

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K141427

**B. Purpose for Submission:**

Modification of previously cleared instrument, assay, and control material.

Changes to assay reagents include the ADP pellet formulation, TRAP pellet channel location and introduction of a no-agonist pellet. The humidity sensor was removed from the instrument, while a port cover was added. The formulation of the control material was also changed.

**C. Measurand:**

Platelet aggregation

**D. Type of Test:**

Automated whole blood platelet aggregation

**E. Applicant:**

Accumetrics, Inc.

**F. Proprietary and Established Names:**

VerifyNow™ PRUtest

**G. Regulatory Information:**

1. Regulation section:

21 CFR 864.5700 – Automated platelet aggregation system

2. Classification:

Class II

3. Product code:

JOZ – System, Automated Platelet Aggregation

4. Panel:

81 Hematology

**H. Intended Use:**

1. Intended use(s):

The VerifyNow™ PRUtest is a whole blood test used in the laboratory or point of care setting to measure the level of platelet P2Y12 receptor blockade. For *in vitro* diagnostic use. For professional use only.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

Not applicable

4. Special instrument requirements:

The VerifyNow™ PRUtest is designed to be run on the VerifyNow instrument

**I. Device Description:**

The VerifyNow™ PRUtest device contains three lyophilized reagent pellets in separate reaction chambers within the test device:

- 1) ADP pellet consisting of a preparation of Fibrinogen and BSA coated beads, adenosine-5-diphosphate (ADP), prostaglandin E1 (PGE1), dye, buffer, and a preservative
- 2) TRAP pellet (internal control) consisting of a preparation of iso-TRAP (Thrombin Receptor Activating Peptide), Fibrinogen and BSA coated beads, buffer, dye, and a preservative
- 3) No-Agonist Pellet (NAP) consisting of a preparation of BSA coated beads, dye, buffer, and a preservative

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Accumetrics, Inc. VerifyNow™ P2Y12 Assay

2. Predicate 510(k) number(s):

K051231

3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Intended Use	A whole blood test used in the laboratory or point of care setting to measure the level of platelet P2Y12 receptor blockade. For in vitro diagnostic use. For professional use only.	Same
Principle of Operation	Fibrinogen-coated microparticles, ADP/PGE1 platelet activation with turbidimetric optical detection of P2Y12-mediated platelet aggregation	Same
Specimen Type	Citrated whole blood	Same
Testing site	Point of care or laboratory	Same
Device Output	P2Y12 Reaction Units (PRU)	Same
Controls	1. Bi-level external/ wet quality control (WQC) 2. Internal control	Same
Calibration	Factory	Same
<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
ADP pellet formulation	80% Fibrinogen/20% BSA	100% Fibrinogen
TRAP control formulation	20 $\mu$ M TRAP & 800 $\mu$ M Par-4	3.4 $\mu$ M TRAP
NAP pellet	No-agonist pellet (NAP) introduced	No-agonist pellet absent
Control Level 1	Contains BSA	Contains fish skin gelatin
Control Level 2	GRP conjugate truncated	GRP conjugate original length
Instrument humidity sensor	Removed	Present

**K. Standard/Guidance Document Referenced (if applicable):**

CLSI C28-A3c Defining, Establishing, Verifying Reference Intervals in the Clinical Lab, Approved Guideline-3<sup>rd</sup> Edition

CLSI EP05-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-2<sup>nd</sup> Edition

CLSI EP09-A3 Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline

CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

IEC 60601-1-2 Medical Electrical Equipment- Part1-2: General Requirements for Safety- Collateral Standard: Electromagnetic Compatibility-Requirements and Tests

**L. Test Principle:**

The patient sample is citrate-anticoagulated whole blood, which is automatically dispensed from the blood collection tube into the test device by the instrument, with no blood handling required by the user. Fibrinogen-coated microparticles are used in the VerifyNow PRU Test device to bind activated platelet GP IIb/IIIa receptors. ADP is incorporated into the assay to activate platelets, and the reagent is formulated to specifically measure P2Y12-mediated platelet aggregation. When the activated platelets are exposed to the fibrinogen-coated microparticles, aggregation occurs in proportion to the number of activated platelet receptors. The VerifyNow PRU Test reports results in P2Y12 Reaction Units (PRU).

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

Reagent precision- whole blood:

Between-lot precision and between-instrument precision were determined in two separate experiments. Duplicate whole blood samples from each of 9 donors covering the device’s measuring range were tested on a minimum of five (5) non-consecutive days on three VerifyNow™ Instruments. A different device lot was tested on each instrument, and lot/instrument combinations were reassigned each day for randomization purposes. Precision estimates in this experiment include the effects of both instrument and lot variability. Within-run, between-run, between-day, and between-lot variability was determined for each donor. With the exception of donor 7, the data met the pre-determined acceptance criteria of ≤10% CV.

Days	Runs/day	Reps	Mean PRU	Within-run		Between-run		Between-day		Between-lot	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
5	1	30	64.1	4.7	7.4	ND	ND	9.6	15.0	0.0	0.0
8	2	96	123.4	9.6	7.8	18.7	15.1	57.6	46.7	0.0	0.0
5	2	60	161.9	6.3	3.9	5.1	3.1	17.7	10.9	0.0	0.0
5	1	30	162.5	10.9	6.7	ND	ND	13.3	8.2	0.0	0.0
5	1	30	190.4	12.0	6.3	ND	ND	15.1	7.9	0.0	0.0
10	2	114	215.6	13.0	6.0	7.3	3.4	16.5	7.6	0.0	0.0
5	1	30	220.6	8.9	4.0	ND	ND	17.0	7.7	0.0	0.0
10	2	118	243.8	10.4	4.2	0.0	0.0	21.9	9.0	0.0	0.0

Days	Runs/day	Reps	Mean PRU	Within-run		Between-run		Between-day		Between-lot	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
10	2	120	289.0	11.2	3.9	7.7	2.7	27.0	9.3	0.0	0.0

No data (ND) for between-run precision for four subjects that had only one run per day. One of these subjects had insufficient blood volume to complete the between instrument experiment.

For evaluation of between-instrument variability, nine donors were tested with one reagent lot in duplicate across all three instruments for a minimum of five days, with two runs per day using a different operator for each run. The between-instrument variability was  $\leq 10\%$ .

Days	Runs/day	Reps	Mean PRU	Within-run		Between-run		Between-day		Between-instrument	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
5	1	30	67.1	7.3	10.8	ND	ND	9.3	13.8	0.0	0.0
5	2	60	115.4	8.8	7.6	20.4	17.7	65.9	57.1	0.0	0.0
5	2	60	159.9	9.3	5.8	4.1	2.5	18.1	11.3	3.6	2.3
5	1	30	160.2	11.2	7.0	ND	ND	11.6	7.3	0.0	0.0
5	1	30	228.7	7.9	3.5	ND	ND	14.4	6.3	8.1	3.5
5	2	54	230.7	14.7	6.4	12.1	5.3	9.2	4.0	7.9	3.4
8	2	89	252.8	13.6	5.4	0.0	0.0	10.4	8.1	9.3	3.7
9	2	101	289.6	10.7	3.7	5.4	1.9	21.4	7.4	11.4	3.9

No data (ND) for between-run precision for four subjects that had only one run per day. One of these subjects had insufficient blood volume to complete the between instrument experiment.

#### Repeatability and Reproducibility- Wet Quality Controls:

Repeatability: Due to changes to the quality control material, repeatability for one lot of Wet Quality Control (WQC) Level 1 and 2 was tested in duplicate with three instruments over 20 non-consecutive days. Within-run, between-run, between-day, and total variability were assessed. Level 2 WQC meet the acceptance criteria of  $\%CV \leq 10\%$ .

n	Mean PRU	Within-run		Between-run		Between-day		Between-lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
240	300.5	13.6	4.5	7.7	2.5	8.3	2.8	13.5	4.5	22.2	7.4

Level 1 WQC simulates a patient samples with highly inhibited platelets and does not produce a significant aggregation profile. The CVs for Level 1 WQC were not calculated because the nominal PRU value is zero. Values generated for Level 1 WQC did not exceed 30 PRU.

Reproducibility: Reproducibility was assessed for one lot of WQC Level 1 and 2 run on three instruments, tested twice a day, in duplicate over six non-consecutive days. The acceptance criterion for WQC Level 2 precision of %CV  $\leq 10\%$  was met. Values generated for Level 1 WQC did not exceed 30 PRU.

n	Mean PRU	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
72	302.7	22.6	7.5	0.0	0.0	8.5	2.8	0.0	0.0	24.2	8.0

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Real time stability: Real time stability studies with control lot 3 are currently ongoing. Three lots of WQC Level 2 controls and three lots of reagent are stored at  $20 \pm 5$  °C. Each of the three lots of WQC Levels 1 and 2 were tested at time 0, 3, 6, and 9 months with each of three reagent lots. The results did not exceed the pre-specified 10% deviation from the initial measurement, supporting a stability claim of 9 months at  $20 \pm 5$  °C for both WQC Level 2 and reagent.

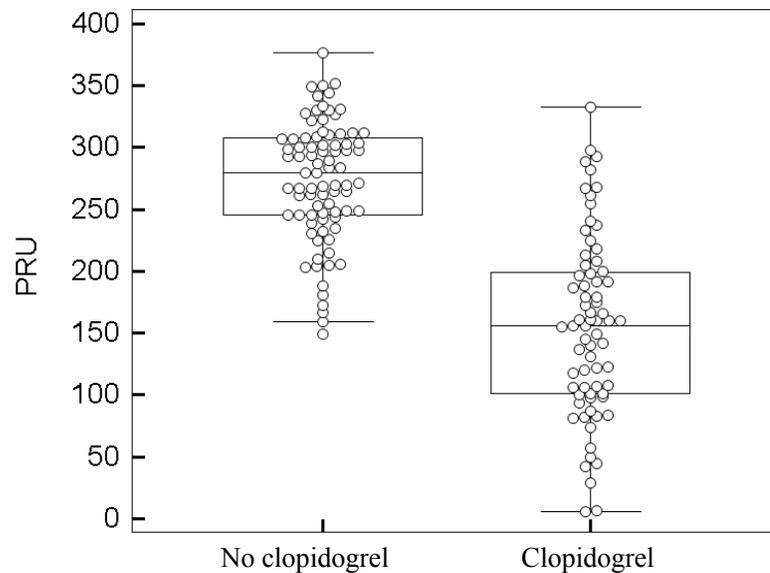
Level 1 WQC simulates a patient samples with highly inhibited platelets and does not produce a significant aggregation profile. The CVs for Level 1 WQC were not calculated because the nominal PRU value is zero. The PRU range of the Level 1 WQC does not exceed 30 PRU in the 9-month testing timeframe.

Isochronous stability reagent stability study with patient samples: In addition to real time stability with the WQC material, blood from two donors and the two levels of WQC material were tested at 3, 6, and 9 months with three (3) lots of reagent stored at both  $-70$  °C and  $20 \pm 5$  °C. The difference in means between whole blood tested with the  $-70$  °C and  $20 \pm 5$  °C conditions was within  $\leq 10\%$  further supporting the 9-month stability claim for VerifyNow™ PRUtest reagent storage at  $20 \pm 5$  °C.

Sample stability: A sample stability study was provided with blood from 11 donors spanning the analytical measuring range (AMR) of the device. Samples were run in duplicate after wait times of  $\leq 1, 5, 10, 20, 30, 60, 120, 240,$  and 300 minutes of storage. The acceptance criterion was defined as PRUtest values that deviated  $< 10\%$  from the one-sided 95% lower confidence boundary of the PRUtest value predicted from the regression line. All but one sample met the VerifyNow™ PRUtest sample stability acceptance criterion for the 10-240 minute timeframe. The failed sample had extremely low PRU values ( $< 20$  PRU). The VerifyNow™ package insert instructs the user to wait a minimum of 10 minutes after blood draw before running the assay.

Shipping stability: Device kits were packaged by facilities personnel responsible for shipping customer material. Effects of initial manual handling, vehicle stacking, loose load vibration, low pressure (high altitude), vehicle vibration, impacts and final manual handling were examined. Two test lots of reagent were subjected to distribution testing. For each donor sample, the mean PRU result must be within  $\pm 10\%$  of the mean of the control (un-shipped) treatment. The mean PRU result for the shipped treatment was within  $\pm 10\%$  of the mean of the control treatment, meeting the pre-determined acceptance criteria.

Expected values: 84 patients with acute coronary syndrome, who are candidates for, but are not receiving a P2Y12 receptor inhibitor and 71 patients receiving aspirin and clopidogrel dual therapy were tested in order to determine expected value ranges. The resulting data and statistical analysis are provided below:



	<b>No P2Y12 Inhibitor</b>	<b>Clopidogrel</b>
N	84	71
Mean	274	156
SD	48	73
Lower (95% CI)	180 (160-200)	6 (0-34)
Upper (95% CI)	376 (358-395)	300 (269-329)
Mean (SD) clopidogrel dose		79 (28)
Mean (SD) ASA dose	137 (103)	167(117)

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

Interference testing was performed in-house to evaluate the effect of common substances in patient samples on the VerifyNow™ PRU Test Assay. The effect on medications and dietary substances commonly present in the intended use population were examined in this study. Blood samples devoid of platelet inhibitor and containing platelet inhibitor were tested pre and post spiking with the test compound. Platelet inhibitor containing samples were spiked with 14 mM MeSAMP to produce PRU values of about 130 PRU. One compound (Prilosec) was tested two hours following ingestion by healthy volunteers. The effect of the potential interferent (spiked or ingested) on the assay result was expressed as percent recovery. Samples were tested in randomized fashion with seven (7) instruments. The following substances were evaluated at the indicated concentrations and met the acceptance criteria of <90% to 110% of baseline PRU Test results:

<b>Substance</b>	<b>Concentration</b>	<b>PASS/FAIL</b>
A3P5PS	100 µM	PASS
Acetaminophen	0.2 and 1.32 mM	PASS
Betamethazone	64 µM	PASS
Caffeine	306 µM	PASS
Captopril	23 µM	PASS
Catechin	86 µM	PASS
Cimetidine	79 µM	PASS
Dipyridamole	20 µM	PASS
Diltiazem	15 µM	PASS
Ethanol	87 mM	PASS
Fish Oil	32 mg/mL	PASS
Pietal (Cilostazol)	14.9 and 60 µM	PASS
Glycosamine HCl	9.40 µM	PASS
Hydrochlorothiazide	20 µM	PASS
Ibuprofen	2.4 mM	PASS
Insulin	3 ng/mL	PASS
Lidocaine	51 µM	PASS
LMW Heparin	1833 U/L	PASS
L-Thyroxine	32 nM	PASS
Nitroglycerin	0.1 µg/mL	PASS
Norfloxetine	7.27 µM	PASS
Norverapamil	4.5 µM	PASS
Oxypurinol	99 µM	PASS
Pravastatin	56 µM	PASS
Propranolol	7.7 µM	PASS
Salicyclic Acid	4.3 mM	PASS
Streptokinase	400 U/mL	PASS
Theophylline	220 µM	PASS
Triglycerides	37 mM	PASS

Warfarin	32 $\mu$ M	PASS
A- Tocopherol	58 $\mu$ M	PASS
DMSO	0.11%	PASS
Celecoxib	8.5 $\mu$ g/mL	PASS
Prilosec	One 20 mg tablet	PASS

*f. Assay cut-off:*

Not applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

A method comparison study, comparing performance of the Accumetrics, Inc. VerifyNow™ PRUtest and the predicate device was executed at three investigational sites with a total of six (6) operators. Citrated whole blood was collected from 119 patients receiving platelet inhibitors. Samples in the low, medium and high ranges of PRU values and covering the AMR of the device were tested.

The ordinary least squares regression analysis results are summarized below:

	<b>n</b>	<b>Slope (95% CI)</b>	<b>Slope p-value</b>	<b>Intercept (95% CI)</b>	<b>Correlation Coefficient R</b>	<b>R<sup>2</sup></b>	<b>Sy x</b>
<b>P2Y12 vs PRU</b>	119	1.01 (0.97–1.05)	0.56	-0.77 (-8.00–6.50)	0.98	0.96	19.0

*b. Matrix comparison:*

Not applicable

3. Clinical studies:

*a. Clinical Sensitivity:*

Not applicable

*b. Clinical specificity:*

Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

A reference range for the VerifyNow™ PRU Test results was determined by testing 152 healthy individuals. The reference range of PRU results from the healthy donor population was determined by calculating the 95% confidence interval (2.5<sup>th</sup> percentile–97.5<sup>th</sup> percentile) in accordance with CLSI C28-A3c and found to be from 182–335 PRU.

N	Mean (PRU)	SD	0.025 quantile (95% CI)	0.975 quantile (95% CI)
152	266	42	182(116-197)	335 (324-354)

**N. Instrument Name:**

Accumetrics, Inc., VerifyNow™ System

**O. System Descriptions:**

1. Modes of Operation:

The operator inserts the sample tube into the test device. This is an individually run, closed-tube assay.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes  or No

3. Specimen Identification:

The VerifyNow™ System's default setting does not require the user to enter a Patient ID, however the system can be programmed to require a Patient ID before an assay can be performed.

4. Specimen Sampling and Handling:

Fresh citrated whole blood samples are required for use with the VerifyNow PRUtest. The sample well contains a needle for sampling directly from a closed vacuum collection tube.

5. Calibration:

The VerifyNow™ PRUtest devices are calibrated at the factory and require no calibration by the user. The calibration information is contained in the barcode on the pouch of each assay device. The barcode must be scanned whenever a new lot of assay devices are to be tested.

6. Quality Control:

Each assay device includes internal controls to determine whether the VerifyNow™ PRUtest result is valid and causes the instrument to report an error message for an invalid test. The negative internal control test is performed during the first five seconds of the assay, before platelet activation and binding to the fibrinogen-coated beads occurs. The positive internal control consists of a reagent channel that is similar to the P2Y12 assay channel, except it uses Thrombin Receptor Activating Peptide (TRAP) instead of ADP as the agonist. The positive internal control test is performed during the active phase of the assay.

The VerifyNow™ PRUtest utilizes VerifyNow™ Wet Quality Controls (WQC) Levels 1 and 2 for verifying the integrity of the VerifyNow™ PRUtest reagents. Controls have been formulated at clinically relevant levels with WQC Level 1 providing low level PRU results. WQC Level 1 is a carbon sol suspension, used to mimic the optical properties of whole blood. WQC Level 2 is designed to provide high level PRU results, comparable to results obtained from patient samples prior to ingestion of clopidogrel. WQC Level 2 is a lyophilized pellet, which is added to the diluent vial and reconstituted before use. WQC Level 2 uses a peptide that binds to fibrinogen to induce aggregation, which simulates the binding of fibrinogen-coated microparticles to platelets in the VerifyNow™ PRUtest. Assigned values for WQC Level 1 ( $\leq 30$  PRU) and Level 2 (228 – 372 PRU) are included in the package insert.

The VerifyNow™ System contains the following internal controls:

- The instrument automatically verifies sample filling, correct fluid transfer and mixing. It also monitors the electronic and mechanical components.
- Each assay device incorporates two levels of quality control to identify invalid test runs caused by random error, reagent degradation or inappropriate blood samples. Before platelet activation and fibrinogen binding begin, the negative internal control performs a test for non-specific aggregation. During the active phase of the assay, the positive internal control channel monitors the reaction and calculates Control Units, which must fall within specified limits.

Electronic Quality Control (EQC) is one quality control mechanism for the VerifyNow™ instrument. It is a reusable device that is inserted by the operator into the assay port and is used to perform a comprehensive testing routine that confirms performance of instrument optics, the pneumatics system, reagent mixing parameters, calibration parameters,

simulates assay testing at two levels to check correct data acquisition and calculation and the required EQC testing frequency. Accumetrics recommends that EQC be run at least on a daily basis, although a preferred testing frequency may be selected by the institution. When the established test interval has elapsed, the user is locked out from running a patient test until the EQC test has been successfully completed.

**P. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**Q. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.