EPI/ADP

ANYSIS™ C/EPI and C/ADP Test Cartridges

INSTRUCTIONS FOR USE

English

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INTENDED USE

The ANYSIS™ C/EPI and C/ADP Test Cartridges are quantitative, whole blood assays used in laboratory or clinical environments to assess platelet function by simulating the process of platelet adhesion and aggregation following a vascular injury. These tests are indicated as an aid in the management of patients who are at an increased risk for bleeding due to platelet dysfunction.

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 collagen-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

The ANYSIS™ C/EPI and C/ADP Test Cartridges are designed to measure platelet function within 10 minutes by simulating the process of platelet adhesion and aggregation following a vascular injury. Common causes of platelet dysfunction include intrinsic factors such as von Willebrand disease (VWD), or exposure to agents such as acetylsalicylic acid (ASA).

All reagents in the ANYSIS™ C/EPI and C/ADP Test Cartridges, i.e. collagen, ADP, and epinephrine, play an important role in the *in-vivo* process of primary hemostasis. Damaged blood vessels expose collagen strands, which mediate the binding of von Willebrand Factor (VWF). Even under high shear conditions, platelets can adhere to VWF, representing the first phase of primary hemostasis. At vascular lesions, high amounts of ADP are released into the blood, while systemic reactions increase the level of epinephrine. All these substances, including collagen, can activate platelets through interaction with specific receptors, thereby enhancing the primary hemostasis process. Such process is simulated by the ANYSIS™ Instrument with reaction concentrations of epinephrine and ADP at 0.5μM and 1.2μM, respectively.

The ANYSIS™ C/EPI and C/ADP Tests are based on platelet aggregation-induced occlusion mechanism to test patients with a suspected bleeding risk. Activated platelets tend to bind to collagen-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. MD decreases with the increase in the number of activated platelets. The reported final MD is 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 reagents adenosine-5-diphosphate (ADP) and epinephrine are each adopted as an agonist to simulate the process of hemostasis *in-vivo*. Therefore, ANYSIS™ C/EPI and C/ADP Test Cartridges can be used in conjunction to assess platelet function for patients suspected of bleeding risk.

GENERAL PRECAUTIONS

- For *in vitro* diagnostic use only.
- The ANYSIS™ Instrument and its components should only be used as directed by the ANYSIS™ User Manual.
- Do not use ANYSIS™ Instrument beyond the expiration dates.
- 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 with the ANYSIS™ User Manual.

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.
- Only use 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™ C/EPI and C/ADP Test Cartridges individually sealed in foil pouches
  - ANYSIS™ C/EPI Test Cartridge contains lyophilized collagen-coated beads and epinephrine.
  - ANYSIS™ C/ADP Test Cartridge contains lyophilized collagen-coated beads and ADP.

MATERIALS REQUIRED BUT NOT PROVIDED

- ANYSIS™ Instrument
- Blood collection tubes with 3.2% buffered sodium citrate
- 100-1000µL 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™ C/EPI and C/ADP Test Cartridges are calibrated at the factory.

INTERPRETATION OF RESULTS

Test results are reported as migration distance (MD), which is determined by the degree of platelet activation by the specific agonist. MD Results are reported as the extent of platelet aggregation in response to platelet activation aided by either EPI or ADP.

EPI Test Criterion

- **≥228 MD** – is associated with increased rates of bleeding risk due to intrinsic platelet dysfunction (e.g. VWD and Glanzmann thrombasthenia) or ASA-mediated platelet dysfunction.

ADP Test Criterion

- **≥190 MD** – is associated increased rates of bleeding risk due to intrinsic platelet dysfunction (e.g. VWD and Glanzmann thrombasthenia).

Results of ANYSIS™ C/EPI and C/ADP Tests 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 Bernard-Soulier Syndrome have not been studied with ANYSIS™ C/EPI and C/ADP Tests. ANYSIS™ C/EPI and C/ADP Test are not intended for use with these types of platelet disorders.
- Certain drugs that inhibit platelet function may affect the results of ANYSIS™ C/EPI and C/ADP Test Cartridges.
- Glycoprotein IIb/IIIa inhibitors abciximab, eptifibatide, and tirofiban significantly affect platelet aggregation. 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

To demonstrate the test performance of ANYSIS™ C/EPI and C/ADP Tests, specimen samples were collected and classified according to the test results of PFA-200 C/EPI and C/ADP, respectively. The establishment of reference ranges for ANYSIS™ C/EPI Test with specimen collected in blood collection tubes with 3.2% buffered sodium citrate incorporated 163 subjects, while that of ANYSIS™ C/ADP Test has been established with 184 subjects. Both specimen groups were composed of ostensibly healthy individuals with no previous history or laboratory results indicative of platelet dysfunction. These individuals were screened either verbally or through medical records for a previous history of hyperlipidemia and/or diabetes. Specimen reference range and clinical characteristics are organized in **Tables 1 & 2.**

The clinical study was designed to compare the following two groups of obtained samples: 1) Negative control (with normal platelet function); 2) positive group (with abnormal platelet function). These groups were classified in concurrence to the test results of PFA-200. For each subject, whole blood sample was collected and tested for the degree of platelet aggregation. 163 subjects were simultaneously tested with ANYSIS™ C/EPI Test and PFA C/EPI test, while 184 subjects were simultaneously tested with ANYSIS™ C/ADP Test and PFA C/ADP test. The test results of Anysis tests were evaluated against those of PFA tests, respectively. Acquired data is displayed in graphic representations. (See **Figure 2 - Figure 5**)

ROC Curve Analysis

A total of 163 measurements for the ANYSIS™ C/EPI Test and 184 measurements for the ANYSIS™ C/ADP Test were evaluated by receiver operating characteristic (ROC) curve analysis, respectively. The purpose of this analysis was 1) to evaluate the ability of MD result to discriminate samples based on the platelet function against the test results of PFA-200, and 2) to calculate the sensitivity and specificity of ANYSIS™ C/EPI and C/ADP Tests for detecting a sample with platelet dysfunction.

ADP and epinephrine both have pivotal roles in the *in-vivo* process of primary hemostasis in response to a vascular lesion, activating specific receptors involved in platelet adhesion and aggregation. **Figures 6 & 7** show the ROC curve analysis, which reveals the area under the curve to be 0.919 (95% confidence interval 0.866-0.956, P<0.0001) for the ANYSIS™ C/EPI and 0.946 (95% confidence interval 0.943-0.998, P<0.0001) for ANYSIS™ C/ADP Test, indicating that both tests have excellent ability to detect platelet dysfunction involved in primary

hemostasis.

With the optimal cutoff of ≥228 MD, the sensitivity and specificity of the ANYSIS™ C/EPI Test were 77.9 and 92.3, respectively. As for the ANYSIS™ C/ADP Test, sensitivity was determined to be 96.8% and the specificity to be 88.7% at the optimal cutoff of ≥190 MD. (See **Tables 3 & 4**)

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™ C/EPI and C/ADP Tests. Precision was assessed with both positive and negative samples. Two lots of cartridges were tested 10 times, respectively. The test results are presented in **Tables 5 & 8**.

Repeatability test was conducted by comparing MD results from two different operators testing on a single whole blood sample. ANYSIS™ C/EPI and C/ADP Tests were conducted 20 times by each operator, respectively. The within-run CV values and the total CV values of the conducted tests are presented in **Tables 6 & 9.**

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™ C/EPI and C/ADP Tests. Each operator tested 20 tests at each site with three prepared samples, switching sites every 10 tests.

Each person then scored the test result as (+) for MD greater than or equal to the assigned cutoff and (-) for MD less than the assigned cutoff. 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 for both ANYSIS™ C/EPI and C/ADP Tests. Test results are summarized in **Tables 7 & 10**.

Interfering Substances

Laboratory testing was performed to determine the effect of several classes of reagents on the ANYSIS™ C/EPI and C/ADP Test results. Organized MD differences before and after reagent effect and the corresponding SD can be found in **Figures 8 & 9**.

- ANYSIS™ C/EPI Test, no interference (therapeutic concentrations/concentration ranges)
  - Human Hemoglobin (2.5 mg/mL)
  - D-(+)-glucose at 100 mg/mL
  - Ethanol (0.25 µL/mL)
  - L-Ascorbic Acid (5.21 mg/mL)
- ANYSIS™ C/EPI Test, slight interference (therapeutic concentrations/concentration ranges)
  - Cholesterol (6 mg/dL)
  - Acetone (20 µL/mL)
  - Acetaminophen (1 mg/mL)
  - Albumin from human serum (200 mg/mL)
- ANYSIS™ C/EPI Test, significant interference (therapeutic concentrations/concentration ranges)
  - Bilirubin (0.2 mg/mL)
  - Triglyceride (12.5 mg/mL)
  - Sodium citrate (5 mg/dL)
  - Sodium chloride (68 mg/mL)
- ANYSIS™ C/ADP Test, no interference (therapeutic concentrations/concentration ranges)
  - Human Hemoglobin (2.5 mg/mL)
  - Acetaminophen (1 mg/mL)
  - Ethanol (0.25 µL/mL)
- ANYSIS™ C/ADP Test, slight interference (therapeutic concentrations/concentration ranges)
  - Albumin from human serum (150 mg/mL)
- ANYSIS™ C/ADP Test, significant interference (therapeutic concentrations/concentration ranges)
  - D-(+)-glucose (100 mg/mL)
  - Sodium citrate (5 mg/dL)
  - Sodium chloride (68 mg/mL)
- Glycoprotein IIb/IIIa inhibitors such as abciximab, eptifibatide, and tirofiban may significantly affect ANYSIS™ C/EPI and C/ADP Test results. See TEST LIMITATIONS section for details.

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

Figure 1: Test cartridge

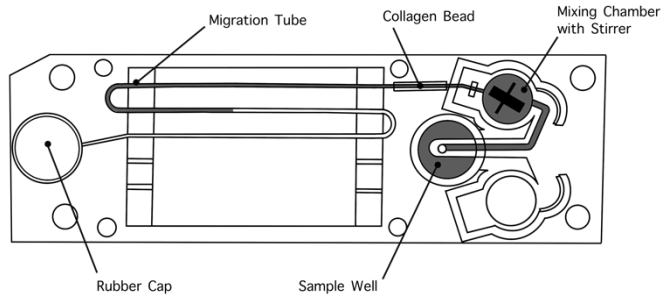


Table 1: Clinical characteristics

	Anysis-C/ADP	Anysis-C/EPI
Gender	72.8% Male; 27.2% Female	67.5% Male; 32.5% Female
Age Range	30-86	30-86
History of diabetes	37.0 %	41.1%
History of hyperlipidemia	56.0 %	52.8%
History of both	25.0 %	27.0%

Table 2: Reference ranges for two groups

	Anysis-C/ADP		Anysis-C/EPI	
Reference Range	Mean±SD	*p*-value	Mean±SD	*p*-value
Negative	153.5±37.8	< 0.001	174.3±34.5	< 0.001
Positive	255.7 ± 28.1		246.7 ± 32.3	

Figure 2: Comparison of EPI-MD values for negative control and positive group

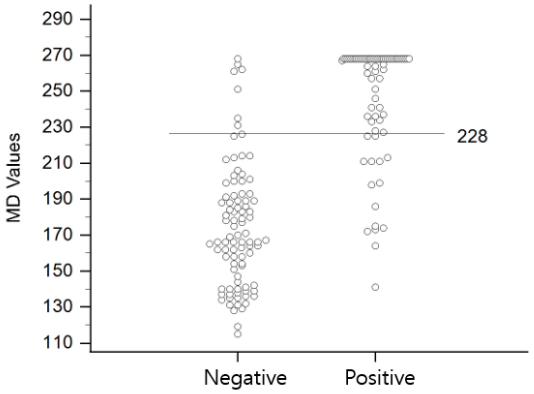


Figure 3: Frequency distributions of EPI-MD results for negative and positive groups

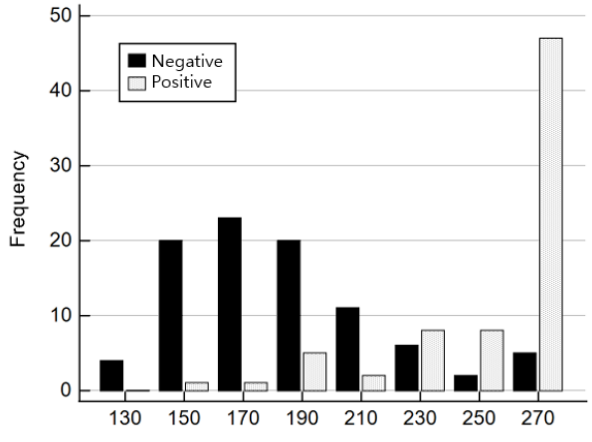


Figure 4: Comparison of ADP-MD values for negative control and positive group

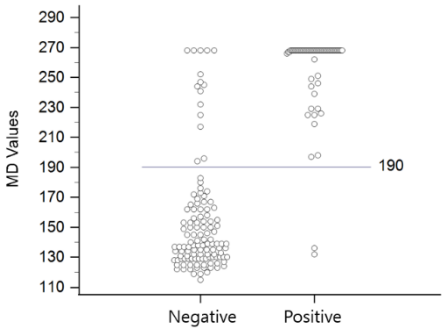


Figure 5: Frequency distributions of ADP-MD results for negative and positive groups

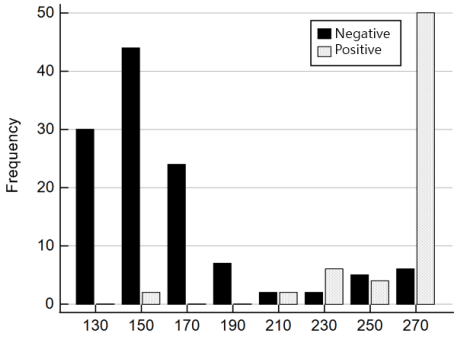


Figure 6: ROC curve analysis of ANYSIS™ C/EPI Test

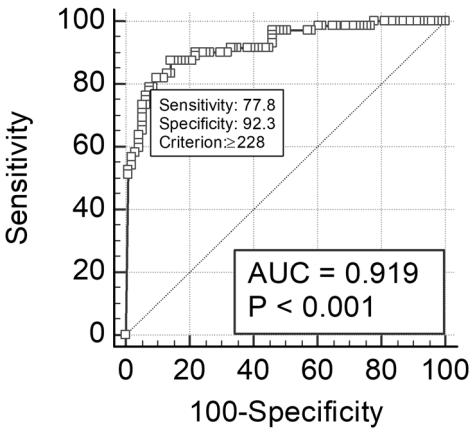


Figure 7: ROC curve analysis of ANYSIS™ C/ADP Test

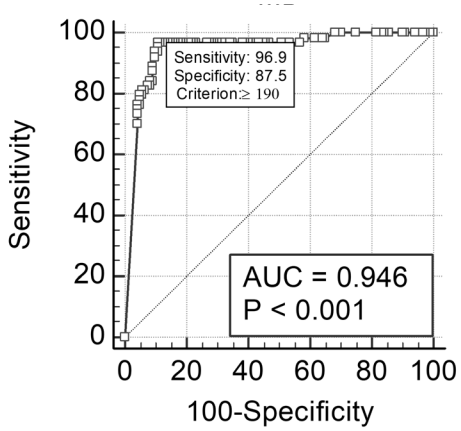


Table 3: Sensitivity and specificity of ANYSIS™ C/EPI Test

Test Result	Positive (n=72)	Negative control (n=91)
POSITIVE ≥228 MD (n)	63	13
NEGATIVE <228 MD (n)	9	78

Sensitivity = 77.8%
Specificity = 92.3%
(*p*-value: <0.0001)

Table 4: Sensitivity and specificity of ANYSIS™ C/ADP Test

Test Result	Positive (n=64)	Negative control (n=120)
POSITIVE ≥190 MD (n)	62	15
NEGATIVE <190 MD (n)	2	105

Sensitivity = 96.9%
Specificity = 87.5%
(*p*-value: <0.0001)

Table 5: Reproducibility of ANYSIS™ C/EPI Test between two lots of cartridges

PRECISION

	Cartridge		MD	SD	%CV*	%RV
NEGATIVE	**Lot**	n	Mean			
<190 MD	**1**	10	186	9.7	5.2	0.5
	2	10	187	10.2	5.5	
POSITIVE						
≥190 MD	**1**	10	268	0.0	0.0	1.9
	2	10	263	13.5	5.1	

Table 6: Reproducibility of ANYSIS™ C/EPI Test between two operators

REPRODUCIBILITY

			Within-Run		Total	
Operator	n	MD	SD	%CV*	%CV*	%RV
	(results)	Mean				
1	20	176	13.0	7.4	8.2	2.2
2	20	180	16.1	9.0		

Table 7: ANYSIS™ C/EPI Test waived testing site performance

		Within-Run			Total	
		n	MD	%CV*	%CV*	%RV
SAMPLE	**SITE**	(results)	Mean			
A (-)	**1**	20	174	8.0	6.9	2.2
	2	20	177	5.7		
B (+)	**1**	20	258	5.2	4.8	0.7
	2	20	259	4.5		
C (+)	**1**	20	266	2.7	1.9	0.6
	2	20	268	0.0		

Table 8: Reproducibility of ANYSIS™ C/ADP Test between two lots of cartridges

	Cartridge		MD	SD	%CV*	%RV
NEGATIVE	**Lot**	n	Mean			
<190 MD	**1**	10	180	13.1	7.3	3.3
	2	10	174	9.3	5.3	
POSITIVE						
≥190 MD	**1**	10	239	18.5	7.8	4.6
	2	10	250	14.7	5.9	

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

Table 9: Reproducibility of ANYSIS™ C/ADP Test between two operators

			Within-Run		Total	
Operator	n	MD	SD	%CV*	%CV*	%RV
	(results)	Mean				
1	20	164	10.6	6.6	6.9	3.8
2	20	163	15.0	7.3		

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

Table 10: ANYSIS™ C/ADP Test waived testing site performance

			Within-Run		Total	
		n	MD	%CV*	%CV*	%RV
SAMPLE	**SITE**	(results)	Mean			
A (-)	**1**	20	163	9.4	8.8	1.1
	2	20	165	8.4		
B (+)	**1**	20	227	9.0	8.8	0.9
	2	20	229	8.9		
C (+)	**1**	20	254	7.8	8.7	2.3
	2	20	248	9.7		

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

Figure 8: ANYSIS™ C/EPI Test interfering and non-interfering substances

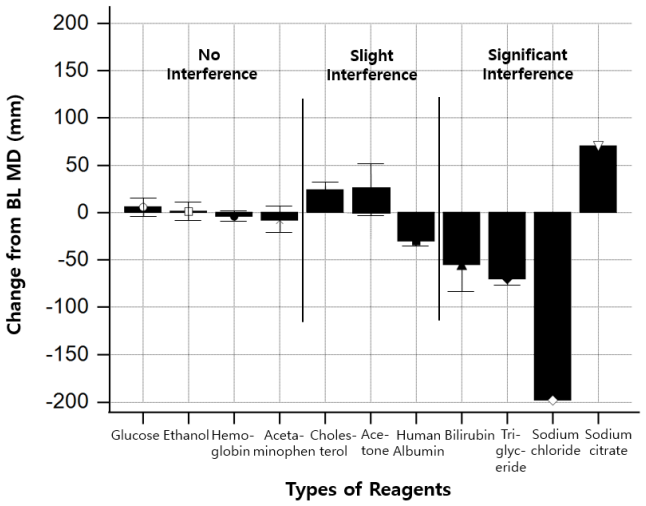
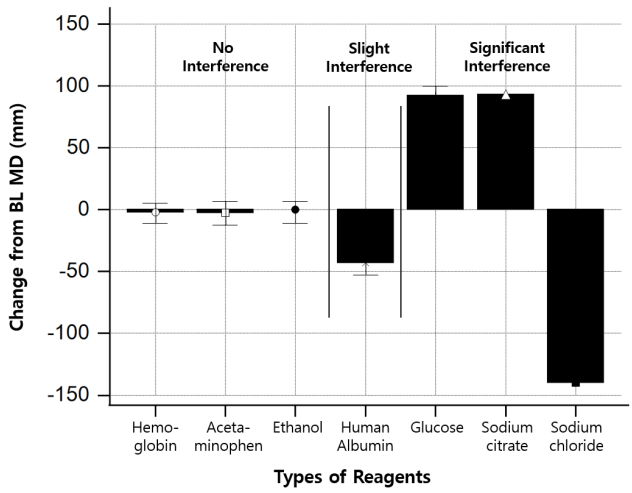
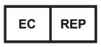


Figure 9: ANYSIS™ C/ADP Test interfering and non-interfering substances



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**

RHEO Meditech

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