

QUANTA Lite™ β_2 GPI IgA ELISA

708675

For *In Vitro* Diagnostic Use

CLIA Complexity: High

Intended Use

QUANTA Lite™ β_2 GPI IgA is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of β_2 GPI IgA antibodies in human serum. The presence of β_2 GPI IgA antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of certain autoimmune disease thrombotic disorders, such as those secondary to systemic lupus erythematosus (SLE) or other lupus-like thrombotic diseases.

Summary and Explanation of the Test

Anticardiolipin antibodies (ACA) have been strongly associated with venous and arterial thrombosis.¹⁻⁵ These findings were first observed during studies on systemic lupus erythematosus, a disease whose many symptoms include thrombosis.^{6,7} Recent studies⁸⁻¹² have shown that a 50kD serum cofactor is required for cardiolipin antibodies, induced in SLE patients, to bind to cardiolipin which has been coated onto plastic plates. The cofactor has been identified as β_2 -glycoprotein I also termed apolipoprotein H.^{8,12,13,14} While β_2 GPI has been known as an *in vitro* inhibitor of the intrinsic blood coagulation pathway,¹⁵ ADP-dependent aggregation,¹⁶ and prothrombinase activity of activated platelets,¹⁷ its physiological function is still unclear.

It has become apparent that anticardiolipin antibody from patients with anti-phospholipid syndrome (APS) recognize a modified β_2 GPI structure and not cardiolipin, native β_2 GPI or an epitope structurally defined by both cardiolipin and β_2 GPI.⁸⁻¹²

Galli et al.⁹ and Viard, et al.¹⁸ individually reported that anti-cardiolipin antibody derived from autoimmune patients (i.e., SLE and/or APS) were directed to the β_2 GPI molecule coated on polystyrene plates. Koike¹¹ and Matsuura¹² showed conclusively that β_2 GPI is indeed the antigen to which many anticardiolipin antibody patients are actually binding and furthermore showed that the phospholipid merely serves to link the β_2 GPI to the solid phase.

There is a well known potential for traditional anticardiolipin antibody tests to produce false positive results due to cross-reactivity of phospholipids with certain infectious disease samples, most notably syphilis, and also with certain other autoantibodies such as double stranded DNA.^{2,3} By eliminating phospholipid from the solid phase and using only β_2 GPI, the test becomes even more specific for detecting potential coagulation problems. This enhanced specificity has been shown conclusively by several groups.^{11,12,16,17,19,20,21,22} The β_2 GPI autoantibody test is a useful and more specific assay to be used in conjunction with the traditionally used anticardiolipin antibody and lupus anticoagulant tests for aiding in diagnosis of thrombosis in at-risk patients.

IgG class β_2 GPI antibodies were found in 17 of 47 SLE patients (36%).¹⁸ Nine of these 47 SLE patients had thrombosis and 8 of these (89%) had β_2 GPI antibody. Hisham, et al.²⁰ found IgG class β_2 GPI antibodies in all 5 patients (100%) with at least 2 documented clinical manifestations of APS. All 5 of these patients were classified as having high levels of β_2 GPI antibodies. Eight of 14 patients (57%) classified as having low levels of β_2 GPI antibodies had at least 1 thrombotic event.

Tsutsumi, et al.²² found IgG and IgM antibodies to β_2 GPI in 10.1% and 5.8% respectively of 308 randomly selected Japanese SLE patients. IgG β_2 GPI antibodies were more common in patients with a history of thrombosis. This group also reported that of 15 patients with recurrent fetal loss 20% were IgG β_2 GPI positive. They also showed that patients with a history of thrombosis were more likely to be positive for IgM β_2 GPI and that levels of IgM β_2 GPI were higher in the group with thrombosis than in those without. Of 15 patients without a history of fetal loss, none were IgM β_2 GPI positive.

Cabiedes, et al.²³ studied 94 patients with SLE, 39 of whom had clinical manifestations of APS. The association of clinical manifestations of APS was more strongly associated with β_2 GPI antibodies than with positivity in conventional ACA test. IgG β_2 GPI antibodies were present in sera from 16 of 18 patients (89%) with APS. Of 22 patients positive for ACA yet without clinical manifestations of APS, none were β_2 GPI positive.

Cerrato, et al.²⁴ found IgG anti- β_2 GPI in 87.5% of a group of 32 patients with thrombotic histories (APS). IgM anti- β_2 GPI was found in 71.9% of this same group of 32 patients.

Sebastiani, et al.²⁵ detected IgG anti- β_2 GPI in 110 patients (20.3%) and IgM in 109 patients (20.1%) out of a total of 542 patients with SLE. Presence of anti- β_2 GPI was strongly associated with ACA ($p < 10^{-5}$). They further showed that IgG and IgM β_2 GPI antibodies were associated with both arterial and venous thrombosis.

Principles of the Procedure

Purified β_2 GPI 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 β_2 GPI IgA 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. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with that of a five point calibration curve. Results are reported out semi-quantitatively in standard IgA anti- β_2 GPI units (SAU).

Reagents

1. Polystyrene microwell ELISA plate coated with a purified β_2 GPI antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to β_2 GPI, prediluted, 1.2mL
3. β_2 GPI IgA ELISA Control, 1 vial of buffer containing preservative and human serum antibodies to β_2 GPI, prediluted, 1.2mL
4. β_2 GPI IgA ELISA Calibrator A, 1 vial of buffer containing preservative and human serum antibodies to β_2 GPI, prediluted, 1.2mL
5. β_2 GPI IgA ELISA Calibrator B, 1 vial of buffer containing preservative and human serum antibodies to β_2 GPI, prediluted, 1.2mL
6. β_2 GPI IgA ELISA Calibrator C, 1 vial of buffer containing preservative and human serum antibodies to β_2 GPI, prediluted, 1.2mL
7. β_2 GPI IgA ELISA Calibrator D, 1 vial of buffer containing preservative and human serum antibodies to β_2 GPI, prediluted, 1.2mL
8. β_2 GPI IgA ELISA Calibrator E, 1 vial of buffer containing preservative and human serum antibodies to β_2 GPI, prediluted, 1.2mL
9. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
10. HRP Wash Concentrate, 1 vial of 40x concentrate - colored red containing Tris-buffered saline and Tween 20, 25mL. Refer to the Methods Section for dilution instructions.
11. HRP IgA Conjugate, (goat), anti-human, 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

Warnings

1. **WARNING:** This product contains a chemical (0.02% chloramphenicol) in the sample diluent, controls, and 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 β_2 GPI IgA ELISA Control, β_2 GPI IgA ELISA Calibrators and ELISA Negative Control should be handled in the same manner as potentially infectious material.²⁶
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. NCCLS Document H18-A2 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 β_2 GPI ELISA microwell plate (12-1 x 8 wells), with holder
- 1 1.2mL prediluted ELISA Negative Control
- 1 1.2mL prediluted β_2 GPI IgA ELISA Control
- 1 1.2mL prediluted β_2 GPI IgA ELISA Calibrator A
- 1 1.2mL prediluted β_2 GPI IgA ELISA Calibrator B
- 1 1.2mL prediluted β_2 GPI IgA ELISA Calibrator C
- 1 1.2mL prediluted β_2 GPI IgA ELISA Calibrator D
- 1 1.2mL prediluted β_2 GPI IgA ELISA Calibrator E
- 1 50mL HRP Sample Diluent
- 1 25mL HRP Wash Concentrate, 40x 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 HRP Wash Concentrate
 Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

Method

Before you start

1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.
3. Prepare a 1:101 dilution of each patient sample by adding 5µL of sample to 500µL of HRP Sample Diluent. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the β₂ GPI IgA ELISA Calibrators, the β₂ GPI IgA ELISA Control or the ELISA Negative Control.
4. Determination of the presence or absence of β₂ 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** β₂ GPI IgA ELISA Calibrator A through E directly from the vial. The five point standard curve has the following values:

Point	Standard IgA β ₂ GPI Units (SAU)
A Prediluted β ₂ GPI IgA ELISA Calibrator A	150.0
B Prediluted β ₂ GPI IgA ELISA Calibrator B	75.0
C Prediluted β ₂ GPI IgA ELISA Calibrator C	37.5
D Prediluted β ₂ GPI IgA ELISA Calibrator D	18.8 or 18.75
E Prediluted β ₂ GPI IgA ELISA 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 ELISA Negative Control and the β₂ GPI IgA ELISA Control to the wells.
 NOTE: Both the β₂ GPI IgA ELISA Control and ELISA Negative Control are pre-diluted and ready to use. The value and acceptable range of the β₂ GPI IgA ELISA Control is printed on the vial label. If the Control fails to fall within the acceptable range printed on the label, repeat the run.
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 **diluted** HRP Wash 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 100 μ L of TMB Chromogen to each well and incubate **in the dark** for 30 minutes at room temperature.
8. Add 100 μ L 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 β_2 GPI IgA ELISA Calibrators, β_2 GPI IgA ELISA Control and ELISA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the β_2 GPI IgA ELISA Calibrators, β_2 GPI IgA ELISA Control, and ELISA Negative Controls 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 β_2 GPI IgA ELISA Calibrator A must be greater than the absorbance of the prediluted β_2 GPI IgA ELISA Control which must be greater than the absorbance of the prediluted ELISA Negative Control.
 - b. The prediluted β_2 GPI IgA ELISA Calibrator A must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
 - c. The β_2 GPI IgA ELISA Control absorbance must be more than twice the ELISA Negative Control or over 0.25.
 - d. The β_2 GPI IgA ELISA Control concentration must be within the range stated on its label.
 - e. The user should refer to NCCLS Document C24-A 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 IgA assay against the log of their concentrations. Use a line of best fit curve plot. Alternatively a log/log plot may be used. The SAU units assigned to the calibrators are found on the calibrator vial.
3. Determine the unknown β_2 GPI IgA concentration in Standard β_2 GPI IgA Units (SAU) from the "X" axis by reading the corresponding absorbance on the "Y" axis.
4. Negative values range from 0-20 units. Positive results are greater than 20 SAU.

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 β_2 GPI IgA antibodies and suggests the possibility of certain autoimmune disease thrombotic disorders, such as those secondary to systemic lupus erythematosus or other lupus-like thrombotic diseases.
2. A negative result indicates no β_2 GPI IgA antibody or levels below the detection limit of the assay cutoff.
3. It is suggested that the results reported by the laboratory should include the statement: "The following results were obtained with the INOVA QUANTA LiteTM β_2 GPI IgA ELISA. β_2 GPI 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 β_2 GPI antibodies in diseases other than SLE is currently under investigation.
2. When negative anti- β_2 GPI titers are found in the presence of clinical indications, a lupus anticoagulant, anticardiolipin or other additional testing is indicated.

3. Diagnosis cannot be made on the basis of anti- β_2 GPI results alone. These results must be interpreted in conjunction with physical findings.
4. Treatment must not be initiated on the basis of a positive anti- β_2 GPI titer alone. Supportive clinical indications must also be present.
5. It is to be expected that some samples can be anticardiolipin positive yet anti- β_2 GPI negative. The anti- β_2 GPI test is a more specific marker of thrombotic risk. The anticardiolipin test can produce false positive results due to cross-reactivity with dsDNA or certain infectious disease antibodies.
6. The assay performance characteristics have not been established for matrices other than serum.

Expected Values and Specific Performance Characteristics

A total of 215 normal samples were tested with the QUANTA Lite™ β_2 GPI IgA ELISA kit. This group was roughly divided between males and females ranging in age from 18 to 58. Seven of these samples (3.2%) were positive for IgA β_2 GPI antibodies. The mean value for this normal population was 2.52 SAU. The table below summarizes the internal and external clinical studies of the QUANTA Lite™ β_2 GPI IgA ELISA test.

Patient Group	Number	Number Positive	(%)
SLE + APS*	14	10	(71.4)
APS	48	26	(54.2)
SLE (No APS)	24	7	(29.2)
Infectious**	30	0	(0.0)
Normals	215	7	(3.2)

*APS = antiphospholipid syndrome

**The infectious group consisted mostly of samples with positive syphilis serology.

Based on the above table, clinical sensitivity of the IgA β_2 GPI antibody test is 58.1%. Specificity and clinical agreement are 94.8% and 87.9% respectively.

Relative Sensitivity and Specificity

Concordance with IgA Cardiolipin

The IgA β_2 GPI kit was compared side by side with an IgA anticardiolipin (ACA) test. Fourteen normal sera, 16 APS patients and 22 SLE sera from patients without obvious thrombotic histories were evaluated. This comparison study can be summarized in the following table.

		IgA β_2 GPI			
		+	-		
IgA	+	6	6**	Relative Sensitivity	50.0%
ACA	+			Relative Specificity	80.0%
	-	8*	32	Relative Efficiency	74.1%

*2 of these patients were from the APS group. The other 6 are from the SLE group.

**4 of these 6 patients was from the APS group and 2 are from the SLE group

Six samples were positive by both methods. All six of these samples were from the APS group. Thirty-two samples were negative by both methods. There were 8 samples β_2 GPI positive yet ACA negative. Two of these were from the APS group and the other 6 were from the SLE group. Three of the six β_2 GPI positive, ACA negative, SLE patients were only weakly positive for β_2 GPI (21, 24 and 29 SAU). There were 6 samples IgA ACA positive yet β_2 GPI negative. Two of these were from the SLE group and 4 were from the APS group.

When regressions are calculated for IgA ACA and β_2 GPI values for the 14 normal sera, the 16 APS patients and the 22 SLE patients an "r" value of 0.825 is produced with a slope of 1.31.

Precision and Reproducibility

Precision and Reproducibility data were calculated by running a negative, high and moderate positive sample six times on six consecutive days. The mean of the negative was 18.2, the moderate positive was 37.2 and the strong positive was 79.6. Within-run and between-run results are tabulated below.

	Negative		Moderate Positive		Strong Positive	
	SD	CV	SD	CV	SD	CV
Overall	0.82	4.4%	1.61	4.3%	4.06	5.1%
Within Run	1.22	6.6%	1.49	4.0%	4.07	5.2%
Between Run	1.22	6.6%	1.50	4.0%	3.40	4.2%

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