

# VerifyNow<sup>®</sup>

## Aspirin

### VerifyNow Aspirin Platelet Reactivity Test

## INSTRUCTIONS FOR USE

### English

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#### INTENDED USE

The VerifyNow Aspirin Test is a qualitative test to aid in the detection of platelet dysfunction due to aspirin ingestion in citrated whole blood for the point of care\* or laboratory setting.

This test is not for use in patients with underlying congenital platelet abnormalities, patients with non-aspirin induced acquired platelet abnormalities or in patients receiving non-aspirin anti-platelet agents (may be used in patients treated with selective COX-2 inhibitors, e.g., celecoxib (Celebrex<sup>®</sup>).

\* Approval for use in the point of care setting may not be available in all countries (e.g., Canada).

#### 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 test device and quality control materials. See Figure 1 for a representation of the test device. Quality control measures include an instrument based electronic quality control (EQC), two levels of wet quality controls (WQC), and internal quality controls. The instrument manages all test sequencing, temperature, reagent-sample mixing and performs self-diagnostics. Upon completion of the test the degree of platelet function is determined and the result is displayed. The device contains a lyophilized preparation of human fibrinogen-coated beads, platelet agonist, and buffer. The whole blood patient sample is automatically dispensed from the blood collection tube into the device by the instrument, with no blood handling required by the user.

#### PRINCIPLE

Aspirin affects platelet function by irreversibly inhibiting the cyclooxygenase-1(COX-1) enzyme involved in the conversion of arachidonic acid to thromboxane A<sub>2</sub>, which ultimately activates the GP IIb/IIIa receptors involved in platelet aggregation. If aspirin has produced the expected anti-platelet effect, such aggregation will not occur. The VerifyNow Aspirin Test incorporates the agonist arachidonic acid to activate platelets. The Aspirin Test is designed to measure 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 GP IIb/IIIa receptors. Light transmittance increases as activated platelets bind and aggregate fibrinogen-coated beads. The instrument measures this change in optical signal caused by aggregation.

## MATERIALS PROVIDED

- 10 (PN: 85053-10) or 25 (PN: 85053) VerifyNow Aspirin Test devices, individually sealed in foil pouches. Each test device contains lyophilized fibrinogen-coated beads, platelet agonist, peptide, bovine serum albumin, stabilizer, and buffer.

## REAGENT STORAGE AND HANDLING

- Test Device Kit Indicator: Each VerifyNow Test 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.
- Store test devices at 2°C to 25°C (36°-77°F).
- If refrigerated, allow test devices to reach room temperature 18° to 25°C (64°- 77°F) prior to use.
- Test device should remain sealed in the foil pouch until ready for use to prevent damage by humidity.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Greiner Bio-One Vacuette® 2ml blood collection tubes containing 3.2% sodium citrate. Greiner Catalog # 454322 or Nipro catalog #NP-CW0185-1 blood collection tubes (1.8ml) containing Sodium Citrate (3.2%).
- VerifyNow Instrument with Electronic Quality Control (EQC).
- VerifyNow Assay WQC, Catalog #85047.

## GENERAL PRECAUTIONS

- For *in vitro* diagnostic use.
- The VerifyNow Instrument and its components should only be used as directed in the User Manual.
- Do not use the VerifyNow Aspirin Test device or WQC materials beyond the expiration date.
- All patient samples should be handled as if capable of transmitting disease.
- Samples should be treated as biohazardous material and handled according to the institution's policies.
- 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 test device as biohazardous material and dispose of in an appropriate manner.

## SAMPLE COLLECTION AND HANDLING

All whole blood samples must be tested from **Greiner Bio-One Vacuette® 2ml** partial fill 3.2% sodium citrate vacuum collection tubes or Nipro catalog #NP-CW0185-1 blood collection tube (1.8 mL) containing 3.2% sodium citrate. Samples should be collected between 2 and 30 hours after ingestion of aspirin. Blood must set a minimum of 30 minutes after collection before test but no longer than 4 hours.

### Instructions for Sample Collection Directly Into Vacuum Collection Tubes:

1. Whole blood may be collected from venous or arterial sites using a 21 gauge or larger needle in partial fill 3.2% citrate vacuum collection tube. Blood samples should be obtained from an extremity free of peripheral venous infusions.

2. Collect a discard tube first (at least 2 mL).
3. Gently invert the sample tube at least 5 times to ensure complete mixing of the contents.

**Special Instructions if blood is obtained from an indwelling catheter:**

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.
2. When using a syringe, transfer blood to the appropriate blood collection tube immediately after collection.
3. Gently invert the sample tube at least 5 times to ensure complete mixing of the contents.

**Sample Collection Precautions:**

- Improper blood collection techniques may cause an Error 24 result. Refer to the Quality Control section of this package insert for additional information.
- If drawing blood for a CBC at the same time as sample collection for VerifyNow Aspirin Test, fill the CBC tube last.
- Do not freeze or refrigerate sample.
- Collection of the blood sample should be performed with care to avoid hemolysis or contamination by tissue factors. Samples with any 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 Manual for complete operating instructions.
2. Open the foil pouch and remove the test device. Test devices should only be handled by finger grip. (See Figure 1)
3. Remove the needle's protective sheath from the test device by pulling directly up on the sheath. Do not twist the sheath as this may remove the needle.
4. At the instrument prompt, insert the test device into the instrument.
5. At the instrument prompt, gently invert the sample tube at least 5 times, and insert onto the needle of the test device. If your instrument has a test port cover, close it now. If not, proceed to Step 6.
6. The instrument will run the test and display the result in approximately five minutes.

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

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

## REPORTED RESULTS

Test results are reported as Aspirin Reaction Units (ARU), which are calculated as a function of the rate of aggregation. Interpretation of results is based on the following assigned cutoffs:

### Interpretation of Results:

≥ 550 ARU - Platelet dysfunction consistent with aspirin has not been detected

< 550 ARU - Platelet dysfunction consistent with aspirin has been detected

Results should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

## INSTRUMENT MESSAGES

Under certain conditions, a test run may be aborted. In this case, the instrument will display an Error or Attention message. Please refer to the VerifyNow User Manual for a more detailed explanation of these messages.

## CALIBRATION

VerifyNow Aspirin Test devices are calibrated at the factory. This calibration information is contained in the barcode on the pouch of each device. The barcode must be scanned whenever a new lot of devices is to be tested. If a new lot of devices is being used, the instrument will prompt the user by displaying a barcode icon after the test device is inserted.

- At the prompt, place the 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 VerifyNow System includes several quality control mechanisms that detect errors due to system failures, adverse environmental conditions, and operator performance.

The manufacturer recommends that an Electronic Quality Control (EQC) be run once per day. This reusable device verifies instrument optics, pneumatics and reagent mixing functions.

The VerifyNow Aspirin Test 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.
- Each test device incorporates two levels of quality control 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. A failure of this test will result in an Attention message (Attention 24) by the VerifyNow Instrument, and no ARU result will be reported.
- During the active phase of the test, the positive internal control monitors the reaction and calculates Control Units, which must fall within specified limits. A failure of the positive control may be indicative of reagent degradation or an abnormal sample. The VerifyNow Instrument will report an Attention 24 or Attention 28 message, and no Aspirin Reaction Units (ARU) will be reported.

In the case of an Attention 24, an Electronic Quality Control (EQC) should be performed to test instrument function. If the EQC passes, the VerifyNow System is functioning normally. In these cases, the problem may be associated with the blood sample and the following causes for Attention 24 should be investigated:

- The patient being tested is on an interfering substance such as abciximab (ReoPro®), eptifibatid (Integrilin®), or tirofiban (Aggrastat®).
- An improper blood collection technique was used to draw the sample.
- The discard tube was used to run the test.
- The patient being tested has a low platelet count, a low hematocrit or an inherited platelet disorder.
- A Wet Quality Control sample was run in Patient Test mode rather than QC mode.

If none of the above can be determined to be the cause of the Attention 24, Wet Quality Control (WQC) Level 2 may be run to confirm the integrity of the test device and the reagents.

In the case of Attention 28, an Electronic Quality Control (EQC) should be performed to test instrument function. If the EQC passes, 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 test.
- The sample was not run within the specified period 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 test device and the reagents.

Wet Quality Control Materials (WQC) are available from Accriva for verifying the integrity of the VerifyNow System. VerifyNow 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 WQC materials are formulated to detect major system failures.

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

The manufacturer recommends that a Level 2 WQC be run once each time a new lot or a new shipment of VerifyNow Aspirin Test kits is received. Control ranges are included in the "Expected Values" section of the control package insert. If the control material does not produce a result within the values stated, repeat the procedure using a new device and WQC material. If the value is still incorrect, call Accriva Technical Support for assistance.

## TEST LIMITATIONS

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

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

Patients with inherited platelet disorders such as von Willebrand Factor Deficiency, Glanzmann Thrombasthenia and Bernard-Soulier Syndrome have not been studied with the VerifyNow Aspirin Test. Patients receiving the following anti-platelet agents may not be tested with VerifyNow Aspirin Test, based on documented interference testing results: GP IIb/IIIa inhibitors, dipyridamole, clopidogrel, non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit COX-1 and/or COX-1, COX-2 enzymes (ibuprofen, naproxen, diclofenac, indomethacin, and piroxicam).

The performance of VerifyNow Aspirin Test on patients with acquired non-drug induced platelet abnormalities is not known.

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 eptifibatid (Integrilin) and tirofiban (Aggrastat). The platelet function recovery time varies among individuals and is longer for patients with renal dysfunction.

VerifyNow Aspirin 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 Accriva. If there are problems related to the VerifyNow System, call Accriva Technical Support at (800) 579-2255 (US) or +1 (858) 263-2502 (Intl).

## PERFORMANCE CHARACTERISTICS

A clinical study was performed with the VerifyNow Aspirin Test on patients at three centers. The study objective was to demonstrate the ability of the VerifyNow Aspirin Test to measure platelet dysfunction due to aspirin. Venous whole blood samples in 3.2% sodium citrate were collected from 65 patients on chronic aspirin therapy (81 mg per day) and 71 patients before and after ingesting 325 mg of aspirin and tested in duplicate with the VerifyNow Aspirin Test. Patients were verbally screened for enrollment into the clinical trial based on stated history of vascular disease or existence of at least two of eight risk factors for developing vascular disease as noted in American Heart Association criteria (current or previous history of smoking, hypertension, hyperlipidemia, family history of vascular disease, post-menopausal females, diabetes mellitus, morbid obesity, or sedentary lifestyle). (See **Table 1**).

Data does not include patients that indicated they were currently on other known anti-platelet agents, or had non-aspirin induced abnormalities of platelet dysfunction, whether acquired or congenital.

VerifyNow Aspirin Test results (duplicates) were evaluated against the presence and absence of aspirin ingestion. See the concordance table (**Table 2**) and dot plot demonstrating the pre- and post-Aspirin results (**Figure 2**).

## EXPECTED VALUES

A reference range of ARU values was calculated from all patients enrolled in the clinical study. Results are illustrated in a non-parametric frequency distribution in **Figure 3**. Values are distributed as pre-aspirin and post-aspirin. The reference range for pre-aspirin samples is 620-672 ARU (2.5 to 97.5 percentile).

## Precision

Simple and complex precision were calculated for VerifyNow Aspirin Test. Simple precision was determined with VerifyNow Test WQC Level 1 and Level 2. Three lots of devices were each tested 20 times on one lot of each level of control. The results are presented in **Table 3**.

Complex precision was calculated using whole blood from a volunteer donor over 20 days for a total of 80 data points. The within-run CV presented in **Table 4** is defined by NCCLS guidelines.

## EXPECTED PERFORMANCE IN WAIVED TESTING SITES

Field studies were conducted at three non-laboratory sites, where 65 people with no laboratory training were chosen from the general population to run the VerifyNow Aspirin Test. Each person read the instructions and then tested three prepared samples at three different ARU levels (**Table 5**).

Each person then scored the test result as (+) for ARU greater than 550 ARU and (-) for ARU less than 550 ARU. At each test site a trained user tested and scored the same samples. All 65

untrained users correctly scored Sample A as negative (-) and Samples B and C as positive (+). There was 100% agreement between the trained and untrained users at all three test sites.

## INTERFERENCE STUDIES

Laboratory testing was performed to determine the effects of several classes of drugs on VerifyNow Aspirin Test results. The following medications may cause a change in platelet function. The following information should be considered for patients who are to be tested with the VerifyNow Aspirin Test.

- **P2Y12 Inhibitors:** Plavix<sup>®</sup>, Ticlid<sup>®</sup>, and Effient<sup>®</sup> are commonly prescribed in conjunction with aspirin. While infrequent, these agents may cause a reduction of ARU in some patients. However, the effect of the P2Y12 inhibitors did not affect the categorization of patients taking aspirin as having platelet dysfunction (i.e. ARU < 550) due to aspirin ingestion. The duration of inhibitory effects varies among these P2Y12 inhibitors. Average durations are listed below:
  - Plavix (up to 5 days)
  - Ticlid (up to 5 days)
  - Effient (up to 10 days)
- **Other Anti-Platelet Agents:** These agents can all inhibit platelet function and may result in a decreased ARU value independent of the effects of aspirin. The duration of inhibitory effects varies among drugs. Average duration times are listed for each drug.
  - Aggrenox (10 days)
  - Persantine (12 hours)
  - Pletal/Cilostazol (12 hours)
- **NSAIDs:** Like aspirin (ASA), NSAIDs have been documented to inhibit platelet function. Unlike ASA, NSAIDs do not irreversibly inhibit platelet function. This may lead to less platelet inhibition by ASA if the NSAID and ASA are taken at the same time. Average duration times for these inhibitory effects are given for each drug.
  - Ibuprofen (Motrin, Advil) (8 hours)
  - Naproxen (Aleve, Anaprox, Naprelan, Naprosyn) (24 hours)
  - Diclofenac (Voltaren, Cataflam) (24 hours)
  - Indocin (24 hours)
  - Feldene (50 hours)
- **GP IIb/IIIa Inhibitors:** Patients who have been administered tirofiban (Aggrastat<sup>®</sup>) or eptifibatid (Integrilin<sup>®</sup>) within two days, or abciximab (ReoPro<sup>®</sup>) within two weeks should not be tested.

Other classes of commonly used drugs were tested with no significant effect on VerifyNow Aspirin Test performance (antioxidants, ACE inhibitors, antiarrhythmics, anticoagulants, antidepressants, insulin, allopurinol, alcohol, beta blockers, bronchodilators, calcium channel blockers, gastrointestinal medications, betamethasone, lovastatin, and the thyroid hormone L-thyroxine). The thrombolytic agent streptokinase showed a measurable inhibition of platelet function, as measured by the VerifyNow Aspirin Test.

Laboratory and clinical testing was performed to assess the effect of the levels of several blood constituents:

Test performance was not affected by hematocrit values between 29-56%, platelet count values of  $\geq 92,000$  platelets per microliter or moderate to extensive blood hemolysis induced by physical manipulation. The degree of hemolysis was determined by visual examination of plasma from

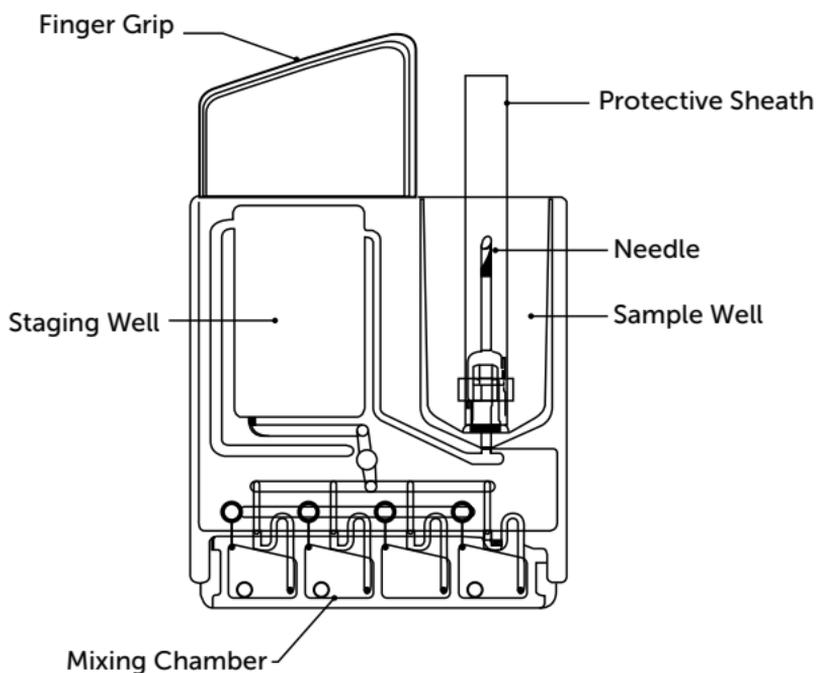
centrifuged samples collected concurrently with VerifyNow Aspirin Test samples.

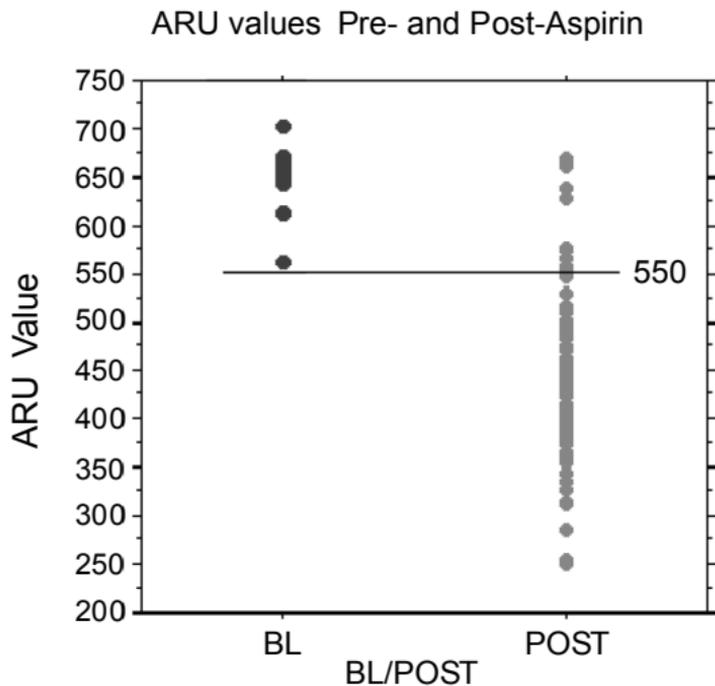
No significant interference was observed on samples studied with triglyceride concentrations up to 577 mg/dL.

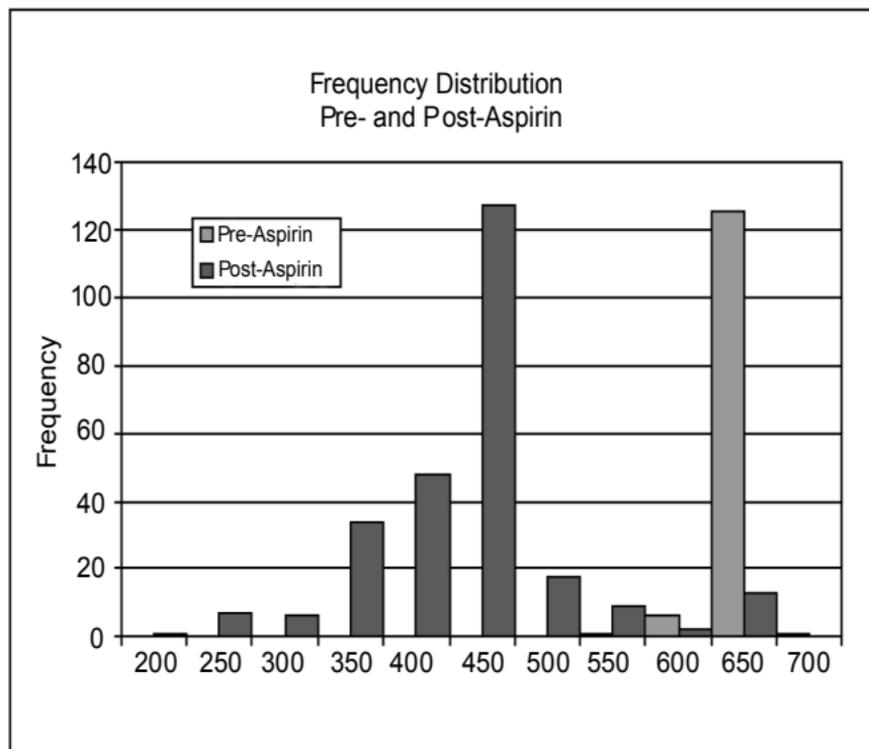
Fibrinogen levels between 164-529 mg/dL were tested with the VerifyNow Aspirin Test. No known relationship exists between performance of VerifyNow Aspirin Test and fibrinogen levels.

## REFERENCE SECTION:

**Figure 1: Test Device**



**Figure 2: Aspirin (ARU) values pre- and post-Aspirin results**

**Figure 3: Aspirin ARU reference ranges**

**Table 1: % Patients enrolled in clinical trial**

<b>% of Patients Enrolled</b>	
History of cardiovascular disease	28.7%
Two risk factors	33.8%
Three risk factors	23.5%
Four or more risk factors	14.0%
<b>Demographics</b>	
Gender	69% Female; 31% Male
Age range	21 to 89 years
<b>Ethnicity</b>	
Caucasian	79.4%
African-American	0.7%
Asian	2.9%
Hispanic	14.7%
Other	2.2%

**Table 2: Reference Range**

<b>Test Result</b>	<b>ASPIRIN STATE</b>	
	<b>PRESENT</b>	<b>ABSENT</b>
POSITIVE <550 ARU	245	0
NEGATIVE ≥ 550 ARU	23	141

Aspirin Present:  
Sensitivity = 91.4%  
Specificity = 100%

**Table 3: Aspirin simple precision results****PRECISION**

Device		ARU	SD	CV*	
Level 1	Lot	Mean			
	1	20	350	0	0
	2	20	350	0	0
	3	20	350	0	0
Level 2					
	1	20	608	12	2.0
	2	20	609	10	1.6
	3	20	618	10	1.6

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

**Table 4: Aspirin complex precision using a single patient****NCCLS Complex Precision Statistics Whole Blood**

		Within-Run			Total	
n	n	ARU	SD	CV%	SD	CV%
(days)	(results)	Mean				
20	80	636	2.7	0.4	3.9	0.6

**Table 5: Waived Testing Site Performance**

Sample	Target ARU Level
A (-)	350
B (+)	625 - 650
C (+)	725 - 750

## BIBLIOGRAPHY

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2. Awry, E H, Loscalzo, J. Aspirin. *Circulation* 2000;101:1206-1218.
3. Stafford, R. Aspirin Use Is Low Among United States Outpatients With Coronary Artery Disease. *Circulation* 2000;101:1097-1101.

## EXPLANATION OF SYMBOLS

	Lot Number
	Catalog Number
	In vitro Diagnostics Device
	Manufacturer
	Use By
	Temperature Limits
	Caution, consult accompanying documents.
	Contains sufficient for < n > tests
	Authorized Representative in the European Community
	WARNING: This symbol indicates a potential biological hazard
	EN - Do not reuse
	Consult Instructions for Use

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d i a g n o s t i c s



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