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DOCUMENT NUMBER:	RIV-PPP-1183
DOCUMENT TITLE:	VerifyNow PRU Test for P2Y12 Receptor Blockade
DOCUMENT NOTES:	

LOCATION:	RIV-rel	**VERSION:**	01
DOC TYPE:	RIV PPP	**STATUS:**	Release

EFFECTIVE DATE:	28 Aug 2024	**NEXT REVIEW DATE:**	28 Aug 2026
RELEASE DATE:	28 Aug 2024	**EXPIRATION DATE:**	

AUTHOR:		**PREVIOUS NUMBER:**	
OWNER:		**CHANGE NUMBER:**	RIV-CR-0435

VerifyNow® PRUTest™ Procedure for P2Y12 Receptor Blockade

Purpose or Principle or Introduction

The VerifyNow PRUTest is designed to measure platelet P2Y12 receptor blockade. Substances known to specifically block the P2Y12 receptor include the thienopyridine class of drugs, including clopidogrel.

The test is based upon the ability of activated platelets to bind fibrinogen. Fibrinogen-coated microparticles aggregate in whole blood in proportion to the number of expressed platelet GP IIb/IIIa receptors. The rate of microbead aggregation is more rapid and reproducible if platelets are activated; therefore, the reagent adenosine-5-diphosphate (ADP/PGE1) is incorporated into the test channel to induce platelet activation without fibrin formation. The reagent is formulated to specifically measure P2Y12-mediated platelet aggregation. Light transmittance increases as activated platelets bind and aggregate fibrinogen-coated beads. The instrument measures this change in optical signal and reports results in P2Y12 Reaction Units (PRU).

Scope

This procedure is intended for trained Clinical Laboratory Scientists (CLS) and Medical Laboratory Technicians (MLT) who can perform the moderately complex VerifyNow PRU test.

Specimen collection and specifications

- Patient Preparation: None required.
- See VerifyNow Operational Procedure for general collection instructions.

Specimen transport

- Specimen must be hand carried to the laboratory and must not be sent via pneumatic tube system.

Specimen stability and storage

- The sample must be incubated at room temperature for 10 minutes prior to testing and can be used up to 4 hours after collection if stored at room temperature (18 to 25 °C). *Do not refrigerate or freeze specimen.*

Specimen rejection

- Samples with evidence of clotting must not be used.

Continued on next page

VerifyNow® PRUTest™ Procedure for P2Y12 Receptor Blockade, Continued

Instrument

VerifyNow analyzer

Reagent

The following are the reagents necessary to perform the assay.

- VerifyNow PRUTest device (reagent device), box of 25 test devices (PN 85225), OneLink item number 10391410. All required reagents are contained within the individually packaged PRUTest device.
  - Each individually sealed VerifyNow PRU test device contains the lot number and expiration date stamped on the foil pouch and contains lyophilized fibrinogen-coated beads, ADP, bovine serum albumin, PGE1, and buffer.
  - Store test devices at 15°C to 25°C (59° - 77°F). The PRUTest product is stable under these conditions until the date indicated on the pouch.
  - Test device should remain sealed in the foil pouch until ready for use to prevent damage by humidity.
  - Once removed from its foil pouch, the test device must be handled only by the finger grip and used immediately.
  - Do not use the VerifyNow PRUTest device beyond the expiration date.

- VerifyNow Assay WQC Kit, Catalog #85047, OneLink item number Box of 6 Diluent Tubes and 6 Pellets.
  - Control materials should be stored at 15°C to 25°C (59° - 77°F).
  - Control material is suitable for use until the expiration date stamped on the label.
  - Test Control Level 1 is ready for use as provided.
  - Do not open the vial containing the Level 2 Control pellet until immediately prior to use.
  - Control material should be used within 15 minutes of reconstitution. Do not freeze.

Materials and supplies

The following contains the list of materials and supplies required.

- Electronic Quality Control (EQC).
- Greiner Bio-One Vacuette® partial fill blood collection tubes (2 mL fill volume) containing 3.2% sodium citrate. Greiner Catalog #454322 or Nipro catalog #NP-CW0185-1 blood collection tube (1.8 mL) containing 3.2% sodium citrate.
- Phlebotomy supplies, including needle of 21 gauge or larger.

Continued on next page

VerifyNow® PRUTest™ Procedure for P2Y12 Receptor Blockade, Continued

Safety

Refer to the safety manual for general safety requirements.

Calibration and Calibration Verification

- VerifyNow PRUTest devices are calibrated by the manufacturer at the factory. This calibration information is contained in the barcode on the pouch of each test device.
- The barcode must be scanned whenever a new lot of test devices is to be tested. The system will not allow a test device to proceed without the lot number calibration information being scanned into the system. If a new lot of test devices is being used, the instrument will prompt the user by displaying a barcode icon after the test device is inserted.
    a) At prompt, place the test device pouch approximately one inch in front of the barcode reader found on the left side of the instrument, so that the light shines on the center of the barcode reader.
    b) An audible beep will be heard when the instrument receives the required information.
    c) The user needs only to perform this action once per lot.
- No additional calibration is performed by the user.
- Calibration Verification is performed by the use of wet Quality Control materials with every new lot of reagent and at specified time intervals (refer to Quality Control Procedures below), with periodic review of QC results by the laboratory technical supervisor. Tests of platelet function are non-linear, and no additional calibration verification is required.

System (Internal) Quality Control for Each Sample Tested

- Each time a test device is run on the VerifyNow Instrument, the instrument verifies the test device expiration date, sample filling, optics performance, correct fluid transfer, and proper mixing. The system controls prevent the user from running an expired test device.
- The system also detects certain other user errors, such as placing the test device or the sample in the instrument at the wrong time, or removing the test device before the testing is complete. These controls prevent reporting of an inaccurate test result.
- The test device internal controls in VerifyNow PRUTest can detect failures of the reagent system due to improper storage or handling conditions.
- Two levels of quality control are incorporated into each test device to identify invalid test runs caused by random errors, reagent degradation, or inappropriate blood samples.
- Before platelet activation and fibrinogen binding begin, the negative internal control performs a test for non-specific aggregation.

Continued on next page

VerifyNow® PRUTest™ Procedure for P2Y12 Receptor Blockade, Continued

- During the active phase of the test, the positive internal control channel monitors the reactive and calculates Control Units, which must fall within specified limits.
- A failure of the negative or positive control displays an "error" message by the VerifyNow instrument, and no PRU result is reported.
- The internal controls will flag an improperly collected or mishandled blood sample, or a blood sample with certain types of interfering substances.
- The test device internal controls detect errors from the reagent system, adverse environmental conditions, and additional types of user errors.

Procedure

Follow the steps below to test patient specimen for PRU.
Refer to the VerifyNow Operational Procedure for comprehensive instructions on the analyzer.

Step	Action
1	The VerifyNow® analyzer should be left on, if turned off, power on the instrument using the power switch at the back panel. The instrument will power on and perform a self-testing routine lasting approximately 30 seconds. This will initiate the following startup checks in preparation of the assay: a. A system program and data memory check to ensure memory integrity b. A system temperature check to ensure the test warming plate reaches and maintains the proper temperature. c. A system checks of proper operating voltages; and d. A system intra-communication validation.
2	After the self-testing is complete, the Start screen will display. Press the Next key to advance to the Main Menu. The instrument should be allowed to warm-up for at least 15 minutes prior to use. A flashing message will display while the instrument is initially warming up. During this time the icon keys are inactive. Warm up message no longer displays when it's ready.
3	If sufficient time has elapsed, the instrument will prompt you to perform an EQC. Refer to VerifyNow Operational Procedure for steps in running EQC. The following checks are performed: a. Instrument optics. b. Pneumatics system that draws the sample into the test device for reaction and measurement. c. Reagent mixing parameters and sample data acquisition. d. Correct calibration parameters.

Continued on next page

VerifyNow® PRUTest™ Procedure for P2Y12 Receptor Blockade, Continued

	If required, enter User ID and Password After a **PASS** has been achieved on EQC, the instrument will be ready to perform an assay.
4	i. When the initial screen displays, press the **Assay** key. ii. Enter the patient ID and then press the Next key. The screen will prompt to insert the assay device. iii. Prepare materials needed – assay device and the sample tube. Do not remove the assay device from the foil pouch until ready to use.
6	Open the foil pouch and remove the test device just before use. Hold the assay device by the finger grip. Note: Each assay device has a finger grip. Avoid handling other surfaces of the assay device. • If WQC is indicated, perform and determine acceptability of WQC.
7	Remove the needle's protective sheath by pulling directly up on the sheath. Do not twist the sheath as this may remove the needle.
8	Open instrument cover. Insert the test device at the instrument prompt. • If this is a new test devices lot, the barcode prompt will display. At prompt, place the test device pouch approximately one inch in front of the barcode reader found on the left side of the instrument, so that the light shines on the center of the barcode reader. An audible beep will signal that the instrument has read the bar code, and the testing will continue.
9	At the instrument prompt, invert the sample tube at least 5 times, and insert onto the needle in the test device. Close instrument cover. CAUTION: Sample is under pressure. Do not remove sample tube from test device. Only remove the test device from the instrument after testing is completed.
10	The instrument will run the test and display the result in less than three minutes.
11	Record or print the sample result.
12	Open instrument cover. Remove the test device by grasping the device finger grip and pulling straight up. Do not remove the tube from the test device. Close instrument cover.
13	Dispose of the entire test device/sample tube in appropriate biohazard waste container.
14	The instrument is ready to test the next sample.

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VerifyNow® PRUTest™ Procedure for P2Y12 Receptor Blockade, Continued

Recalling Patient Results

In order to enable the recall of specific patient results, the instrument must be configured to require a Patient ID to be entered before each test is performed.

1. If required, enter User ID and Password.
2. Choose the second option (Button next to file folder) from the Main Screen.
3. Enter the Patient ID and press the arrow for Next.
4. A VerifyNow PRUTest result is indicated by a "y".
5. Record or print the results. The most recent result is displayed, along with the date stamp indicating the date and time the test was performed. To toggle to other results, use the left and right arrow key.
6. If you choose the PRINT ALL icon, everything displayed below the blinking cursor will print.

Expected Values or Reference Range

- Pre-drug reference range: 208-418 PRU
- PRU values <208 (low end of reference range) are specific evidence of a P2Y12 inhibitor effect.

Interpretation / Results / Alert Values

- Lower PRU levels are associated with expected antiplatelet effect.
- P2Y12 result values >=208 PRU are indicative of a decreased response to P2Y12 inhibitors.

Test Limitations and Method Notes

- To minimize problems during specimen handling, test performance and reporting of test results, the area where testing is performed must contain the proper workbench space, ventilation, utilities, and supplies necessary for conducting the type and volume of testing performed.
- Place the VerifyNow Instrument on a clean, firm, level surface without vibration. Avoid placement near sources of heat or cold, incandescent lighting or direct sunlight.
- The VerifyNow Instrument operates at ambient temperature (18-32°C or 64-90°F) and up to 85% humidity without condensation.
- The lyophilized agent is hygroscopic and can degrade after prolonged exposure to room air. Therefore, the test device should be used shortly after removal from the foil pouch.
- Store reagents and quality control material according to the package directions.
- Delays in testing or difficulty of specimen collection may result in spurious values. Do not test any sample that is clotted, too old, hemolyzed or that has been mishandled or mislabeled.

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VerifyNow® PRUTest™ Procedure for P2Y12 Receptor Blockade, Continued

- Blood should be collected from a freely flowing venipuncture to maintain the integrity of the specimen. After the blood sample is collected, wait 10 minutes before performing PRUTest. Samples assayed prior to 10 minutes or four or more hours after collection may result in spurious PRU values or errors messages, e.g., "attention" or "error" messages.
- When results are not with the expected limits, the possibility of improper sample collection or handling should be investigated. Repeat the test using a new test 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 PRUTest. The VerifyNow PRUTest is not intended for use with these types of platelet disorders.
- Patients with a known history of platelet counts <100 x109/L have not been studied.
- Assay performance was not affected by hematocrit values between 33–52%, and platelet count values between 119,000–502,000/µL. Patient samples having hematocrit values outside of this range may generate an error message of "Attention 28". If message persists with a new test device and well mixed sample, suggest recollection, and cancel in Cerner using reason "Technical Error, Test Not Performed".
- Patients who have been treated with Glycoprotein IIb/IIIa inhibitor drugs should not be tested until platelet function has recovered. This time period is approximately 14 days after discontinuation of drug administration for abciximab (ReoPro) and up to 48 hours for eptifibatide (Integrilin) and tirofiban (Aggrastat). The platelet function recovery time varies among individuals and is longer for patients with renal dysfunction.
- The VerifyNow PRUTest results should be interpreted in conjunction with other clinical and laboratory data available to the clinician.

Controlled Documents

The following controlled documents support this procedure.

VerifyNow Operational Procedure

Non-Controlled Documents

The following non-controlled documents support this policy.

Werfen. VerifyNow Assay Wet Quality Control (WQC), Package Insert, English, VN1018NEU 01, San Diego, CA.

Werfen. 2022. VerifyNow Operator Manual, 000VN5006EN.C 02, English, 04/2022. San Diego, CA.

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VerifyNow® PRUTest™ Procedure for P2Y12 Receptor Blockade, Continued

Werfen. 2017. VerifyNow PRUTest-Platelet Reactivity Test Device Package Insert, VN1016WEU 02, 03/17. San Diego,

Werfen. 2018. VerifyNow Whole Blood Platelet Reactivity Testing System Reference Guide, MVN0045 01, 06/18. Bedford, MA.

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Regional Parent Document Reference Number: SCPMG-PPP-0592 Rev: 01

Signature Manifest

Document Number: RIV-PPP-1183 **Revision:** 01
Title: VerifyNow PRU Test for P2Y12 Receptor Blockade
Effective Date: 28 Aug 2024

All dates and times are in Pacific Standard Time.

Coagulation Regional Docs

Operations Director Approval

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