes
  - Genetic polymorphisms-ASA
  - Genetic polymorphisms-Plavix: CYP3A and CYP2C19

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## Urinary 11-dehydrothromboxane B<sub>2</sub>

- Metabolite not formed by platelet
- High concentration
- Longer circulating half-life
- Minimal pre-analytical variables
- Specimen stable 72 hours at room temperature
- Major clinical outcomes study to support the test
- Standardization of test
  - to outcome studies
  - between laboratories
  - Disadvantages: Liver disease, renal disease may affect results

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## Comparison of The 24-hour Sensitivity of Four Platelet Function Assays to A Single Aspirin

DL McGlasson, G Fritsma, M Chen, Z Knight, M Dobbs, 59<sup>th</sup> Clinical Research Squadron and Department of Neurology Wilford Hall Medical Center, Lackland AFB, TX and University of Alabama Birmingham, Division of Laboratory Medicine, Birmingham, AL

## Aspirin Response Assays

- Assays that measure platelet response to aspirin may predict aspirin's cardioprotective effect
- We compared four methods for monitoring 24-hour platelet inhibition in healthy subjects by a single 81 mg and 325 mg (standard child and adult) aspirin dose
- We anticipated these assays would reveal a greater 24-hour anti-platelet aspirin effect of 325 mg compared to 81 mg
- We further anticipated that the assays were comparable in their ability to detect the aspirin effect

Introduction

## Subjects and Procedure

- Fifty normal healthy volunteers were enrolled. None had taken aspirin or other NSAIDs for  $\geq 14$  days
- 20 females, mean age 33.1 (18-51)
- 30 males, mean age 36.6 (20-58)
  1. Baseline citrated whole blood and urine
  2. Subjects observed to ingest a single 81 mg aspirin
  3. Citrated blood and urine obtained 24 hours after dosing
- Process repeated  $\geq 14$  days with single 325 mg aspirin

Materials and Methods

## Chronolog WBA<sup>®</sup>

- Records whole blood platelet aggregation by impedance
- Whole blood platelet aggregation is chosen as the reference method for aspirin response detection
- 10  $\mu$ L of aggregation agonists 1.0  $\mu$ g/mL collagen (Coll) and 0.5 mM arachidonic acid (AA) were added to 1:1 saline/whole blood suspensions
- Post-aspirin aggregation impedance  $\leq 8$  ohms indicates anticipated aspirin response

Materials and Methods

## 11-dehydro Thromboxane B<sub>2</sub>

- Urine 11-dehydrothromboxane B<sub>2</sub> (11-DHT) is an end product of the platelet arachidonic acid prostaglandin pathway whose urine concentration reflects in vivo platelet activity
- Aspirin inhibits the prostaglandin pathway and decreases urine 11-DHT production
- $\geq 50\%$  11-DHT reduction from baseline indicates aspirin effect
- Urine 11-DHT is measured using random urine when normalized to urine creatinine

Materials and Methods

## Verify/Now®

- Arachidonic acid (AA)-impregnated cartridge aggregates platelets
- Aggregation time interval expressed as aspirin reaction units (ARUs)
- Post-aspirin aggregation impedance  $\leq 550$  ARUs indicates response

Materials and Methods

## Siemens Healthcare Diagnostics PFA-100®

- Records platelet-induced whole blood interval to occlusion of an agonist-impregnated cartridge aperture producing closure time (CT)
- Specimens first assayed with ADP/collagen impregnated cartridges
- If ADP/collagen CT  $\leq 145$  s, aspirin effect was assessed by epinephrine/collagen (EPI/Coll) impregnated cartridges
- EPI/Coll CT  $\geq 175$  seconds is anticipated aspirin response

Materials and Methods

## 24-Hour Response to 81 mg and 325 mg Aspirin: Means

	Chronolog WBA® Aggregometry Reference Method		11-DHT	VerifyNow®	Dade-Behring PFA-100®
	1.0 ug Coll	0.5 mM AA			
Baseline 81 mg	20.5 $\Omega$	19.1 $\Omega$	978.4 pg/mg	643.7 ARU	136.2 CT
24-h Response to 81 mg	16.1 $\Omega$ *	2.1 $\Omega$ *	510.7 pg/mg*	600.7 ARU*	170.0 CT*
Baseline 325 mg	18.2 $\Omega$	18.1 $\Omega$	884.5 pg/mg	646.2 ARU	130.5 CT
24-h Response to 325 mg	13.6 $\Omega$ *	1.9 $\Omega$ *	349.1 pg/mg*	465.3 ARU*	258.2 CT*

In all assays, 81 mg to 325 mg baselines are not significantly different at  $p < 0.05$   
\*All aspirin responses significant at  $p < 0.05$

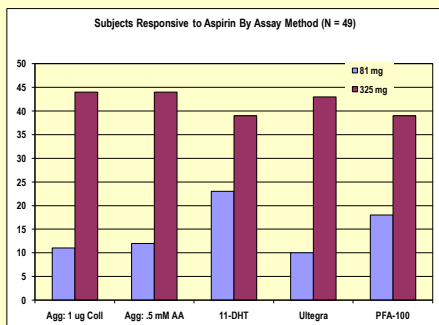
Results

## 24-Hour Response to 81 mg and 325 mg Aspirin: Action Limits

	Chronolog WBA® Aggregometry Reference Method		11-DHT	VerifyNow®	SiemensPFA -100®
	1.0 ug Coll	0.5 mM AA			
N = 49					
Action Limit	$\geq 8 \Omega$		$\leq 50\%$ Reduction	$\geq 550$ ARU	EPI/COLL CT $\leq 175$ s
Response to 81 mg Aspirin	11 (22.4%)	12 (24.5%)	23 (46.9%)	10 (20.4%)	18 (36.7%)
Response to 325 mg Aspirin	44 (89.8%)	44 (89.8%)	39 (80.0%)	43 (87.8%)	39 (80.0%)

Results

## 24-Hour Response to 81 mg and 325 mg Aspirin: Action Limits



Results

## 24-hour Response to 325 mg Aspirin

- There was no significant gender effect at baseline or 24 hours for 11-DHT and VerifyNow in either the 81 or 325 mg arm (data not displayed)
- The systems equivalently recorded an average 85.5% 24-hour individual subject responses to 325 mg aspirin relative to action limits

Results

## 24-hour Response to 81 mg Aspirin

- The systems recorded a significant mean reduction of platelet function 24 hours after a single dose of 81 or 325 mg aspirin
- The ratio of individual subject responses to 81 mg aspirin relative to action limits averaged 30.2%
- The 11-DHT individual subject responses to 81 mg aspirin, 46.9%, is the most sensitive
- The Dade-Behring PFA-100 individual subject responses to 81 mg aspirin, 36.7%, is the second most sensitive

Discussion

## Predictive Values of Methods

- The predictive value of 11-DHT, VerifyNow, and PFA-100 compared to aggregation, averages 39% at 81 mg aspirin
- The predictive values of 11-DHT and VerifyNow compared to aggregation at 325 mg aspirin are 86.8% and 93.0%, respectively
- 11-DHT and VerifyNow duplicate the reference method's ability to identify the 24-hour platelet response to 325 but not 81 mg aspirin
- These data may be confirmed using a 7-day dosage schedule

Results

## Analysis

- Platelet inhibition across 3 assays seems to be dose dependent (81mg vs 325 mg) at 24 hours.
- Out of 38 individuals whose WBPA showed no significant changes at 81 mg, 31 of those individual become responders at 325 mg.
- % of aspirin resistance may be high in this study secondary to one time dose effect. If subjects were to take aspirin on daily basis, % of aspirin resistance may drop.
- Initial responders may develop aspirin tolerance according to some studies when taking aspirin chronically.

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## Comparison of Four Commercial Platelet Function Assays' Ability to Detect Response to 7 Days of Aspirin at 81 and 325 mg Doses

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## Aspirin Response

- We compared the ability of four commercial platelet function assays to detect the 7-day aspirin (ASA) response in normal subjects taking 81 or 325 mg
- Laboratory detection of inadequate ASA-induced platelet suppression may indicate physiological insensitivity, called "aspirin resistance"
- ASA resistance is a recognized cause of failed ASA therapy and may predict arterial thrombosis risk
- We anticipated the assays would reveal a dosage effect for 325 mg compared to 81 mg ASA
- We anticipated the assays are comparable in their ability to detect ASA response

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## Materials and Methods

- We consented forty-five normal healthy volunteers. None had taken ASA or other NSAIDs for  $\geq 14$  days
  - 22 females, mean age 33.1 (18-51)
  - 23 males, mean age 36.6 (20-58)
- 1. Baseline 3.2% Na citrate whole blood and urine
- 2. Subjects provided a single 81 mg aspirin for 7 days
- 3. Na citrate whole blood and urine obtained 24 hours after final dose
- Repeated  $\geq 14$  days with single 325 mg ASA for 7 days

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## Mean Responses to 7-Day ASA at 81 mg and 325 mg

	Chronolog WBA® WBPA Reference Method		11-DHT	Accumetrics Verify/Now®	Siemens PFA-100®
	1 ug/mL Coll	500 µM AA			
Baseline pre-81 mg	20.0 Ω	16.8 Ω	538.0 pg/mg	634.5 ARU	138.8 CT
7-d response to 81 mg	6.0 Ω*	3.2 Ω*	161.7 pg/mg*	436.3 ARU*	226.7 CT*
Baseline pre-325 mg	21.3 Ω	19.6 Ω	642.4 pg/mg	647.6 ARU	121.6 CT
7-d response to 325 mg	4.1 Ω*	1.0 Ω*	206.7 pg/mg*	425.8 ARU*	250.0 CT*

No pre-81 mg to pre-325 mg baselines are significantly different at p < 0.05  
\*All 7-day responses significant at p < 0.05

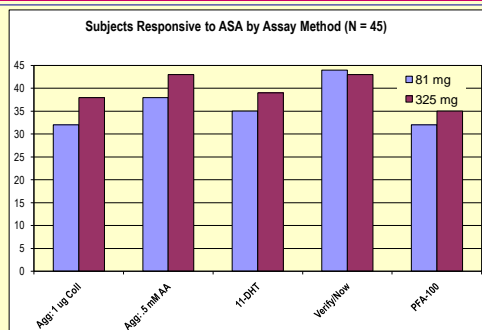
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## Percent 7-Day Response to 81 mg and 325 mg ASA by Action Limits

N and (%) ASA Responders					
N = 45	Chronolog WBA® WBPA Reference Method		11-DHT	Accumetrics Verify/Now®	Siemens PFA-100®
	1 ug/mL Coll	500 µM AA			
<b>Action Limit</b>	<b>≥ 8 Ω aggregation</b>		<b>≤ 50% reduction</b>	<b>≤ 550 ARU</b>	<b>EPI/COLL CT ≥ 175 s</b>
Response to 81 mg ASA	32 (71.1)	38 (84.4)	35 (77.8)	44 (97.8)	32 (71.1)
Response to 325 mg ASA	38 (84.4)	43 (95.6)	39 (86.7)	43 (95.6)	35 (77.8)

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## Numerical 7-Day Response to 81 mg and 325 mg ASA by Action Limits



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## Discussion

- Mean platelet response to ASA at 81 or 325 mg ASA for 7 days for all platforms were significant
- Verify/Now is the most sensitive to 81 mg and 325 mg ASA
- WBPA using 1.0 µg/mL collagen, 11-DHT and PFA-100 detected the most instances of ASA resistance
- Positive predictive values were comparable for 11-DHT, PFA-100, and Verify/Now at 81 and 325 mg
- These data provide support for these methods to use in clinical settings to distinguish aspirin responders vs. non responders
- *We recommend continued testing on clinical populations to confirm the dosage effect and compare platforms to clinical outcomes*

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## Potential study limitations

- Other possible mechanisms of clinical aspirin resistance
  - Patient non-compliance and underdosing
  - COX 2 expression inducing production of THX-A2
  - Glycoprotein IIb/IIIa polymorphism
  - Erythrocyte/Leukocyte/platelet interaction
  - Elevated fibrinogen and vonWillebrand's factor
  - Type II Diabetics do not respond as well to ASA or Plavix
  - Cigarette smoking and hypercholesterolemia
- Platelet inhibition may not be constant over an extended time with a fixed dose of Aspirin
- Aspirin resistance in the single dose study may be higher because of one time dose effect. Percent of aspirin resistance may be reduced if given aspirin on daily basis.
- Some people might show biochemical platelet inhibition at baseline without administration of antiplatelet drugs.

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## Clinical Implications

- For individuals who do not respond to 81mg ASA when tested by these methods, titrating up aspirin dose may be needed to achieve sufficient platelet inhibition over several days and retest
- For those who are aspirin non-responders when testing by these methods with 325 mg aspirin (including urine 11-dehydrothromboxane), alternate anti-platelet therapy may be indicated
- Initial responders may develop aspirin tolerance according to some studies when taking aspirin chronically.
- There are needs for randomized double blind studies to show that by giving alternate anti-platelet therapy in patients with a history of vascular events on ASA and shown to be biochemically ASA resistant, the risk of further events is decreased when compared to those individuals continued with aspirin

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**COMPARISON OF THE DETECTION OF P2Y12-RECEPTOR BLOCKADE IN PRE-ANGIOCATH SUBJECTS WITH CARDIOVASCULAR DISEASE BY LIGHT-TRANSMITTANCE AND WHOLE-BLOOD AGGREGOMETRY, VERIFY NOW® P2Y12 AND INNOVANCE® PFA P2Y**

David L. McGlasson, MS, MLS (ASCP) CM,  
Anand D. Shah, MD.  
Wilford Hall Medical Center, Lackland AFB, TX

Funding for meeting registration to present this poster was received from Siemens Healthcare Diagnostics, Inc.

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## INTRODUCTION

- Our purpose is to determine the accuracy with which a new technology from Siemens Healthcare Diagnostics, Inc. can quantify the effects of the anti-platelet medication clopidogrel on platelet function.
- In this study we compared the results of the INNOVANCE® PFA P2Y\* (P2Y), a new test cartridge for the PFA-100® system to the following test systems:
- Light transmittance aggregometry (LTA) with 20  $\mu\text{M}$  ADP and whole blood aggregometry (WBA) using 5 and 10  $\mu\text{M}$  ADP performed on a Chrono-Log 700 platelet aggregometer.
- Verify Now® P2Y12 cartridge by Accumetrics

\*Not available for sale in the US

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## INTRODUCTION: Continued

- We anticipate that patients receiving clopidogrel as anti-platelet therapy for coronary artery disease will demonstrate platelet inhibition (when tested with P2Y) using the PFA-100® system.
- The performance characteristics of the P2Y cartridge used in this protocol have not been established for the US.

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## MATERIALS AND METHODS

- Blood was collected with 3.2% and 3.8% sodium citrate from 102 subjects with cardiovascular disease after receiving clopidogrel (6-24 hours post loading) with 300 mg [n=35] or 600 mg [n=7] or after 7 days of 75 mg daily [n=60].
- P2Y12 receptor blockade was detected with P2Y using a cut-off of >106 seconds. (provided by manufacturer for this study)
- Only the P2Y was tested with 3.2 and 3.8% sodium citrated blood.

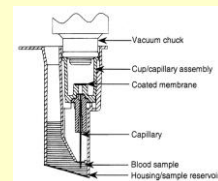
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## MATERIALS AND METHODS: continued

- The VerifyNow (VNP) system was only tested with 3.2% sodium citrate.
- Cut off for the VNP was >20% inhibition. (provided by manufacturer in personal communication)
- Cut-off using LTA with 20  $\mu\text{M}$  ADP on the Chrono-Log 700 platelet aggregometer was <50% amplitude (in-house method validation).
- WBA with 5  $\mu\text{M}$  ADP <5 ohms and 10  $\mu\text{M}$  <8 ohms (in-house method validation).

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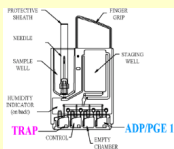
## PFA-100® Cartridge Schematic



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## VerifyNow® P2Y12

- **RAPID**
  - Result available in <3 minutes
- **EASY**
  - Whole blood - no sample preparation
  - Automatic sampling from closed tube
  - Factory calibrated reagents
- **ACCURATE**
  - More specific than optical aggregometry
  - Can measure % platelet inhibition without weaning patient off drug
- **COST-EFFECTIVE**
  - Reimbursement
  - CPT code 85576 (2 times)
  - FDA cleared



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## RESULTS

- Sensitivity is determined by dividing the number of true positives (TP) by the TP plus the false negatives (FN) X 100% (TP/TP+FN).
- Detection rates of P2Y12-receptor blockade for each method are shown in the table below:

Method	PFA P2Y 3.2%	PFA P2Y 3.8%	VN P2Y12	LTA 20 µM	WBA 5 µM	WBA 10 µM
Sensitivity	59%	95%	60%	88%	89%	72%

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## RESULTS: Continued

- Concordance is the agreement between two methods cut-offs usually expressed in percent (%)
- The total concordance for this set of post drug patients was computed and the results are as follows:

	VN P2Y12	WBA 5 µM	WBA 10 µM	LTA 20 µM
<b>P2Y 3.2%</b>	71%	64%	65%	69%

	VN P2Y12	WBA 5 µM	WBA 10 µM	LTA 20 µM
<b>P2Y 3.8%</b>	71%	90%	90%	76%

	WBA 5 µM	WBA 10 µM	LTA 20 µM
<b>VN P2Y12</b>	68%	67%	72%

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## DISCUSSION

- The P2Y 3.8% results of 95% compares favorably with the results obtained in both WBA ADP concentrations. The P2Y 3.2% data compares closely with the VerifyNow® cartridge system.
- Concordance with the VerifyNow® cartridge system was favorable at 71% for both P2Y sodium citrate concentrations.
- However, when comparing with WBA the 3.8% citrate results with the P2Y cartridge was 90%.
- The INNOVANCE® PFA-P2Y agrees favorably with other methods for detection of P2Y12 receptor blockade induced by clopidogrel.

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## Introduction

### Effects of Elevated Fibrinogen, Factor VIII, von Willebrand Antigen, and Immunologic von Willebrand Factor the INNOVANCE® PFA P2Y Cartridge

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- The INNOVANCE® PFA-100 P2Y® (PFA P2Y, ADP receptor) cartridge closure time (CT) is used to detect platelet inhibition by clopidogrel (Plavix).
- The purpose of this protocol was to determine if elevated levels of Fg, FVIII:C, VWF:Ag and VWF would affect CTs in PFA P2Y system.
- Elevated levels of Fg, FVIII:C, VWF:Ag and VWF have been implicated in thromboembolic disease.
- Our protocol sought to determine if shortened PFA P2Y CTs correlated with elevated levels of Fg, FVIII:C, VWF:Ag and VWF.

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## Materials and Methods

- Subjects were screened and gave informed consent.
  - 101 were admitted to the study.
- Subjects were 18–90 years of age and scheduled to receive clopidogrel due to a history of cardiovascular or cerebrovascular disease, or at least two of eight risk factors for developing vascular disease based on American Heart Association criteria.
- Risk factors included a history of smoking, hypertension, hyperlipidemia, family Hx of vascular disease, post-menopausal females, diabetes mellitus, morbid obesity or sedentary lifestyle.
- Subjects were excluded if they were less than 18 years of age or receiving any platelet function inhibitor medication or herbal supplement.
- Other exclusions were pregnant or nursing females, inherited or acquired von Willebrand disease or participation in any other clinical trial. All subjects had a platelet count >100,000/mcL and a hematocrit >30%.

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98

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## Results

- Mean plasma levels were: Fg: 381.0 mg/dl; FVIII:C: 169%; VWF:Ag: 161%, VWF: P2Y: 210%.
- Linear regression gave a parameter estimate of –0.71.
- Logistic regression yielded the results shown in the table:

Parameter	p Values are significant at p < 0.05		
	Col/EPI	Col/ADP	PFA P2Y
Fg	0.61	0.63	0.68
FVIII:C	0.68	0.88	0.50
VWF:A	0.87	0.88	0.07
g			

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## Discussion

- The mean values for the coagulation parameters are all above the upper limits of our normal ranges with the exception of the Fg value which is at the upper end of the normal range.
- The population we studied may be at risk for occurrence of thrombotic events based on these results.
- Neither Col/EPI nor the Col/ADP CTs correlated with elevated factor levels.
- PFA P2Y CTs showed a significant inverse correlation with VWF by logistic regression analysis (p<0.01).

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## Discussion

- Elevated VWF appears to correlate with shorter PFA P2Y CTs despite clopidogrel therapy.
- The linear regression parameter estimate for VWF shows that for every per cent (%) the VWF value changes, the CT changes by 0.7 seconds.
- We speculate that like elevated VWF, short PFA P2Y CTs may signal risk of thrombotic events in this population.
- The INNOVANCE® P2Y cartridge is currently awaiting FDA clearance.

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- VerifyNow Accumetrics is based in San Diego, CA, United States
- Chrono-Log is based in Havertown, PA, United States

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## ANTIPLATELET THERAPY AND SURGERY? When to stop meds?

- Discontinuing therapy prior to surgery: 20% incidence of ischmia
- Continuation of drugs: 35% increased risk of bleeding
- ACCP guidelines (2011): Stop ASA or clopidogrel 7-10 days prior to surgery
- Patients pre-op for CABG: stop at least 7 days for clopidogrel and 7 days for prasugrel .
- Consider genotyping for CYP219
- Perform platelet function testing

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## ANTIPLATELET THERAPY AND SURGERY? When to stop meds? Cont.

- Society for Thoracic Surgeons Society guidelines (2011): Discontinue P2Y12 receptor inhibitors 3 days prior coronary revascularization.
- Consider POCT for platelet ADP response to ID P2Y12 non-responders.
- ACCF/AHA guidelines (JACC 2011;57:1920-59). Withdraw clopidogrel 7 days prior to CABG and 5 days with prasugrel. Suggest genotyping for CYP2C19 and platelet function testing.

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