

# VerifyNow®

IIb/IIIa

14320-WEB.C

## Instructions for Use

## Instructions pour l'usage

## Anwendungsvorschriften

## Istruzioni per uso

## Instrucciones para el uso

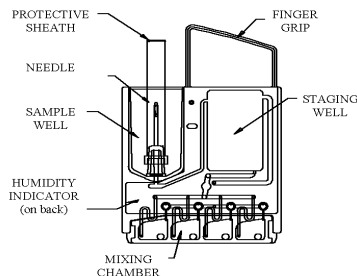


VerifyNow® IIb/IIIa Assay  
**Accu:metrics®**

Customer Support  
Accumetrics (USA) 1 800-643-1640  
Accumetrics (Outside USA) +1 858-643-1600  
MDSS +1 858-643-1600

### INTENDED USE

The VerifyNow IIb/IIIa Assay is a semi-quantitative, whole blood platelet function assay used to measure glycoprotein (GP) IIb/IIIa receptor blockade in patients treated with abciximab or eptifibatid. VerifyNow IIb/IIIa Assay results should be interpreted in conjunction with other clinical and laboratory data available to the clinician.



### PRODUCT DESCRIPTION

The VerifyNow System is a turbidimetric based optical detection system, which measures platelet-induced aggregation. The system consists of an instrument, a disposable assay device and quality control materials. Quality control measures include an instrument based electronic quality control (EQC), two levels of wet quality controls (WQC), internal quality controls, and shipping controls. The instrument controls all assay sequencing, temperature, reagent-sample mixing and performs self-diagnostics. The degree of platelet function is determined and the result is displayed.

The assay device contains a lyophilized preparation of human fibrinogen-coated beads, thrombin receptor activating peptide (iso-TRAP), and buffer. The patient sample is anticoagulated whole blood, which is automatically dispensed from the blood collection tube into the assay device by the instrument, with no blood handling required by the user.

### PRINCIPLE

The VerifyNow IIb/IIIa Assay is designed to assess platelet function based upon the ability of activated platelets to bind fibrinogen. Fibrinogen-coated microparticles aggregate in whole blood in proportion to the number of unblocked platelet GPIIb/IIIa receptors.

The rate of microbead aggregation is more rapid and reproducible if platelets are activated; therefore the reagent iso-TRAP is incorporated into the assay to induce platelet activation without fibrin formation. Light transmittance increases as activated platelets bind and aggregate fibrinogen-coated beads. The instrument measures this change in optical signal and reports results in Platelet Aggregation Units (PAU).

### MATERIALS PROVIDED

- 25 VerifyNow IIb/IIIa assay devices individually sealed in foil pouches. Each assay device contains lyophilized fibrinogen-coated beads, iso-TRAP, bovine serum albumin, and buffer.

### REAGENT STORAGE AND HANDLING

- Assay Device Kit Indicator: Each VerifyNow Assay kit has a temperature indicator on the outside of the packaging. The user is instructed to inspect the indicator upon receipt of the kit. If the indicator has changed color, the kit has been exposed to elevated temperature, and a Wet Quality Control (WQC) Level 2 must be run to ensure that the reagents are performing properly. The Temperature Indicator detects errors due to adverse environmental conditions.
- For extended storage, maintain assay devices at 2° to 8°C. Alternatively, these assay devices may be stored for up to 8 weeks at room temperature (18 to 25°C) or (64° to 77°F), but not beyond the expiration date printed on the pouch. When removed from refrigeration, write the date to discard on the pouches and/or kit box.
- Allow assay devices to reach room temperature prior to use.
- Assay devices should remain sealed in the foil pouches until ready for use to prevent damage by humidity.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Sodium citrate should not be used as an anticoagulant when measuring samples from patients treated with eptifibatid.

The following tubes may be used with the VerifyNow System:

- Greiner Bio-One Vacuette® partial fill blood collection tubes:
  - 3.2% sodium citrate, 2 mL fill volume (Greiner catalog # 454321/454322).
  - Lithium heparin: 3 mL fill volume (Greiner catalog # 454082/454244) or 2 mL fill volume (Greiner catalog # 454237)
- VENOSAFE partial fill blood collection tubes:
  - 3.2% sodium citrate, 1.8 mL or 2.7 mL fill volumes
  - Lithium heparin, 2 mL or 3 mL fill volumes
- VerifyNow Instrument with Electronic Quality Control (EQC).
- VerifyNow Assay WQC, Catalog #85047.

### PRECAUTIONS

- For *in vitro* diagnostic use.
- The VerifyNow Instrument and its components should only be used as directed in the User's Manual.
- Do not use the VerifyNow IIb/IIIa assay device or WQC materials beyond the expiration date.
- All patient samples should be handled as if capable of transmitting disease.
- The reagents are manufactured with a material purified from human plasma that was found negative for all communicable diseases tested, including HIV-1, HIV-2, Hepatitis B surface antigen (HBsAg) and HCV. Handle assay devices as biohazardous material and dispose of in an appropriate manner.

### SAMPLE COLLECTION AND HANDLING

Instructions for Sample Collection From Indwelling Catheters:

1. Whole blood samples that are obtained from an indwelling catheter should be collected after sufficient discard (approximately 5 mL) has been drawn to clear the line. Ensure indwelling catheter is free of clots. **Transfer blood to the blood collection tube immediately after collection.**
2. Gently invert the sample tube at least 5 times to ensure complete mixing of the contents.
3. Assay the sample within 15 minutes of collection as instructed in the Test Procedure.

Instructions for Peripheral Samples:

1. Whole blood may be collected from venous or arterial sites using a 21 gauge or larger needle in an appropriate blood collection tube. Blood samples should be obtained from an extremity free of peripheral venous infusions.
2. Collect a discard tube first (approximately 2 mL).
3. Gently invert the sample tube at least 5 times to ensure complete mixing of the contents.
4. Assay the sample within 15 minutes of collection as instructed in the Test Procedure.

### SAMPLE COLLECTION PRECAUTIONS

- Fresh whole blood samples in the appropriate collection device are required for use with the VerifyNow Instrument.
- If drawing blood for a CBC at the same time as sample collection for VerifyNow IIb/IIIa Assay, fill the CBC tube last.
- Do not freeze or refrigerate samples.
- Collection of the blood sample should be performed with care to avoid hemolysis or contamination by tissue factors. Samples with evidence of clotting should not be used.
- Always ensure collection tubes are filled to the indicated fill volumes. At altitudes greater than 2500 feet above sea level, blood collection tubes may not fill to the specified volume, which results in an incorrect ratio of blood to anticoagulant. Users at these elevations should refer to their facility's blood collection protocols for instructions to properly fill blood collection tubes.
- Samples should be collected and handled according to the institution's policies and procedures pertaining to biohazardous material.

### TEST PROCEDURE

1. Refer to the VerifyNow System User's Manual for complete operating instructions.
2. Open the foil pouch and remove the assay device. Assay devices should only be handled by finger grip.
3. Verify that the humidity sensor is not the same color on both sides.
4. Remove the needle's protective sheath by pulling directly up on the sheath. Do

not twist the sheath as this may remove the needle.

- At the instrument prompt, insert the assay device into the instrument.
- At the instrument prompt, gently invert the sample tube at least 5 times, and insert onto the needle in the assay device. If your instrument has an assay port cover, close it now. If not, proceed to Step 7.
- The instrument will run the test and display the result in less than two minutes.

**CAUTION:** Sample is under pressure. Do not remove sample tube from assay device. Only remove assay device from the instrument after assay is completed.

- Remove the assay device by grasping the assay device finger grip and pulling straight up. Do not remove the tube from the assay device. Dispose of the entire assay device/sample tube in appropriate biohazard waste container.

**REPORTED RESULTS**

The VerifyNow IIb/IIIa Assay reports patient results in Platelet Aggregation Units (PAU), which are calculated as a function of the rate and extent of aggregation. The VerifyNow instrument also allows the user to calculate the percent inhibition for a given patient provided that a baseline PAU value was obtained prior to administration of drug.

The use of GP IIb/IIIa inhibitors such as abciximab and eptifibatid has been shown to be useful as an antithrombotic strategy. In patients receiving abciximab or eptifibatid and in animal models of coronary thrombosis with abciximab, the ex vivo platelet aggregation response was virtually abolished when GP IIb/IIIa blockade was maintained at steady state >80%. Further study has shown that with all GP IIb/IIIa inhibitors, including abciximab and eptifibatid, platelet function inhibition >95% at 10 minutes after the start of therapy was associated with a significant decrease in the incidence of a major adverse cardiac event.

**INSTRUMENT MESSAGES**

Under certain conditions, an assay run may be aborted. In this case, the instrument will display an error or attention message. Please refer to the User's Manual for a more detailed explanation of the error messages.

**CALIBRATION**

VerifyNow IIb/IIIa assay devices are calibrated at the factory. This 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 is to be tested. If a new lot of assay devices is being used, the instrument will prompt the user by displaying a barcode icon after the assay device is inserted.

- At prompt, place the assay device pouch in front of the barcode reader found on the left side of the instrument, so that the barcode on the pouch lines up with the barcode reader.
- An audible beep will be heard when the instrument receives the required information.
- The user needs only to perform this action once per lot.

**QUALITY CONTROL**

- The manufacturer recommends that an Electronic Quality Control (EQC) be run once per day. This reusable device verifies instrument optics, pneumatics and reagent mixing.
- The VerifyNow System also contains the following internal controls
  - The instrument automatically verifies sample filling, correct fluid transfer and mixing. It also monitors the electronic and mechanical components.
  - To prevent random error, test results of two assay channels are automatically compared and must agree within specific limits.
  - Each assay device contains an internal humidity sensor. If the assay device has been degraded due to excessive humidity, the sensor will be the same color on both sides. If this occurs, the assay device should not be used.

In case of an Attention 28, an Electronic Quality Control (EQC) should be performed to test instrument function. If the EQC is OK, the VerifyNow® System is functioning normally. In these cases, the problem may be associated with the blood sample and the following causes for Attention 28 should be investigated:

- The patient being tested has a hematocrit outside of the applicable range.
- An improperly mixed sample was used to run the assay.
- The sample was not run within the specified amount of time.

If none of the above can be determined to be the cause of the Attention 28, Wet Quality Control (WQC) Level 2 may be run to confirm the integrity of the assay device and the reagents.

Wet Quality Controls are available from Accumetrics for verifying the integrity of the VerifyNow System. VerifyNow Assay WQC Level 1 and Level 2 are formulated at clinically relevant levels and can be used as part of a laboratory quality control program.

The manufacturer recommends that a Level 2 WQC be run once each time a new lot or a new shipment of VerifyNow IIb/IIIa Assay kits is received or every 30 days.

VerifyNow Assay WQC (Catalog #85047) is available from Accumetrics.

For question regarding WQC materials call Customer Support for assistance.

**ASSAY LIMITATIONS**

Delays in testing or difficulty of specimen collection may result in spurious values.

The lyophilized reagent is hygroscopic and can degrade after prolonged exposure to room air. Therefore, the assay device should be used shortly after removal from the foil pouch.

When results are not within the expected limits, the possibility of improper sample collection or handling should be investigated. Repeat the test using a new test assay device and sample.

Patients with inherited platelet disorders such as von Willebrand Factor Deficiency, Glanzmann Thrombasthenia and Bernard-Soulier Syndrome have not been studied with the VerifyNow IIb/IIIa Assay. VerifyNow IIb/IIIa Assay is not intended for use with these types of platelet disorders.

Patients with a known history of platelet counts <100 x10<sup>9</sup>/L have not been studied.

The VerifyNow IIb/IIIa assay has not been compared to platelet aggregometry in patients with a 30% decrease in PRP platelet count.

VerifyNow IIb/IIIa Assay test results should be interpreted in conjunction with other clinical and laboratory data available to the clinician.

**SERVICE**

The VerifyNow Instrument is not intended to be serviced by the user. Instruments in need of repair are required to be returned to Accumetrics. If there are problems related to the VerifyNow System, call Accumetrics' Customer Support at (800) 643-1640.

**REFERENCE RANGES**

VerifyNow IIb/IIIa Assay was performed in clinical studies at 9 centers on patients undergoing percutaneous coronary intervention. Testing was performed on samples from 173 patients with planned treatment with abciximab (173 baseline and 173 post abciximab) and 114 patients with planned treatment with eptifibatid (114 baseline and 97 post eptifibatid).

The following table presents the mean PAU values at Baseline (prior to abciximab or eptifibatid administration), ≥80% and ≥95% inhibition.

	Abciximab			Eptifibatid		
	PAU Baseline	≥80%	≥95%	Baseline	≥80%	≥95%
n	173	173	130	114	97	50
Mean	224	9	2	199	11	3
SD	54	20	4	40.6	9.4	3
Reference Range	125-330	0-44	0-13	136-288	0-31	0-10

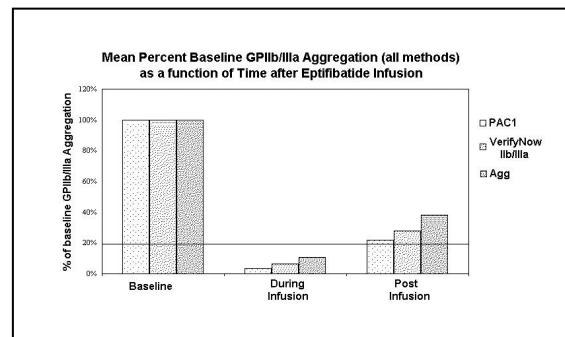
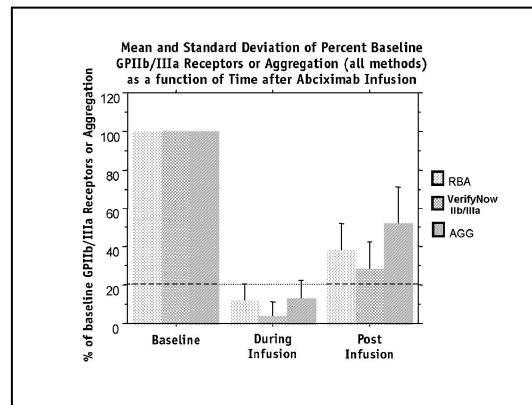
Reference ranges were calculated using NCCLS guidelines. The following demographic characteristics were considered: gender, ethnicity, current smoker, and previous abciximab use. The mean baseline PAU was consistent across all groups, and no statistically significant bias was demonstrated in any of the demographic variables tested. An analysis of the clinical data and the associated demographic information showed that there was no need to create separate reference ranges based upon demographic variables. As has been shown, there is no overlap between the baseline and inhibition reference ranges, which provides the clinician with an unambiguous interpretation.

**PERFORMANCE CHARACTERISTICS**

Studies evaluating VerifyNow IIb/IIIa Assay in patients treated with abciximab compared VerifyNow IIb/IIIa Assay to an 125 I-abciximab Receptor Blockade Assay (RBA) which quantitates the molecules bound per platelet and platelet aggregometry using a CHRONO-LOG Optical Aggregometer with 20 μM ADP as the agonist to detect the maximal slope over a five minute period. The unit of measure for the VerifyNow IIb/IIIa Assay is PAU and is based upon both the rate and extent of platelet and fibrinogen coated beads binding over a defined period.

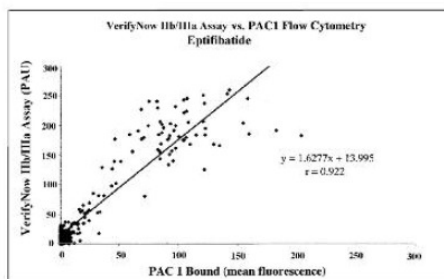
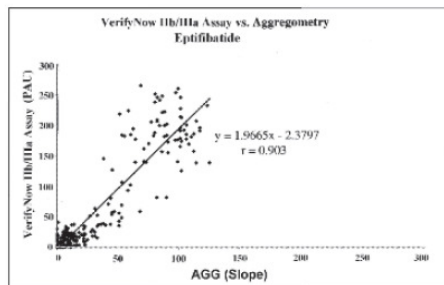
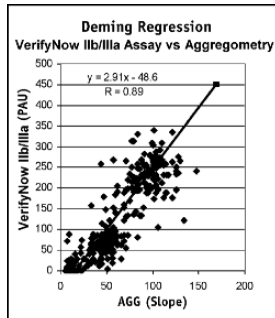
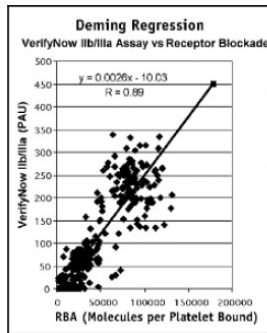
Studies evaluating VerifyNow IIb/IIIa Assay in patients treated with eptifibatid compared VerifyNow IIb/IIIa Assay to the same platelet aggregometry assay described above and a platelet flow cytometric assay measured as mean fluorescence units.

The clinical trials were designed to obtain samples at three time points: 1) Baseline, prior to abciximab or eptifibatid administration; 2) During Infusion, within 1 hour following bolus administration and 3) Post Infusion, 24 hours after bolus or at the time of discharge, whichever occurred first, to assess recovery of platelet function. For each patient, a baseline, during infusion and post infusion (24-hour) sample was measured by each test method and the results recorded. The following graphs show the time course of platelet inhibition for the three methods as individual points and mean +/- standard deviation, respectively, and illustrate the agreement of the three methods.



## Method Correlation

Correlation between methods was assessed by regression analysis based on data from patients treated with abciximab and eptifibatid. The data was analyzed to determine the correlation of VerifyNow Iib/Illa Assay with RBA and platelet aggregometry in abciximab treated patients and with PAC1 Flow Cytometry and platelet aggregometry in eptifibatid treated patients. Data points for the clinical analysis were reported in raw units for each assay: PAU for VerifyNow Iib/Illa Assay; slope for aggregometry; molecules per platelet bound for RBA; and mean fluorescence for PAC1 Flow Cytometry.

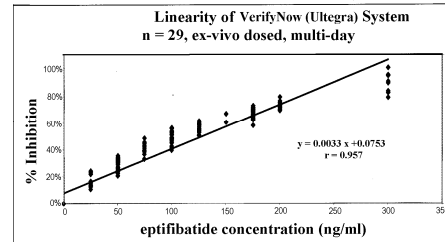


## Analytical Sensitivity

VerifyNow Iib/Illa Assay was run (n=6 per patient) on sixteen patients dosed with abciximab and measured at 10 minutes post bolus dose. The analytical sensitivity of the assay was determined to be 8 PAU (2 X SD).

## Linearity

The following graph shows the linearity of the VerifyNow System. The testing consisted of multiple donors ex-vivo dosed at nine eptifibatid concentrations over multiple days with multiple replicates. The linearity data show that the relationship between eptifibatid concentration and % inhibition is not linear.



## Precision

Simple and complex precision for both abciximab and eptifibatid were calculated according to NCCLS precision guidelines using three lots of VerifyNow Iib/Illa assay devices and two instruments for abciximab and two lots of assay devices and three instruments for eptifibatid. Whole blood was drawn from volunteer donors over 20 days. The within-run CV shown here is defined by NCCLS as the within-run precision value.

### Complex Precision Statistics (Abciximab) as Defined by NCCLS

Description	Complex Precision Whole Blood		Mean	Within-Run (Simple)		Total	
	n (days)	n (results)		SD	CV %	SD	CV %
Female Age 26	20	80	188.3	9.8	5.2	18.7	9.9
Female Age 41	20	80	187.0	9.1	4.9	26.1	13.9
Male Age 29	20	80	224.8	11.1	4.9	25.9	11.5

### Complex Precision Statistics (Eptifibatid) as Defined by NCCLS

Description	Complex Precision Whole Blood		Mean	Within-Run (Simple)		Total	
	n (days)	n (results)		SD	CV %	SD	CV %
Male Age 32	20	78	147.8	9.2	6.2	13.9	9.4
Male Age 28	20	77	185.3	12.2	6.6	16.0	8.6

Simple precision was determined for the VerifyNow Iib/Illa Assay wet quality control Level 2. Three lots of assay devices were each assayed 20 times using a single lot of control. This study was later repeated with a new control lot. The results are shown in the following table.

### Lot to Lot Cartridge Variation

Device Lot	n	PAU Mean	SD	CV*
1	20	202	12	6.0
2	20	180	15	8.3
3	20	176	17	9.9

\*The manufacturer's specification for the coefficient of variation is <10%.

## Interfering Substances

Clinical study data were evaluated to assess the effect of potentially interfering medications on PAU values. Baseline PAU values were compared for patients with and without the following medications present, and no differences were observed: aspirin, ticlopidine, clopidogrel, heparin, warfarin, acetaminophen, NSAIDs, beta-adrenergic blockers, Ca++ channel blockers, statins and nitrates.

Aspirin and heparin were further studied because they are commonly co-administered with GP Iib/Illa therapy. Varying concentrations of both drugs were evaluated in in-vitro studies, and no significant effect on PAU values was observed. These data are consistent with data reported by Colter using an earlier prototype version of the VerifyNow/Ib/Illa Assay.

The effect of platelet counts, hematocrit values and fibrinogen concentration were studied in two clinical trials. Assay performance was not affected by platelet count values between 98,000 and 376,000 platelets per microliter, or hematocrit values between 23 and 45%. No assay interference was observed when samples with fibrinogen levels between 104 and 617 mg/dL were tested with the VerifyNow Iib/Illa assay. Moderate lipemia was found to induce an increase in PAU values by 16%.

Another intravenous GP Iib/Illa inhibitor, tirofiban (Aggrasta<sup>®</sup>) is approved for use in patients with unstable angina. Patients who have been treated with tirofiban may be sent to the cardiac catheterization lab for PCI. The VerifyNow Iib/Illa Assay can detect platelet inhibition by this drug; therefore VerifyNow Iib/Illa Assay results for these patients should be interpreted with care. A sample taken prior to abciximab (ReoPro<sup>®</sup>) or eptifibatid (Integrilin<sup>®</sup>) administration

cannot be used to establish a "baseline" or uninhibited result if a GP IIb/IIIa inhibitor was administered within the past 10 days.

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**EXPLANATION OF SYMBOLS**



Lot Number



Catalog Number



In Vitro Diagnostic Use



Authorized Representative in the European Community



Origin-Human



Manufacturer



Use By



Temperature Limits



Caution: Consult accompanying documents



Contains sufficient for 25 tests



Contains sufficient for 10 tests



Accumetrics, Inc.<sup>®</sup>  
San Diego, CA 92121  
Accumetrics (US): 1-800-643-1640  
Accumetrics (Outside USA): +1-858-643-1600



MDSS GmbH  
Schiffgraben 41  
30175 Hannover, Germany



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US Pat. D 409, 758 and Others Pending.