AccuCardia™ ELISA Test System

IUO

REF ACC6301I

INTENDED USE

The AccuCardia™ ELISA Test System is an ELISA assay for the quantitative determination of sPLA2-IIA protein levels in human plasma; to be used in conjunction with clinical evaluation for assessing cardiovascular disease risk.

SIGNIFICANCE AND BACKGROUND

Cardiovascular disease (CVD) accounts for nearly half of the noncommunicable diseases worldwide, and is a leading cause of global death (1, 2). In 2008, the World Health Organization estimated that 17.3 million people died from CVD; the majority of these deaths being attributed to myocardial infarction (MI) and stroke (2 - 4). The severe clinical consequences of CVD, such as MI and stroke, are typically caused by atherosclerotic lesions; which ultimately disrupt the flow of blood to vital organs such as the heart and/or brain (5, 6). Close examination of these lesions reveal complex arterial structures consisting of connective tissue elements, inflammatory cells, lipids, and debris (5, 7). In recent years, research has shown that phosholipase A2 (PLA2) enzymes are key factors involved in atherosclerotic CVD; thus, they have emerged as promising biomarkers (8 - 11).

PLA2 enzymes are a family of proteins that catalyze the hydrolysis of phospholipids at the sn-2 position, yielding pro-inflammatory lysophospholipids and fatty acids (12 - 14). Secreted PLA2 (sPLA2) proteins make up a subgroup of this family, and consist of 10 calcium-dependent extracellular enzymes with relatively low molecular masses (8). A notable member of this subgroup, termed sPLA2-IIA, has been the focus of numerous basic research and clinical studies aimed at investigating its role in, and association with, cardiovascular conditions such as coronary artery disease (CAD) and atherosclerosis (8, 9). For example, several reports have linked increased plasma levels of sPLA2-IIA with recurrent events and adverse outcomes in patients with stable CAD (15, 16). Recently, additional studies have also shown that increased levels of sPLA2-IIA are predictive of recurrent cardiac events and death in patients presenting with acute coronary syndromes, such as MI and unstable angina (17, 18).

PRINCIPLE OF THE ASSAY

The AccuCardia test is a dual monoclonal antibody sandwich ELISA assay designed to quantitatively detect sPLA2-IIA protein in human plasma. A brief overview of the test procedure is as follows:

1. Test plasma, Calibrators, and Controls are diluted in Sample Diluent, then transferred to a microtiter plate containing immobilized anti-sPLA2-IIA antibody.
2. The diluted samples are incubated for one hour in the antibody-coated microwells. Sample-derived sPLA2-IIA is bound to the plate via interaction with the immobilized antibody. After incubation, the wells are washed to remove other plasma components.
3. A solution containing horse radish peroxidase (HRP)-conjugated anti-sPLA2-IIA antibody is then added to each well, and the plate is incubated again for one hour. After incubation, the plate is washed to remove unbound HRP conjugate.
4. The microwells containing immobilized sPLA2-IIA and HRP-conjugate are incubated for 10 minutes with peroxidase substrate solution, and hydrolysis of the substrate produces a color change.
5. After 10 minutes, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution is proportional to the amount of sPLA2-IIA protein in the original test sample.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label.

PLATE		1. Plate: 96 wells configured in twelve, 1 x 8-well, strips coated with a sPLA2-IIA specific antibody. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ		2. Conjugate: sPLA2-IIA specific HRP-conjugated antibody. One, 15mL, white-capped, amber bottle. Ready to use (pH 7.5 ± 0.1).
CONTROL	I	3. Low Positive Control: One **green**-capped vial. Lyophilized.
CONTROL	II	4. Medium Positive Control:: One **red**-capped vial. Lyophilized.
CAL	A	5. Calibrator A: One **white**-capped vial. Lyophilized.
CAL	B	6. Calibrator B: One **yellow**-capped vial. Lyophilized.
CAL	C	7. Calibrator C: One **orange**-capped vial. Lyophilized.
CAL	D	8. Calibrator D: One **blue**-capped vial. Lyophilized.
CAL	E	9. Calibrator E: One **clear**-capped vial. Lyophilized.
CAL	F	10. Calibrator F: One **purple**-capped vial. Lyophilized.
DIL	SPE	11. Sample Diluent: One, 30mL green-capped bottle. Ready to use (pH 7.5 ± 0.1).
SOLN	TMB	12. TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN	STOP	13. Stop Solution: One, 15mL, red-capped, bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF	10X	14. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (clear solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTES:

1. **The Stop Solution and Wash Buffer are not test system/lot number dependent and may be used interchangeably with any of the ZEUS ELISA™ Test Systems.**
2. **Test System also contains:**
   a. **Component Label containing lot specific information inside the Test System box.**
   b. **Package Insert providing instructions for use.**

PRECAUTIONS

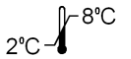
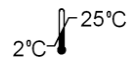
1. For investigational use only. Performance characteristics for this product have not been established.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.

4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HbsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (19).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 – 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
8. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
9. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
10. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
11. Dilution or adulteration of these reagents may generate erroneous results.
12. Do not use reagents from other sources or manufacturers.
13. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
14. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
15. Avoid microbial contamination of reagents. Incorrect results may occur.
16. Cross contamination of reagents and/or samples could cause erroneous results.
17. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
18. Avoid splashing or generation of aerosols.
19. Do not expose reagents to strong light during storage or incubation.
20. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
21. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
22. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
23. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
24. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
25. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Software capable of computing results using a 4-parameter logistic curve-fitting equation
3. Incubator capable of 37°C incubation.
4. Sample dilution plate
5. Pipettes capable of accurately delivering 10 – 200µL.
6. Multichannel pipette capable of accurately delivering 50 – 200µL.
7. Reagent reservoirs for multichannel pipettes.
8. Wash bottle or microwell washing system.
9. Distilled or deionized water.
10. One liter graduated cylinder.
11. Serological pipettes.
12. Disposable pipette tips.
13. Paper towels.
14. Laboratory timer to monitor incubation steps.
15. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening – strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
	Reconstituted Calibrators/Controls are stable for up to 30 days.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrators, Positive Controls, TMB, Sample Diluent
	Stop Solution: 2 – 25°C Wash Buffer (1X): 20 – 25°C for up to 7 days, 2 – 8°C for 30 days. Wash Buffer (10X): 2 – 25°C

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Store samples at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, plasma may be stored between 2 - 8°C, for no longer than 48 hours. If delay in testing is anticipated, store test plasma at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause cause protein degradation and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory (20).
4. Use only freshly drawn and properly refrigerated/frozen **EDTA-plasma** obtained by approved aseptic venipuncture procedures (21, 22). Avoid using hemolyzed, lipemic, or bacterially contaminated plasma.

ASSAY PROCEDURE

1. **Setup**
   a. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
   b. If using the kit for the first time, reconstitute the lyophilized Calibrators and Controls as follows:
      i. Add 250µL of distilled water to each vial.

ii. Allow the freshly reconstituted vials to sit for 10 - 15 minutes at room temperature.
iii. Vortex before use.

NOTE: Once reconstituted, the Calibrators and Controls should be used within 60 days.

c. Prepare 1X Wash Buffer by adding the contents of the10X Wash Buffer bottle to 900mL of deionized water. Mix by gentle inversion.

d. Determine the number of microwells needed. For each run, wells must be assigned for duplicate analysis of six Calibrators, Control I and Control II. Assign additional wells for duplicate analysis of patient samples. Check software and reader requirements for the correct Calibrator/Control configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

EXAMPLE PLATE SET-UP						
	1	2	3	4	5	6
A	Calibrator A	Calibrator A	Patient Specimen 1	Patient Specimen 1	Patient Specimen 9	Patient Specimen 9
B	Calibrator B	Calibrator B	Patient Specimen 2	Patient Specimen 2	Etc.	
C	Calibrator C	Calibrator C	Patient Specimen 3	Patient Specimen 3		
D	Calibrator D	Calibrator D	Patient Specimen 4	Patient Specimen 4		
E	Calibrator E	Calibrator E	Patient Specimen 5	Patient Specimen 5		
F	Calibrator F	Calibrator F	Patient Specimen 6	Patient Specimen 6		
G	Control I	Control I	Patient Specimen 7	Patient Specimen 7		
H	Control II	Control II	Patient Specimen 8	Patient Specimen 8		

2. **Procedure**

a. Dilute each Calibrator, Control, and patient specimen 1:11 in Sample Diluent (i.e.: 25µL sample + 250µL Sample Diluent).

b. To individual wells, add 100µL of each diluted Calibrator, Control, and patient specimen in duplicate according to the ELISA plate setup map shown above. (Note: Ensure the samples are properly mixed, and use a different pipette tip for each sample)

c. Incubate the plate uncovered at 37 ± 1°C for 60 ± 5 minutes. **NOTE: Do NOT seal the plate during incubation.**

d. Wash the microwell strips five times.

i. **Manual Wash Procedure**:

1. Vigorously shake out the liquid from the wells.
2. Fill each microwell with 350µL of Wash Buffer. Make sure no air bubbles are trapped in the wells.
3. Repeat steps 1 and 2, for a total of five washes.
4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.

ii. **Automated Wash Procedure:**

If using an automated microwell wash system, set the dispensing volume to 350µL/well. Set the wash cycle for five washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

e. Add 100µL of the Conjugate to each well at the same rate and order as the specimens.

f. Incubate the plate uncovered at 37 ± 1°C for 60 ± 5 minutes. **NOTE: Do NOT seal the plate during incubation.**

g. Wash the microwell strips according to step d within this procedure.

h. Add 100µL of TMB to each well at the same rate and order as the specimens.

i. Incubate the plate at room temperature (20 - 25°C) for 10 minutes ± 1 minute.

j. Stop the reaction by adding 100µL of Stop Solution to each well at the same rate and order as the TMB. Positive samples will turn from blue to yellow.

k. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

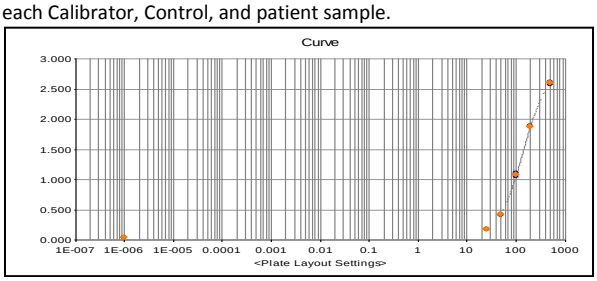
1. Reconstitute Calibrators and Controls .
2. Dilute Calibrators, Controls and samples 1:11 in Sample Diluent
3. Add diluted Calibrators, Controls, and samples to duplicate microwells - 100µL/well.
4. → *Incubate 60 ± 5 minutes at 37° ± 1°C.*
5. Wash five times.
6. Add Conjugate - 100µL/well.
7. → *Incubate 60 ± 5 minutes at 37° ± 1°C.*
8. Wash five times.
9. Add TMB - 100µL/well.
10. → *Incubate 10 ± 1 minute at 20 - 25°C.*
11. Add Stop Solution - 100µL/well.
12. Read immediately.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrators, Controls, and samples must be run in duplicate.
2. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
3. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.
4. The Low and Moderate Positive Controls are intended to monitor for substantial reagent failure. The Controls should yield ng/mL values within the lot-specific ranges listed on the component label provided with each kit.
5. If either, or both, of the Controls fall outside the ranges listed on the lot-specific label, the assay results are invalid, and testing should be repeated.

INTERPRETATION OF RESULTS

1. Enter the lot-specific ng/mL value for each Calibrator into the appropriate section of the desired 4-parameter logistic curve fitting software. The lot-specific Calibrator values are listed on the component label provided with each kit. NOTE: Be sure to program the software to calculate the mean ng/mL value using the O.D. values derived from duplicate analysis of each Calibrator, Control, and patient sample.



2. Specimens with results exceeding the highest Calibrator will be reported as "greater than X ng/mL" by the 4-parameter curve fitting software. If desired, samples that yield a "greater than Xng/mL" result at the recommended 1:11 dilution may be repeat tested after serial dilution in Sample Diluent. Once the diluted sample reaches the quantitative range of the assay, and receives a valid ng/mL value, the dilution factor can be used to back-calculate the actual value.
Example: *4p software assigns a ">525 ng/mL" result to a patient sample. To begin retesting, the plasma sample is diluted 1:11 in Sample Diluent, per the standard assay procedure. Then the 1:11-diluted sample is serially diluted three times (1:2, 1:4, and 1:8) in Sample Diluent. The results after serial dilution and retesting are as follows: 1:2 = ">525 ng/mL", 1:4 = 350 ng/mL, 1:8 = 175ng/mL.* **Calculations:** *Measured value x Dilution factor = Actual ng/mL, so 350 X 4 = 1400 ng/mL* **or** *175 x 8 = 1400 ng/mL.*
3. Test results should be interpreted in conjunction with the patient's clinical evaluation and, potentially the results of other diagnostic procedures.

LIMITATIONS OF THE ASSAY

1. Only **EDTA-plasma** should be used with the assay. Lithium Heparin plasma has been demonstrated to be incompatible with the assay. Serum, and plasma derived from blood collection tubes containing other anticoagulants, have not been fully tested/validated, and thus should be avoided.
2. Whole blood is not an appropriate sample type for this assay.

PERFORMANCE CHARACTERISTICS

1. **Limits of Blank, Detection, and Quantitation**
   a. **Limit of Blank (LOB) for Calibrator Diluent = 7.03 ng/mL**
   Two technicians each analyzed 60 replicates of calibrator diluent, totaling 120 replicates.
      i. Average ng/mL from 2 independent runs (n = 120 data points) = 3.99 ng/mL
      ii. Standard deviation from 2 independent runs (n = 120 data points) = 1.85
      iii. LOB = Avg + 1.645(StdDev) = 3.99 + 1.645(1.85) = 7.03 ng/mL
   b. **Limit of Blank (LOB) for EDTA-plasma = 7.15ng/mL**
   Five different units of EDTA-plasma (deficient for sPLA2-IIA) were assayed by two technicians on 5 different days. On each day, 12 assay replicates were performed for each plasma unit, totaling 300 replicates.
      i. Average ng/mL from 5 independent runs (n = 300 data points) = 4.55 ng/mL
      ii. Standard deviation from 5 independent runs (n = 300 data points) = 1.58
      iii. LOB = Avg + 1.645(StdDev) = 4.55 + 1.645(1.58) = 7.1 ng/mL
   c. **Limit of Detection (LOD) = 10 ng/mL, Limit of Quantitation (LOQ) = 22 ng/mL**
   The LOD for the assay was determined using EDTA-plasma containing both recombinant and endogenous sPLA2-IIA at various levels, and was considered valid if ≥ 95 % of the replicates tested at a given level yield values greater than the established LOB of 7.15 ng/mL. For each level of sPLA2-IIA, a minimum of 50 replicates were assayed by two technicians. The LOQ was also determined, and was selected based upon defined precision criteria (i.e. CV values of 15% or less) for samples harboring a given level of recombinant and/or endogenous sPLA2-IIA.

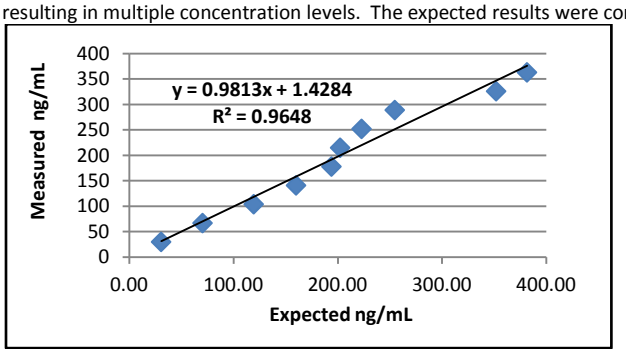
Recombinant sPLA2-IIA Spiked into Normal Plasma

sPLA2-IIA spike level	% of Replicates Higher than LOB	Valid LOD	CV Less than 15 %	Valid LOQ
10 ng/mL	100	Yes	No	No
15 ng/mL	100	Yes	Yes	Yes
20 ng/mL	100	Yes	Yes	Yes
25 ng/mL	100	Yes	Yes	Yes

Endogenous sPLA2-IIA from Patient Samples

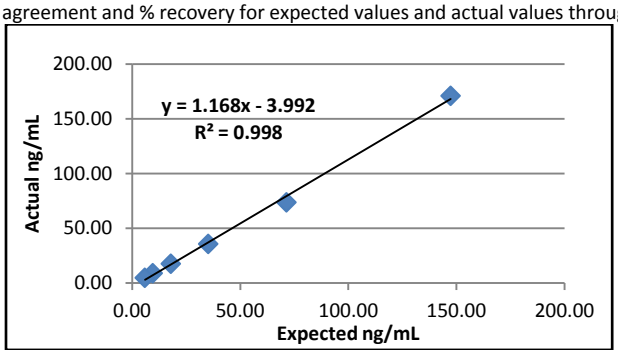
Approximate sPLA2-IIA level	% of Replicates Higher than LOB	Valid LOD	CV Less than 15 %	Valid LOQ
10 – 15 ng/mL	100	Yes	No	No
15 – 20 ng/mL	100	Yes	No	No
20 – 25 ng/mL	100	Yes	Yes	Yes

2. **Linearity and Assay Range**
   a. **Plasma Pools -** A plasma sample spiked with high levels of sPLA2-IIA was diluted with a plasma pool containing low levels of endogenous sPLA2-IIA, resulting in multiple concentration levels. The expected results were compared to the actual results, and the outcomes are shown below.



Sample	Expected Value (ng/mL)	Measured Value (ng/mL)
Pool 1	30	30.64
Pool 2	67	70.37
Pool 3	104	119.42
Pool 4	141	160.05
Pool 5	178	194.01
Pool 6	215	202.33
Pool 7	252	222.88
Pool 8	289	254.73
Pool 9	326	351.93
Pool 10	363	381.54
Pool 11	400	* ND

   b. **Dilutional Recovery -** A plasma sample containing high levels of endogenous sPLA2-IIA was serially diluted in the sample diluent. Diluted samples were assayed in duplicate, and resultant data were analyzed for agreement and % recovery for expected values and actual values throughout the dilution series.



Dilution Factor	Expected Value ng/mL	Measured value ng/mL
Neat	NA	>525.000
1:2	NA	341.80
1:4	170.90	147.35
1:8	73.68	71.43
1:16	35.71	35.24
1:32	17.62	17.95
1:64	8.97	9.58
1:128	4.79	5.91

   c. **Assay Range -** Together, the Plasma Pool, Dilutional Recovery, LOD, and LOQ studies support a quantitative range of 22 – 360 ng/mL and a qualitative range of 10 – 500 ng/mL.

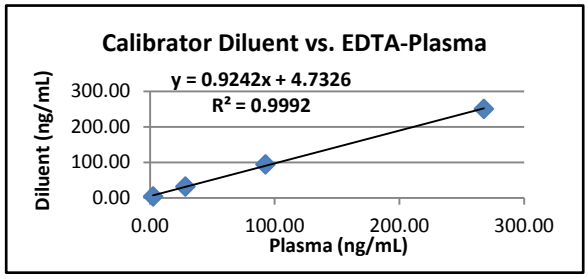
3. **Precision**

Plasma samples containing endogenous sPLA2-IIA at various levels were assayed twice a day for twenty days.

sPLA2-IIA Plasma Levels	Mean	Within-Run		Within -Day		Between-Run		Total	
	(ng/mL)	StD	%CV	StD	%CV	StD	%CV	StD	%CV
Negative	5.3	0.2	6.0	1.0	34.7	0.3	6.7	2.3	43.9
High Negative	20	0.5	2.5	1.1	8.7	0.5	2.2	1.8	9.4
Borderline Positive	38	1.1	3.0	1.5	5.8	0.7	1.8	2.8	7.4
Low Positive	71	1.8	2.6	3.1	6.7	1.0	1.4	4.9	7.0
Moderate Positive	138	4.9	3.5	3.6	5.5	2.7	1.9	8.8	6.3
High Positive	184	7.6	4.2	6.4	6.6	5.9	3.2	15.0	8.1

4. **Calibrator Diluent vs. EDTA-plasma**

Recombinant sPLA2-IIA was spiked into Calibrator Diluent, and 3 different EDTA-plasma units, at target concentrations of approximately 300, 100, and 30 ng/mL. Non-spiked and spiked plasma/diluent were assayed in triplicate, and the agreement between ng/mL values were evaluated for respective matrices. As shown below, the ng/mL values obtained from Calibrator Diluent and EDTA-plasma are in good agreement (R^2 = 0.9992).



sPLA2-IIA Level	Calibrator Diluent Mean ng/mL	EDTA-Plasma Mean ng/mL (n=3 donors)
0	2	4
Low	28	32
Medium	93	94
High	268	251

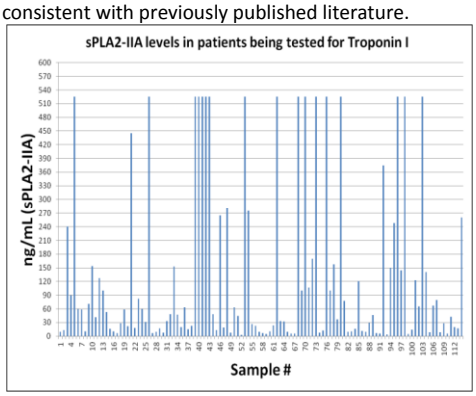
5. **Hook Effect**

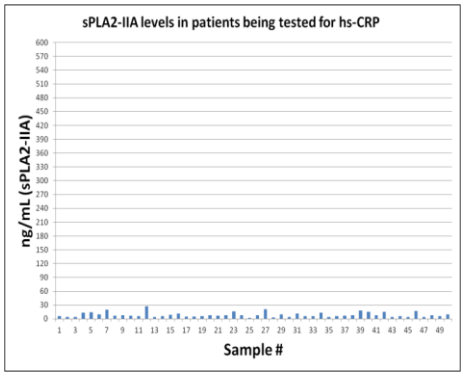
Recombinant sPLA2-IIA was spiked into Calibrator Diluent to a concentration of 5000 ng/mL, then serially diluted to near the LOD of 10 ng/mL. Diluted samples were assayed in triplicate. As shown below, no false negatives were observed, demonstrating that the assay is not susceptable to a 'hook effect' up to at least 5000 ng/mL of sPLA2-IIA.

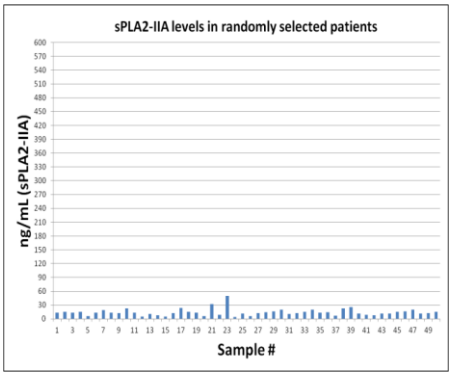
Targeted Spike (ng/mL)	Replicate A (ng/mL)	Replicate B (ng/mL)	Replicate C (ng/mL)	Mean Measured (ng/mL)	Expected (ng/mL)	% Recovery
5,000	**>525.000**	**>525.000**	**>525.000**	NA	NA	NA
2,500	**>525.000**	**>525.000**	**>525.000**	NA	NA	NA
1,250	**>525.000**	**>525.000**	**>525.000**	NA	NA	NA
625	**>525.000**	**>525.000**	**>525.000**	NA	NA	NA
312.5	366	327	311	334.68	NA	NA
156.25	183	171	158	170.79	167.34	102.06
78.125	86	90	81	85.60	85.40	100.24
39.0625	44	45	44	44.29	42.80	103.49
19.53	20	21	20	20.17	22.15	91.09
9.76	9	10	9	9.42	10.09	93.44
0	2	2	2	2.18	NA	NA

6. **Clinical Sample Testing**

sPLA2-IIA levels were measured using plasma samples from patients being tested for Troponin I (n=114), hs-CRP (n =50), or a randomly selected population (n=50). As shown below, sPLA2-IIA plasma levels are elevated in patients being tested for Troponin I (i.e. Acute Coronary Syndrome category); a finding that is consistent with previously published literature.

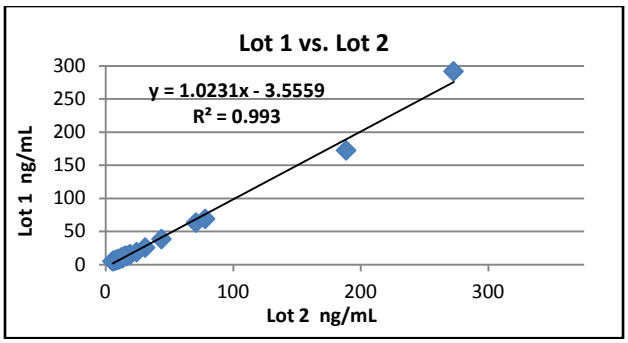






7. **Lot-to-Lot Comparison**

Plasma samples (n=32) containing various levels of endogenous sPLA2-IIA were assayed using two different lots of AccuCardia tests. After plotting the respective data, linear regression analysis demonstrates that data are in good agreement (R^2 = 0.993).



8. **Interference/Cross Reactivity**

The analysis was performed on normal human plasma spiked with various relevant endogenous and exogenous substances. For each substance/ concentration, testing was performed using 0, 50, and 150 ng/mL of sPLA2-IIA. The respective test concentrations of the various substances are listed below:

Substance	High	Low
Albumin (Human)	50 mg/mL	35 mg/mL
Bilirubin	0.15 mg/mL	0.01 mg/mL
Cholesterol	2.5 mg/mL	1.5 mg/mL
*Hemolysate	5 mg/mL	0.5 mg/mL
Intralipids	7.5 mg/mL	3 mg/mL
Triglycerides	5 mg/mL	1.5 mg/mL
Acetylsalicylic acid	0.652 mg/mL	0.252 mg/mL
Acetaminophen	0.2 mg/mL	0.0197 mg/mL
L-ascorbic acid	0.0662 mg/mL	0.0132 mg/mL
Atorvastatin	0.22 mg/mL	0.022 mg/mL
Tolbutamide	0.64 mg/mL	0.064 mg/mL

*** Hemolysate was prepared and tested according to Appendix G of CLSI document EP7**

Outcome: None of the above-listed substances exhibited significant positive or negative interference on assay performance (i.e. all yielded between 80 - 120% signal relative to the respective matrix control values) at both the high and low levels of spiked sPLA2-IIA.

Other Interference/Cross Reactivity Testing: Extensive testing was also performed with HAMA/Rf-spiked plasma samples, and yielded no significant increased or reduced signals.

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