

# QUANTA Lite® ACA IgA III

708635

For *In Vitro* Diagnostic Use

CLIA Complexity: High

## Intended Use

QUANTA Lite™ ACA IgA III is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgA cardiolipin antibodies in human serum. The presence of cardiolipin antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in assessing the risk of thrombosis in individuals with Systemic Lupus Erythematosus (SLE) or lupus-like disorders.

## Summary and Explanation of the test

Anticardiolipin antibodies (ACA) have been strongly associated with venous and arterial thrombosis.<sup>1</sup> These findings were first observed during studies on systemic lupus erythematosus, (SLE) a disease whose many symptoms include thrombosis. Of the many autoantibodies found in SLE, two were found to be directed against phospholipids such as cardiolipin.<sup>2</sup>

While most reports of anticardiolipin antibodies center on IgG and/or IgM class antibodies<sup>3,4</sup> some recent studies indicate that elevated levels of IgA class anticardiolipin antibodies are also found frequently in patients with SLE and related disorders.<sup>5,6,7,8</sup> In these studies IgA values were higher in patients with vascular complications and thrombocytopenia.<sup>7</sup>

## Principles of the Procedure

Purified cardiolipin antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any cardiolipin antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgA conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgA to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgA, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. After stopping the enzymatic production of colored product, the presence or absence of cardiolipin antibody is determined by comparing the sample optical density with that of a five point calibration curve. Results are reported out semi-quantitatively in standard IgA anti-cardiolipin units (APL).

## Reagents

1. Polystyrene microwell ELISA plate coated with a purified cardiolipin antigen and bovine  $\beta_2$  GPI (12-1 x 8 wells), with holder in foil package containing desiccants
2. ACA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to cardiolipin, prediluted, 1.2mL
3. ACA IgA III Control, 1 vial of buffer containing preservative and human serum antibodies to cardiolipin, prediluted, 1.2mL
4. ACA IgA III Calibrator A, 1 vial of buffer containing preservative and human serum antibodies to cardiolipin, prediluted, 1.2mL
5. ACA IgA III Calibrator B, 1 vial of buffer containing preservative and human serum antibodies to cardiolipin, prediluted, 1.2mL
6. ACA IgA III Calibrator C, 1 vial of buffer containing preservative and human serum antibodies to cardiolipin, prediluted, 1.2mL
7. ACA IgA III Calibrator D, 1 vial of buffer containing preservative and human serum antibodies to cardiolipin, prediluted, 1.2mL
8. ACA IgA III Calibrator E, 1 vial of buffer containing preservative and human serum antibodies to cardiolipin, prediluted, 1.2mL
9. ACA III Sample Diluent, 1 vial – colored pink containing PBS-buffered saline, protein stabilizers and preservative, 50mL
10. ACA III PBS Concentrate, 1 vial of 20x concentrate - colored red containing PBS-buffered saline, 50mL. Refer to the Methods Section for dilution instructions.
11. HRP IgA Conjugate, (goat), anti-human IgA, 1 vial – straw colored containing buffer, protein stabilizers and preservative, 10mL
12. TMB Chromogen, 1 vial containing stabilizers, 10mL
13. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

## Warnings

1. **WARNING:** This product contains a chemical (0.02% chloramphenicol) in the conjugate known to the State of California to cause cancer.
2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the ACA IgA Control III, ACA IgA III Calibrator A through E and ACA Negative Control should be handled in the same manner as potentially infectious material.<sup>10</sup>

3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

## Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. Substitution of components other than those provided in this system may lead to inconsistent results.
3. Incomplete or inefficient washing and insufficient liquid removal from the ELISA well strips will cause poor precision and/or high background.
4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
5. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and liquid removal from the wells of the ELISA strips, the photometer used to measure the results, and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
6. Strict adherence to the protocol is recommended.
7. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
8. Unacceptably low absorbencies may be observed following **two** or more uses from a single bottle of HRP conjugate over a period of time. It is important to follow all recommended HRP conjugate handling procedures to prevent this occurrence.
9. Chemical contamination of the HRP conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol or detergent will cause degradation of the HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.

## Storage Conditions

1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.
3. Diluted wash buffer is stable for 1 week at 2-8°C.

## Specimen Collection

This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Following collection, the serum should be separated from the clot. CLSI (NCCLS) Document H18-A3 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

## Procedure

### Materials provided

- 1 Cardiolipin ELISA microwell plate (12-1 x 8 wells), with holder
- 1 1.2mL prediluted ACA Negative Control
- 1 1.2mL prediluted ACA IgA III Control
- 1 1.2mL prediluted ACA IgA III Calibrator A
- 1 1.2mL prediluted ACA IgA III Calibrator B
- 1 1.2mL prediluted ACA IgA III Calibrator C
- 1 1.2mL prediluted ACA IgA III Calibrator D
- 1 1.2mL prediluted ACA IgA III Calibrator E
- 1 50mL ACA III Sample Diluent
- 1 50mL ACA III PBS Concentrate, 20x concentrate

- 1 10mL HRP IgA Conjugate, (goat), anti-human IgA
- 1 10mL TMB Chromogen
- 1 10mL HRP Stop Solution, 0.344M Sulfuric Acid

## Additional Materials Required But Not Provided

Micropipets to deliver 5, 100, 200-300 and 500µL  
 Disposable micropipet tips  
 Test tubes for patient sample dilutions, 4mL volume  
 Distilled or deionized water  
 1L container for diluted ACA III PBS Concentrate  
 Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

## Method

### Before you start

1. **IMPORTANT: Bring all reagents and samples to room temperature (20-26°C) and mix well.**
2. Dilute the ACA III PBS Concentrate 1:20 by adding the contents of the ACA III PBS Concentrate bottle to 950mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 4.0mL of the concentrate to 76mL of distilled or deionized water for every 16 wells that will be used.
3. Prepare a 1:101 dilution of each patient sample by adding 5µL of sample to 500µL of ACA III Sample Diluent. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the ACA IgA III Calibrators A through E, ACA IgA III Control and ACA Negative Control 1:101.
4. Determination of the presence or absence of cardioliipin antibodies requires two wells for each of the calibrators and controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.
5. Preparation of standard curve: For points A through E of the 5 point standard curve, use **PREDILUTED** ACA IgA III Calibrators A through E directly from the vial. The five point standard curve has the following values:

Point		Phospholipid Units (APL)
A	Prediluted ACA IgA III Calibrator A	150.0
B	Prediluted ACA IgA III Calibrator B	75.0
C	Prediluted ACA IgA III Calibrator C	37.5
D	Prediluted ACA IgA III Calibrator D	18.8 or 18.75
E	Prediluted ACA IgA III Calibrator E	9.4 or 9.375

### Assay procedure

1. **ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE (20-26°C) PRIOR TO BEGINNING THE ASSAY.** Place the required number of microwells/strips in the holder. **Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor.**
2. Add 100µL each of the five calibrators, the diluted patient samples, the ACA Negative Control and the ACA IgA III Control to the wells.  
 NOTE: Both the ACA IgA III Control and ACA Negative Control are pre-diluted and ready to use. The value and acceptable range of the ACA IgA III Control is printed on the vial label. If the Control fails to fall within the acceptable range printed on the label, repeat the run. If upon repeat testing the Control falls outside the stated range, call INOVA Technical Service for assistance. It is recommended to run all samples in duplicate.
3. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.
4. Wash step: Thoroughly aspirate the contents of each well. Add 200-300µL of the **diluted** ACA III PBS buffer to all wells then aspirate. Repeat this sequence twice more for a total of three washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well after each washing step. Maintain the same sequence for the aspiration as was used for the sample addition.
5. Add 100µL of the HRP IgA Conjugate to each well. Conjugate should be removed from the bottles using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. **TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.** Incubate the wells for 30 minutes as in step 3.
6. Wash step: Repeat step 4.
7. Add 100µL of TMB Chromogen to each well and incubate **in the dark** for 30 minutes at room temperature.
8. Add 100µL of HRP Stop Solution to each well. Maintain the same sequence and timing of HRP Stop Solution addition as was used for the TMB Chromogen. Gently tap the plate with a finger to thoroughly mix the wells.
9. Read the absorbance (OD) of each well at 450nm within one hour of stopping the reaction. If bichromatic measurements are desired, 620nm can be used as a reference wavelength.

## Quality Control

1. The ACA IgA III Control, the ACA IgA III Calibrators and the ACA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the ACA IgA III Control, the ACA IgA III Calibrators and the ACA Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at  $\leq -20^{\circ}\text{C}$ .
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated.
  - a. The absorbance of the prediluted ACA IgA III Calibrator A must be greater than the absorbance of the prediluted ACA IgA III Control, which must be greater than the absorbance of the prediluted ACA Negative Control.
  - b. The prediluted ACA IgA III Calibrator A must have an absorbance greater than 1.0 while the prediluted ACA Negative Control absorbance cannot be over 0.2.
  - c. The ACA IgA III Control absorbance must be more than twice the ACA Negative Control or over 0.25.
  - d. The ACA IgA III Control concentration must be within the range stated on its label.
  - e. The user should refer to CLSI (NCCLS) Document C24-A3 for additional guidance on appropriate QC practices.

## Calculation of Results

1. Determine the mean value for all duplicate readings.
2. Plot the mean absorbance of the Calibrator curve for the ACA IgA III assay against the log of their concentrations. Use a line of best fit curve plot. Alternatively a log/log plot may be used. The APL units assigned to the calibrators are found on the calibrator vial.
3. Determine the unknown ACA APL concentration from the "X" axis by reading the corresponding absorbance on the "Y" axis.

## Interpretation of Results

The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

1. A positive result indicates the presence of cardiolipin IgA antibodies and can be used in conjunction with other serological tests and clinical findings to aid in assessing the risk of thrombosis in individuals with Systemic Lupus Erythematosus (SLE) or lupus-like disorders.
2. Results should be expressed in APL units. Based on an evaluation of 486 normal samples and 139 positive cardiolipin IgG, IgM and/or IgA samples a suggested cutoff of 12 APL has been made. It is suggested that each laboratory establish their own normal range. *Even when using a calibration curve there is still variation in the results at low levels and false positive results are frequent.*<sup>9</sup> For this reason we suggest that values ranging from 12 to 20 APL, inclusive, be considered indeterminate, with positive results assigned to patient samples  $>20$  APL. Harris and Pierangeli suggest an alternate semi-quantitative method of expressing results.<sup>9</sup> We suggest that values of 20-80 APL be considered as low to medium positive and above 80 APL as high positive results.
3. A negative result indicates no cardiolipin IgA antibody or levels below the detection limit of the assay.
4. It is suggested that the results reported by the laboratory should include the statement: "The following results were obtained with the INOVA QUANTA Lite® ACA IgA III ELISA. Cardiolipin IgA values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgA levels cannot be correlated to an endpoint titer."

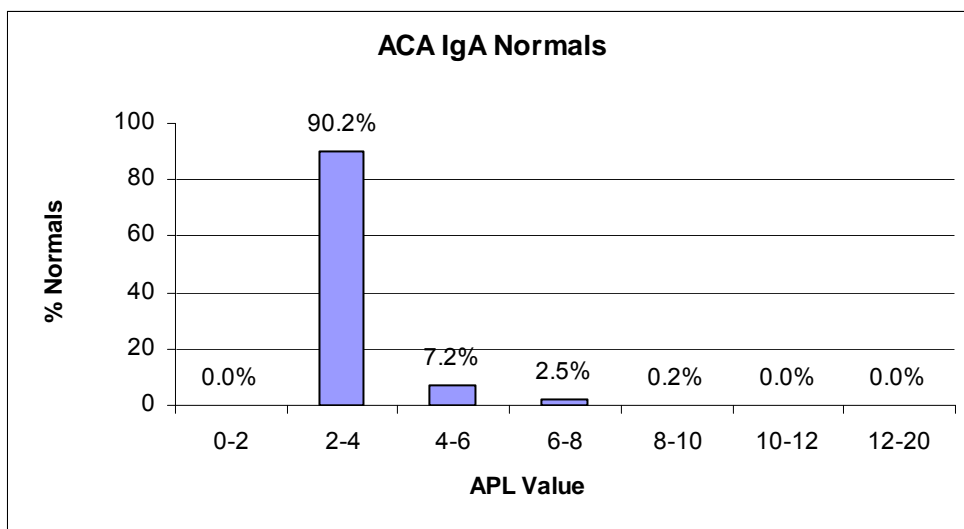
## Limitations of the Procedure

1. The clinical significance of ACA in diseases other than SLE is currently under investigation.
2. When negative ACA titers are found in the presence of clinical indications, a lupus anticoagulant or other additional testing such as anti- $\beta_2$  GPI may be indicated.
3. Diagnosis cannot be made on the basis of ACA results alone. These results must be interpreted in conjunction with physical findings.
4. Treatment must not be initiated on the basis of a positive ACA titer alone. Supportive clinical indications must also be present.
5. A high percentage of confirmed active or seropositive syphilis patients will have elevated ACA levels. Confirmatory procedures should be performed to rule out syphilis.
6. ACA can appear transiently during many infections. Patients positive for ACA should be retested following an appropriate wait.
7. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
8. The assay performance characteristics have not been established for matrices other than serum.
9. Results of this assay should be used in conjunction with clinical findings and other serological tests.
10. If the ACA III PBS Concentrate is not warmed up and mixed well prior to use, inconsistent results may be seen.

## Expected Values

### Normal Range

486 random normal donor samples were assayed for ACA IgA. None were found within the indeterminate range of 12-20 GPL. All 486 normals (100%) were less than or equal to 10 APL.



## Relative Sensitivity and Specificity

### Correlation with INOVA Diagnostics' Second Generation ACA IgA

A total of 625 samples were tested on both the second generation ACA IgA and the third generation ACA IgA. These samples include the 486 normals mentioned in the Normal Range study as well as 139 samples from patients known to be cardiolipin IgG, IgM and/or IgA positive. The results are summarized below.

		QUANTA Lite® ACA IgA III (third generation)					
		-	I	+			
ACA IgA ELISA second generation	-	600	3	0	Relative Sensitivity	100%	
	I*	3	8	1	Relative Specificity	99.5%	
	+	0	0	10	Relative Efficiency	98.9%	

\*Indeterminate

## Precision and Reproducibility

The between run precision and reproducibility of the assay was measured by running two replicates each of a strong positive and a negative in four separate assays on four consecutive days. The within run precision and reproducibility of the assay was measured by running sixteen replicates each of a strong positive and a weak positive in a single assay. The mean value, standard deviation and coefficient of variation for each sample are summarized below.

	Strong Positive			Negative		
	Mean APL	SD	%CV	Mean APL	SD	%CV
Overall	86.8	7.1	8.0%	3.6	0.4	9.9%
Within Run	79.9	3.3	4.2%	4.0	0.8	18.6%
Between Run	93.8	11.0	11.8%	3.1	0.1	1.3%

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