Anysis

P2Y12

ANYSIS™ P2Y12 Test Cartridges

INSTRUCTIONS FOR USE

English

TECHNICAL SUPPORT
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INTENDED USE

ANYSIS™ P2Y12 Test Cartridge is a quantitative, whole blood test used in laboratory or clinical environments to measure the level of platelet P2Y12 receptor inhibition. The test is indicated as an aid in the management of patients who have been treated with P2Y12 inhibitors by identifying patients who are at increased risk for potential thrombotic events. The P2Y12 receptor inhibition would result in reduced rates of thrombosis and increased rates of bleeding.

For *in vitro* diagnostic use, professional use only.

SUMMARY AND EXPLANATION

The ANYSIS™ Instrument measures platelet aggregation-induced occlusion and provides a migration distance (MD) as a result. The ANYSIS™ Instrument consists of the test instrument and disposable test cartridges. See **Figure 1** for a representation of the test cartridge. Quality control measures are internally included in the test instrument. The instrument controls all test sequencing, temperature, reagent-sample mixing, and performs self-diagnostics.

Each single-use test cartridge contains a lyophilized preparation of human fibrinogen-coated beads and platelet agonist. After loading an anticoagulated (citrated) blood sample, the remained process of the testing is automatically conducted, and the degree of platelet aggregation as a result is displayed.

PRINCIPLE OF THE TEST

ANYSIS™ P2Y12 Test Cartridge is designed to measure platelet P2Y12 receptor blockade within 10 minutes. Typical reagents to block the P2Y12 receptor are ticagrelor, clopidogrel and prasugrel. The ANYSIS™ P2Y12 Test is based on platelet aggregation-induced occlusion mechanism. Activated platelets tend to bind to fibrinogen-coated microparticles, which are densely packed in a microtube and subsequently recruit additional activated platelets. Accumulated platelet aggregation in the microbeads section leads to occlusion of blood flow. Then, ANYSIS™ Instrument determines the final migration distance (MD) of blood flow in a microtube. The MD decreases with increasing number of activated platelets. The instrument measures and reports the final MD in a millimeter unit, which does not require any calculations or conversions. The platelet adhesion and aggregation to thousands of microbeads packed in a microtube result in rapid and reproducible results if the platelets are activated.

The reagent adenosine-5-diphosphate (ADP) is adopted as an agonist to induce platelet activation, whereas Prostaglandin E1 (PGE1) is included as an antagonist to inhibit P2Y1 receptor. Therefore, The ANYSIS™ P2Y12 Test Cartridge is designed to evaluate the specific P2Y12-mediated platelet aggregation. The reaction concentrations of ADP and PGE1 utilized by the ANYSIS™ P2Y12 Test Cartridge are 2μM for ADP and 0.6nM for PGE1, respectively.

GENERAL PRECAUTIONS

- For *in vitro* diagnostic use, professional use only.
- The ANYSIS™ Instrument and its components should only be used as directed by the ANYSIS™ User Manual.
- Do not use the ANYSIS™ P2Y12 Test Cartridge beyond the expiration date.
- All patient samples should be handled as if capable of transmitting disease. Use universal precautions.
- The reagents are manufactured with a material purified from human plasma that was found negative for all communicable diseases tested. Handle test cartridge as biohazardous material and dispose them in an appropriate manner.
- In case there are visible defect to the test cartridge or the test cartridge packaging, do not use the test cartridge.
- In case the test results are extremely out of the expected test range, it is possible either the test cartridge or the instrument is out of order. Consult to the ANYSIS™ User Manual to troubleshoot.

REAGENT STORAGE AND HANDLING

- Store test cartridges at 2 °C to 8 °C (36 °F to 46°F). Do not freeze.
- Allow test cartridges to reach room temperature, 18 °C to 25 °C (64 °F to 77 °F), prior to use.
- Test cartridge should remain sealed in the foil pouch until ready for use to limit the exposure of lyophilized reagents to the humidity. Use the test cartridge immediately after unsealing it from the foil pouch.
- Test cartridges are stable until the expiration date printed on the outer box.

SAMPLE COLLECTION AND PROCEDURE

1. Whole blood may be collected from venous sites using a 21 gauge or larger (e.g. 18-20 gauge) needle in an appropriate blood collection tube (citrate tube). Blood samples should be obtained from an extremity free of peripheral venous infusions.
2. Collect a discard tube first (approximately 2 mL). The discard tube must not contain EDTA.
3. Gently invert the sample tube 5 times to ensure complete mixing of the contents.
4. Blood must equilibrate at room temperature (18 °C to 25 °C) for a minimum of 30 minutes after collection before testing, but no longer than 4 hours. Do not place the sample in a water bath or on a rocker plate.

SAMPLE COLLECTION PRECAUTIONS

- Improper blood collection techniques may lead to inaccurate results.
- Use only 21 gauge or larger bore needles for blood collection or transfer.
- Blood samples should be kept upright prior to testing and avoid prolonged contact with the rubber stopper on the blood collection tube.
- Avoid use of a rocker or pneumatic tube transport system.
- Collection of the blood sample must be performed with care to avoid hemolysis or contamination by tissue factors. Samples with evidence of clotting should not be used.
- The first collection tube must be discarded (approximately 2 mL).
- Fresh whole blood samples must be used within 4 hours of collection.
- Always ensure blood collection tubes are filled to the indicated fill volumes. At altitudes greater than 850 meters 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 altitudes should refer to their facility’s blood collection protocols or blood collection tube manufacturer’s recommendations for instructions to properly fill blood collection tubes.
- Do not freeze or refrigerate blood samples.
- All patient samples should be handled as if capable of transmitting disease.
- Universal precautions should be followed.

TEST PROCEDURE

1. After turning on the ANYSIS™ Instrument, enter the user ID and password. The instrument will automatically begin the Self-Test. If the test fails, the indicated problem must be fixed, and the test be re-run. If the Self-Test passes, move onto the next step.
2. When ready to begin testing, click “TEST”

3. Enter the sample information. Then, select the test type that corresponds to the test cartridge that will be used.
4. Open the foil pouch and remove the test cartridge. Test cartridges should only be handled at the end near the rubber cap. Then, gently insert the test cartridge halfway into the instrument.
5. Carefully invert the sample tube 5 times, and slowly pipette 200 µL of whole blood sample as to prevent air bubbles. Push the test cartridge completely into the test instrument.
6. The test is now ready to begin. Press “START“, and wait for the test to complete. Once completed, test result will be displayed in MD (mm), and the cartridge will automatically slide out from the instrument.

⚠	**The cartridge is mechanically engaged. Do not remove the test cartridge from the Anysis Instrument during a test.**

7. Promptly remove the test cartridge. Dispose of the entire test cartridge/sample in appropriate biohazard waste container.

Refer to the ANYSIS™ User Manual for complete operating instructions.

MATERIALS PROVIDED

- 20 ANYSIS™ P2Y12 Test Cartridges individually sealed in foil pouches. Each test cartridge contains lyophilized fibrinogen-coated beads, ADP, and PGE1.

MATERIALS REQUIRED BUT NOT PROVIDED

- ANYSIS™ Instrument
- Blood collection tubes with 3.2% buffered sodium citrate
- 100-1000 µL pipette and corresponding pipette tips

QUALITY CONTROL

To ensure instrument performance, the manufacturer recommends that a Self-Test (ST) be run once per day. This self-test verifies the instrument optics, pneumatics, temperature, and mixing. Refer to the Quality Control section of the ANYSIS™ User Manual for instructions on running ST.

TROUBLESHOOTING

Under certain conditions, the instrument may display an ERROR message. Refer to the ANYSIS™ User Manual for a more detailed explanation of these messages. For additional troubleshooting, contact your local distributor or Anysis Technical Support. Technical Support at: (telephone) +82 (2) 537-5111; (e-mail) techsupport@any-sis.com.

CALIBRATION

ANYSIS™ P2Y12 Test Cartridges are calibrated at the factory.

INTERPRETATION OF RESULTS

Test results are reported as migration distance (MD), which is determined by the amount of P2Y12 receptor-mediated platelet aggregation and is represented as a function of the rate and extent of platelet aggregation due to activation via ADP.

- **≥225 MD** – represents specific evidence for the presence of a pharmacodynamic antiplatelet effect of a P2Y12 inhibitor.
- **≥225 MD** – is associated with reduced rates of thrombosis and increased rates of bleeding due to the presence of the P2Y12 inhibitor effect.

ANYSIS™ P2Y12 Test results should be interpreted in conjunction with all other clinical and laboratory data available to the clinician.

It is the responsibility of the Laboratory Director to either confirm the suitability of the recommended cutoff or to select alternative cutoffs or decision points that are appropriate for the patient population to be tested.

TEST LIMITATIONS

- The lyophilized reagent is hygroscopic and can degrade after prolonged exposure to room air. Therefore, the test cartridge should be used immediately 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 cartridge 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 ANYSIS™ P2Y12 Test. ANYSIS™ P2Y12 Test is not intended for use with these types of platelet disorders.
- Certain drugs that inhibit platelet function may affect the results of the ANYSIS™ P2Y12 Test
- Glycoprotein IIb/IIIa inhibitors such as abciximab, eptifibatide, and tirofiban have been studied to significantly affect platelet aggregation, therefore the MD result. Patients who have been treated with Glycoprotein IIb/IIIa inhibitor drugs should not be tested until platelet function has recovered.
- Drugs that irreversibly affect platelet function may be detected up to 14 days after ingestion.

PERFORMANCE CHARACTERISTICS

A clinical study was designed and conducted to demonstrate the performance of ANYSIS™ P2Y12 Test. The establishment of reference ranges with specimen collected in 3.2 % buffered sodium citrate has been performed on a specimen group with 63 individuals who were actively ingesting P2Y12-inhibitor and 62 individuals who were not ingesting P2Y12-inhibitor. This specimen group was composed of ostensibly healthy individuals with no previous history or laboratory results indicative of platelet dysfunction induced by intrinsic platelet defects, or VWD. These individuals were screened either verbally or through medical records for a previous history of hyperlipidemia and diabetes. Specimen reference range and clinical characteristics are organized in **Tables 1 & 2.**

This clinical study was designed to compare the two groups of obtained samples: 1) Negative control and 2) Positive group. The negative control was the samples that satisfied the following criteria: VerifyNow PRU (≥194) and without P2Y12-inhibitor ingestion; while the positive group was the samples that satisfied the following criteria: VerifyNow %INH (≥10) and with P2Y12-inhibitor ingestion. For each subject, whole blood sample was collected and tested for the degree of platelet aggregation. All 125 subjects were simultaneously tested with the ANYSIS™ P2Y12 Test and VerifyNow P2Y12 tests. Recorded MD results were evaluated against the presence (positive) and absence (negative) of P2Y12 ingestion. Acquired data is organized in graphic representations. (See **Figures 2** & **Figure 3**)

ROC Curve Analysis

A total of 125 measurements were pooled and evaluated by receiver operating characteristic (ROC) curve analysis. The purpose of this analysis was to evaluate the ability of the MD result to discriminate an on-treatment sample from a P2Y12 inhibitor-free sample, in terms of sensitivity and specificity. The P2Y12 inhibitor elicits its antiplatelet effect specifically through its action on the platelet P2Y12 receptor. The MD result is specific for P2Y12 receptor blockade. Therefore, the ability to detect an on-treatment sample reflects the ability of the MD result to identify the presence of an antiplatelet effect of a P2Y12 inhibitor.

Figure 4 shows the ROC curve evaluation, which reveals the area under the curve to be 0.984 (95% confidence interval 0.943-0.998, P<0.0001), indicating that the test has excellent ability to identify the presence of an antiplatelet effect of a P2Y12 inhibitor. At the optimal cutoff of MD **≥**225, the sensitivity was calculated to be 96.8% and the specificity to be 88.7%. (See **Table 3**)

It is the responsibility of the Laboratory Director to either confirm the suitability of the recommended cutoff or to select alternative cutoffs or decision points that are appropriate for the patient population to be tested.

Precision

Precision test was completed to assess the repeated performance of ANYSIS™ P2Y12 Test. Precision was assessed with both positive and negative samples. Two lots of cartridges were each tested 10 times. Two lots of cartridges were tested 10 times, respectively. The test results are presented in **Table 4**.

Repeatability test was conducted by comparing MD results from two different operators testing on a single whole blood sample. ANYSIS™ P2Y12 Test was conducted 20 times by each operator, respectively. The within-run CV values and the total CV values of the conducted tests are presented in **Table 5**.

Expected Testing Performance in Waived Test Sites

In order to demonstrate consistent performance in waived test sites, field studies were conducted at two different sites, where two operators performed the ANYSIS™ P2Y12 Test. Each operator tested 20 trials at each site with three prepared samples, switching sites every 10 trials.

Each person then scored the test result as (+) for MD greater than or equal to 225 MD and (-) for MD less than 225 MD. Both users correctly scored Sample A as negative (-) and Samples B and C as positive (+). There was 100% agreement between the two operators at all test sites. Test results are summarized in **Table 6**.

Interfering Substances

Laboratory testing was performed to determine the effect of several classes of reagents on ANYSIS™ P2Y12 Test results. Organized MD differences before and after reagent effect and the corresponding CV can be found in **Figure 5**.

- The following compounds (therapeutic concentrations/concentration ranges) did not exhibit any apparent interference or cross-reactivity:
    - Human Hemoglobin (2.5 mg/mL)
    - Heparin (6 IU/mL)
    - Ethanol (0.25 µL/mL)
    - L-Ascorbic Acid (5.21 mg/mL)
- The following compounds (therapeutic concentrations/concentration ranges) exhibited slight interference or cross-reactivity:
    - Albumin from human serum (200 mg/mL)
    - Acetaminophen (1 mg/mL)
    - D-(+)-glucose (100 mg/mL)
    - Cholesterol (6 mg/dL)
- The following compounds (therapeutic concentrations/concentration ranges) exhibited significant interference or cross-reactivity:
    - Sodium citrate (5 mg/dL)
    - Sodium chloride (68 mg/mL)
- Glycoprotein IIb/IIIa inhibitors such as abciximab, eptifibatide, and tirofiban may significantly affect ANYSIS™ P2Y12 Test results. See TEST LIMITATIONS section for details.

As with all laboratory tests, ANYSIS™ P2Y12 Test results should be interpreted in the context of all available laboratory and clinical information.

Figure 1: Test cartridge

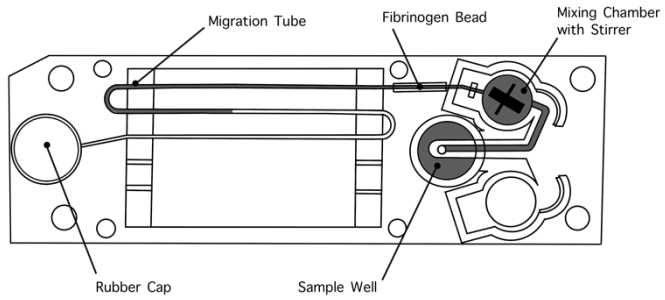


Table 1: Clinical characteristics

Gender	64.0 % Male; 36.0 % Female
Age Range	30 - 86
History of diabetes	33.6 %
History of hyperlipidemia	52.0 %
History of both	17.6 %

Table 2: Reference ranges for two groups

	Mean ± SD	***p*-value**
Without P2Y12-Inhibitor Ingestion (n = 62)	182.1 ± 30.6	< 0.0001
With P2Y12-Inhibitor Ingestion (n = 63)	264.9 ± 11.9	

Figure 2: Comparison of MD values without-P2Y12 ingestion (negative control) and with-P2Y12 ingestion (positive group)

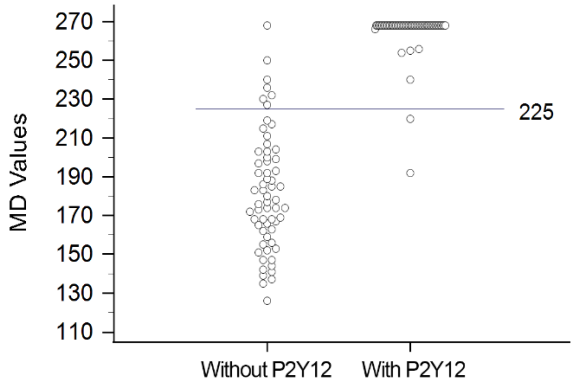


Figure 3 Frequency distributions of MD without-P2Y12 ingestion (negative control) and with-P2Y12 ingestion (positive group)

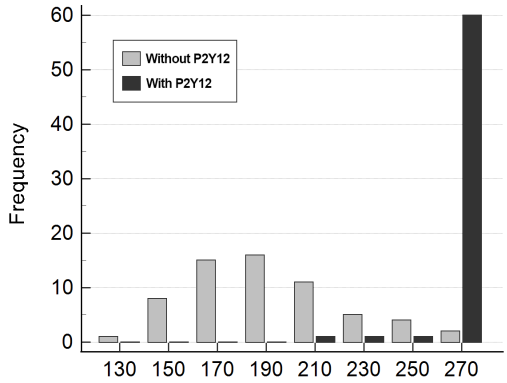


Figure 4: ROC curve analysis

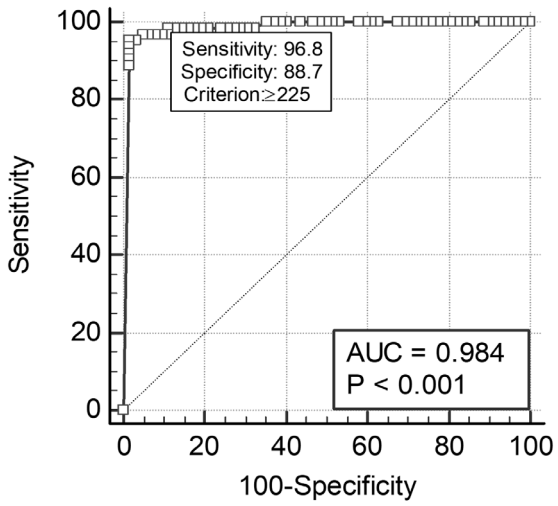


Table 3: Sensitivity and specificity

Test Result	P2Y12 PRESENT (POSITIVE) (n=63)	P2Y12 ABSENT (NEGATIVE) (n=62)
POSITIVE ≥225 MD	61	7
NEGATIVE <225 MD	2	55

Sensitivity = 96.8%
Specificity = 88.7%
(*p*-value: <0.0001)

Table 4: Reproducibility of ANYSIS™ P2Y12 Test between two lots of cartridges

PRECISION

	Cartridge		MD	SD	%CV*	%RV
NEGATIVE	**Lot**	n	Mean			
<225 MD	**1**	10	153	10.9	7.1	2.8
	2	10	158	12.2	7.7	
POSITIVE						
≥225 MD	**1**	10	261	11.4	4.4	0.2
	2	10	260	16.0	6.2	

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

Table 5: Reproducibility of ANYSIS™ P2Y12 Test between two operators

REPRODUCIBILITY

			Within-Run		**Total**	
Operator	n	MD	SD	%CV*	%CV*	%RV
	(results)	Mean				
1	20	266	8.0	3.0	4.1	2.5
2	20	259	12.3	4.8		

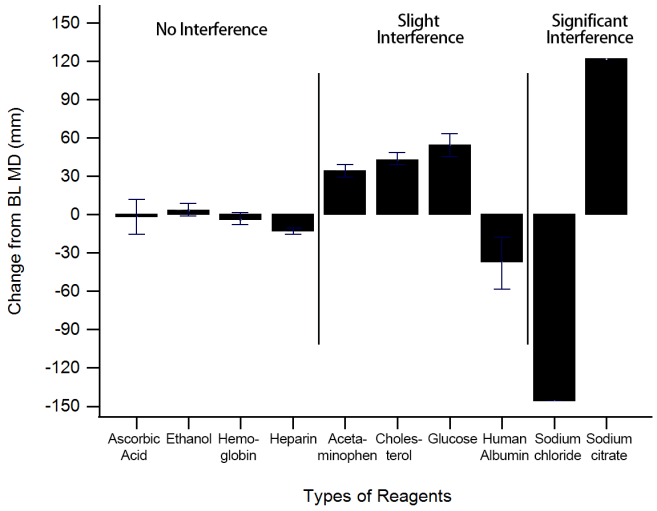
*The manufacturer's specification for the coefficient of variation is ≤10%.

Table 5: Waived testing site performance

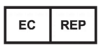
			Within-Run		Total	
		n	MD	%CV*	%CV*	%RV
SAMPLE	**SITE**	(results)	Mean			
A (-)	**1**	20	138	5.2	6.3	5.3
	2	20	145	6.4		
B (+)	**1**	20	268	0.3	3.0	0.9
	2	20	266	4.3		
C (+)	**1**	20	259	6.5	5.4	2.1
	2	20	264	4.0		

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

Figure 5: Interfering and non-interfering substances



EXPLANATION OF SYMBOLS

ISO 15223-1:2016 Medical devices — Symbols to be used with medical device labels, labelling and information to be supplied — Part 1: General requirements			
5.1.1 	**Manufacturer**	5.1.3 	**Date of manufacture**
5.1.4 	**Use by date**	5.1.5 	**Batch code**
5.1.6 	**Catalogue number**	5.1.7 	**Serial number**
5.3.7 	**Temperature limits**	5.4.1 	**Biological risks**
5.4.2 	**Do not re-use**	5.4.3 	**Consult instructions for use**
5.4.4 	**Caution**	5.5.1 	**In-vitro diagnostic medical device**
5.5.5 	**Contains sufficient for < n > tests**		**CE certification**
	**European representative**		**Waste electrical and electronic equipment**

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