QUANTA Flash® aCL IgG Reagents

Inova Diagnostics
A Werfen Company

For *In Vitro* Diagnostic Use. CLIA Complexity: Moderate

REF **701230** **Rx Only**

Intended Use

Fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-cardiolipin (aCL) IgG antibodies in human citrated plasma and serum on the BIO-FLASH® instrument as an aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory and clinical findings.

Summary and Explanation of the Test

Anti-cardiolipin (aCL) antibodies belong to a heterogeneous family of antiphospholipid (aPL) antibodies, which are autoantibodies directed against anionic phospholipids or protein-phospholipid complexes. Persistently elevated levels of aPL antibodies are associated with an increased risk for vascular thrombosis and obstetrical complications. This association is known as antiphospholipid syndrome, classification proposed by Harris in 1987[1]. Assays for the determination of aCL IgG and IgM antibodies, anti-β2 glycoprotein-1 (anti- β2GP1) IgG and IgM antibodies and lupus anticoagulant antibodies are the aPL assays defined in the revised classification criteria determined by the International Committee for the diagnosis of APS in the meeting held in 2006 in Sydney, Australia[2,3]. Both the aCL and anti-β2GP1 assays contain human β2 glycoprotein-1 (β2GP1) on the solid phase.

β2 glycoprotein-1, also called apolipoprotein H, is a 44 kDA glycoprotein with 5 domains that is present in plasma. The fifth domain contains a cluster of positively charged amino acids which is responsible for the binding of anionic phospholipids. The mechanism by which aCL and anti-β2GP1 antiphospholipid antibodies recognize β2GP1 is unclear. Two main theories have been proposed; the first known as “dimerization theory” considers that one antibody must bind two β2GP1 molecules to obtain increased avidity[4], and the second “cryptic epitope theory” considers that the aCL and anti-β2GP1 antiphospholipid binding epitope is only exposed when β2GP1 binds to a negatively charged surface or negative molecules such cardiolipin[5,6]. This epitope is located in domain I[7].

Principles of the Procedure

The QUANTA Flash aCL IgG assay is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with cardiolipin and human purified β2GP1 which capture, if present, the aCL antiphospholipid antibodies from the sample. After incubation, magnetic separation, and a wash step, a tracer consisting of an isoluminol-labeled anti-human IgG antibody is added and may bind with the captured aCL IgG on the particles. After a second incubation, magnetic separation, and wash step, reagents that trigger the luminescent reaction are added, and the emitted light is measured as relative light units (RLUs) by the BIO-FLASH optical system. The RLUs are directly proportional to the aCL IgG concentration in the sample.

The QUANTA Flash aCL IgG assay utilizes a 4 Parameter Logistic Curve (4PLC) fit data reduction method to generate a Master Curve. The Master Curve is predefined and lot dependent and it is stored in the instrument through the cartridge barcode. With the measurement of calibrators, the predefined Master Curve is transformed to a new, instrument specific 4PLC Working Curve. The concentration values of the calibrators are included in the calibrator tube barcodes.

Reagents

The aCL IgG kit consists of:

1. QUANTA Flash aCL IgG Reagent Cartridge containing the following reagents for 100 determinations. The reagents are in phosphate or borate buffer and may contain bovine serum albumin or fetal bovine serum, bovine cardiolipin, human ß2GP1, mouse monoclonal IgG, stabilizers and preservative:
   a. 1 vial of magnetic particle suspension coated with bovine cardiolipin and human purified B2GP1.
   b. 1 vial of Assay Buffer.
   c. 1 vial of Tracer consisting of anti-human IgG antibody labeled with isoluminol.
   d. 1 empty vial.

2. aCL IgG Calibrator 1 vial containing: 1 x 1 mL barcoded tube of a solution with aCL IgG in saline solution containing fetal bovine serum, stabilizers and preservative.

3. aCL IgG Calibrator 2 vial containing: 1 x 1 mL barcoded tube of a solution with aCL IgG in saline solution containing fetal bovine serum, stabilizers and preservative.

The calibrators are lot dependent and they cannot be used with other lots of reagents.

Precautions and Warnings

1. The human derived material in this product was tested by FDA approved methods and found nonreactive for Hepatitis B Surface Antigen (HBsAg), anti-HCV and HIV 1/2 antibodies. Handle as if potentially infectious[8].
2. All reagents contain less than 0.1% sodium azide that may form explosive azides in metal plumbing. Use proper disposal procedures.
3. Avoid contact with skin and eyes (S 24/25). Do not empty into drains (S 29). Wear suitable protective clothing (S 36).
4. Harmful by ingestion (R 22). In case of ingestion, obtain medical attention (S 46).
5. This product is For *In Vitro* Diagnostic Use.

∆Supplemental hazard information:

aCL IgG Cartridge:

Magnetic Microparticles: Up to 10.6% of the mixture consists of component of unknown acute toxicity (dermal, inhalation) for the human health and unknown hazard to the aquatic environment.

Tracer: Up to 5.1% of the mixture consists of component of unknown acute toxicity (oral, dermal, inhalation) for the human health and unknown hazard to the aquatic environment.

Assay Buffer/Sample Diluent: None

aCL IgG Calibrator 1/Calibrator 2: Up to 5% of the mixture consists of component of unknown acute toxicity (oral, dermal, inhalation) for the human health and unknown hazard to the aquatic environment.

Storage Conditions

1. Unopened reagents and calibrators are stable until the expiration date shown on the cartridge and tube labels when stored at 2-8°C. Do not freeze.
2. Opened reagent cartridges should be stored onboard the instrument. The BIO-FLASH software monitors the onboard (in-use) expiration as well as the reagent lot expiration (shelf-life) of the reagent cartridge. The system will not allow use of a cartridge which has expired. aCL IgG Calibrator 1 & 2 - Stability after opening on board the BIO-FLASH System is 3.5 hours.
3. For optimal stability remove calibrators from the system immediately after calibration, and store them at 2-8°C capped in the original vial.

Specimen Collection

Plasma: Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Refer to CLSI Document H21-A5 for further instructions on specimen collection, handling and storage[9]. Thaw frozen samples rapidly at 37°C. After thawing the assay must be performed within 2 hours.
Serum: Following collection, the serum should be separated from the clot. Follow CLSI Document H18-A4 recommendations for storage conditions for samples[10].
Centrifuge specimens containing visible particulate matter before testing.

Procedure

Materials Provided

1 QUANTA Flash aCL IgG Reagent Cartridge
1 QUANTA Flash aCL IgG Calibrator 1
1 QUANTA Flash aCL IgG Calibrator 2

Additional Materials Required But Not Provided

BIO-FLASH instrument with operating computer
BIO-FLASH Triggers (Part Number: 3000-8204)
BIO-FLASH System Rinse (Part Number: 3000-8205)
BIO-FLASH Cuvettes (Part Number: 3000-8206)
BIO-FLASH Sample Diluent (Part Number: 3000-8207)
QUANTA Flash aCL IgG Controls (Part Number: 701232)

Using the BIO-FLASH Chemiluminescent Analyzer

1. Refer to the operator's manual provided with the BIO-FLASH system for detailed operating instructions of the BIO-FLASH chemiluminescent analyzer and the BIO-FLASH software. For additional information and for troubleshooting problems with this assay, contact Inova Diagnostics, Inc. technical service at the address or telephone number found at the end of this Direction Insert.
2. To empty the solid waste container, open the waste drawer. Remove the solid waste container and dispose of properly. Replace the solid waste container, close the waste drawer, and click **Yes** in the **Empty Waste Drawer** window.
3. To replace the triggers, click the **Bulks Inventory F9** button (upper right).
   a. In the **Inventory – Bulks** screen, click the **Triggers** button on the left. A new window will pop up titled **Add Triggers – Remove old bottles.**
   b. Open and remove the waste drawer on the BIO-FLASH instrument. Dispose of any cuvettes in the dry waste drawer. Click **Yes** on the **Empty Waste Drawer** window. Remove the trigger bottles from their holders and click the **Next** button. Unscrew the old trigger bottles from their caps and replace with new triggers. Be sure to do them one at a time, and match the color-coded caps (white to white and red to red).
   c. Follow the instructions in the new window **Add Triggers – Add Trigger 2 bottle.** Once the barcode has been accepted, place Trigger 2 into the color-coded white holder. Click **Next**.
   d. Follow the instructions in the window **Add Triggers – Add Trigger 1 bottle.** Once the barcode has been accepted, place Trigger 1 into the color-coded red holder. Click **Finish.** Replace and close the waste drawer.
4. To replace the System Rinse container, click the **Bulks Inventory F9** button (upper right corner). In the **Inventory – Bulks** screen, click the **Sys. Rinse** button. In the new window **Add System Rinse – Remove bottles**, click **Next.** Follow the instructions in the new window **Add System Rinse – Add bottle.** Once the barcode has been accepted, click **Finish** if necessary.
5. To empty the Fluid Waste Container, from the **Inventory – Bulks** screen, click the **Fluid Waste** button. Remove and dispose of the fluid waste. Click **Next**. Once the empty bottle has been replaced, click **Finish**.

6. To load the **Sample Diluent,** place the bottle in any of the three round spots present on the sample carousel, with the barcode on the label facing out, and close the lid to allow the instrument to scan it.

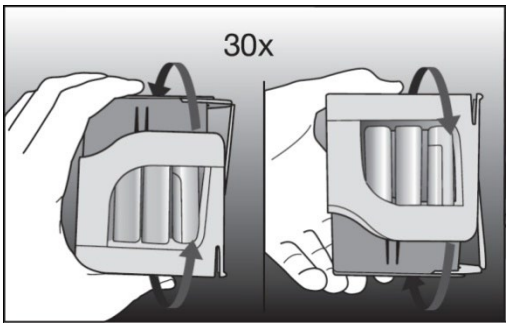
Method

Reagent Cartridge Preparation

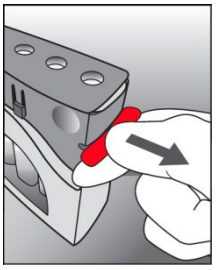
The first time the reagent cartridge is to be used, the following steps must be followed to accurately install the cartridge onto the BIO-FLASH instrument. Note: Do not use the reagent cartridge if any signs of damage are observed.

QUANTA Flash aCL IgG Reagent Cartridge: Microparticles settle during shipment and storage and require mixing to resuspend.

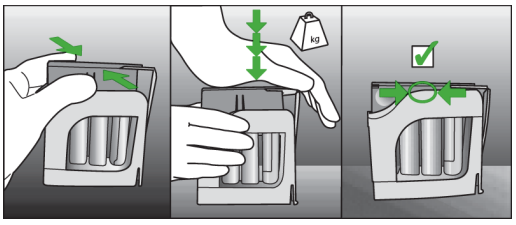
1. The first time that the cartridge is used, gently invert the cartridge 30 times, avoiding the formation of foam. Check for the complete resuspension of the microparticles. If the microparticles are not totally resuspended continue to invert the cartridge until the microparticles have been completely resuspended. If the microparticles do not resuspend, DO NOT USE THE CARTRIDGE.



2. Once the microparticles have been resuspended, place the reagent cartridge on a solid surface to remove the red pull-tab. Hold the reagent cartridge in place with one hand. With your other hand, firmly grasp the red pull-tab on the back of the reagent cartridge and pull it out completely.



3. Press the two tabs on the sides of the piercing cap (grey part) and apply downward pressure to the top portion of the reagent cartridge until it snaps down into a locked position. The tabs should no longer be visible. DO NOT INVERT THE OPEN CARTRIDGE.



4. Carefully place the reagent cartridge into any open slot on the reagent carousel of the BIO-FLASH instrument. Once the cartridge is placed into the reagent carousel, the instrument performs additional periodic mixing of the microparticles.

Assay Calibration

1. Each new lot of reagent cartridge must be calibrated prior to first time use. The software will not allow a new lot to be used until it is calibrated. Refer to the BIO-FLASH operator's manual for complete assay procedure instructions.
2. The QUANTA Flash aCL IgG Calibrators must be mixed by gentle inversion several times be for use to ensure homogeneity of the calibrator. Avoid foam formation.
3. Once the calibration is validated, the reagent cartridge lot on which the calibration was performed is ready for use.

Programming and Running Samples

1. Press the **Worklist** button at the top of the screen and select the **Racks** tab at the bottom.
2. Select the sample rack to be used by highlighting the rack on the screen or by scanning its barcode with the handheld barcode reader. Scan or type in the sample name, select the sample type, container type (tube/cup) and select IgG_aCL_100 from the assay panel. Repeat these steps for all samples.
3. Load a bottle of Sample Diluent on the sample carousel, if necessary.
4. Load the samples into the selected positions in the sample rack and load the rack into the sample carousel of the instrument.
5. If all required materials are on-board the instrument, the start icon will be available, in green, at the top of the screen. Press the start icon to begin the run.

Quality Control

The QUANTA Flash aCL IgG Controls (sold separately - Inova Item Number **#701232**) contains both aCL IgG High and Low Controls. Refer to the **QUANTA Flash® aCL IgG Controls** Direction Insert for detailed instructions on how to input the unit value and standard deviation of each control into the software, as well as how to run the controls. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls are recommended to be run once every 8-hour shift when the assay is used. Refer to Westgard *et al* for identification and resolution of out-of-control situations[11].

Traceability

The reported values were determined over multiple runs on the BIO-FLASH System using specific lots of reagents and against an in-house standard. Following the recommendations of the International Committee for the diagnosis of APS in the meeting held in Sydney[2], the units of the in-house standard for the aCL IgG antibodies have been correlated with the reference chimeric antibody HCAL[12].

Sample matrix

Twenty-one paired citrated-plasma/serum samples were analyzed with the aCL IgG assay. The concentration values were compared by Passing and Bablok regression using the plasma sample values as reference (X-axis), and the correlation coefficient calculated with Pearson correlation. The slope and intercept were respectively 1.04 and -0.22 and the correlation (r) 1.000.

Calculation of Results

A Master Curve is produced for each new lot of QUANTA Flash aCL IgG. This four parameter logistic curve is encoded in the barcode of each reagent cartridge. Once a reagent cartridge has been calibrated, a machine specific working curve will be used to convert the relative light units (RLU) to chemiluminescent units (CU).

Interpretation of Results

The QUANTA Flash Assay is capable of detecting small differences in patient populations. Each laboratory should establish its own normal range based upon its own controls and patient population according to their own established procedures.

aCL IgG results are reported in CU. These units have been established assigning 20 CU to the response of the upper limit of normality range (ULNR) of 252 citrated plasmas from a blood bank (99th percentile). 1 CU corresponds to 16.3 ng/mL of the HCAL chimeric antibody.

Please Note: The BIO-FLASH automatically accounts for the differences in dilution between plasma and serum samples. Reported serum sample values do not need to be corrected by a factor. Refer to the BIO-FLASH operator's manual for additional information.

Limitations of the Procedure

A definitive clinical diagnosis cannot be made on the basis of an aCL IgG positive result, and patient history and clinical findings must also be considered. When an aCL IgG negative result is found in the presence of clinical indications, other aPL assays must be performed as recommended in the revised classification criteria determined by the International Committee for the diagnosis of APS in the meeting held in 2006[2].

aCL IgG results on the BIO-FLASH are not affected by hemoglobin up to 500 mg/dL, bilirubin up to 18 mg/dL, triglycerides up to 1250 mg/dL, heparin (Low Molecular Weight and Unfractionated) up to 2 IU/mL and rheumatoid factor (RF) up to 500 IU/mL.

In a cross-reactivity study, 10 positive samples of each of the following conditions were tested with the QUANTA Flash aCL IgG assay: rheumatoid factor, antinuclear antibodies (ANA) and syphilis (positives for the rapid plasma reagin test, RPR).

This assay has not been validated for pediatric populations.

Patient group	N	n (positive)	**% aCL IgG Assay Positive**
RF	10	0	0.0%
ANA	10	1	10.0%
RPR	10	0	0.0%

Expected Values

A normal range study was performed using healthy adult blood bank citrated plasma donor samples run with QUANTA Flash aCL IgG reagents and calibrators. Following the recommendations of the International Committee in Sydney[2], the threshold for positive aCL IgG antibodies was established at the 99th percentile.

System	**N**	**Upper Limit Normal Range (CU)**
BIO-FLASH	252	20.0

Due to many variables which may affect results, each laboratory should establish its own normal range.

Method Comparison with Predicate Device

The samples used in the clinical study that were within the compared methods' test ranges were measured in a method comparison study with a commercially available FDA cleared ELISA assay. Percent Positive, Negative and Overall Agreement were:

Method Comparison (N = 136)		ELISA Assay			Percent Agreement (95% confidence)
		Positive	Negative	Total	
QUANTA Flash aCL IgG CIA	Positive	28	25	53	Pos. Agree = 80.0% (63.1%-91.6%)
	Negative	7	76	83	Neg. Agree = 75.2% (65.7%-83.3%)
	Total	35	101	136	Total Agree = 76.5% (68.4%-83.3%)

The precision, correlation and clinical study results were obtained using specific lots of reagents and controls.

Clinical Sensitivity and Specificity

An outcome study was performed on 321 frozen citrated plasmas. These plasmas were from 6 different groups, including selected individuals diagnosed as primary APS (PAPS), secondary APS (SAPS), systemic lupus erythematosus (SLE) but not APS and SLE-like by standard objective tests. The fifth group was patients with cardiovascular disorders but not classified in the previous four groups. A group of apparently healthy people was also included. The results summarized below are based on a cut-off of 20 CU:

Patient group	N	n (Positive)	% Positive
PAPS	23	13	56.5%
SAPS	69	37	53.6%
SLE	115	9	7.8%
SLE-like	5	0	0.0%
Others	6	1	16.7%
Normals	103	0	0.0%

Considering positive results in patient groups PAPS and SAPS as true positives, the clinical sensitivity, specificity and overall percent agreement were:

System	N	Sensitivity (95% CI)	Specificity (95% CI)	% Agreement (95%CI)
BIO-FLASH	321	54.3% (43.6%-64.8%)	95.6% (92.1%-97.9%)	83.8% (79,3%-87.7%)

Precision and Reproducibility

All data presented were obtained using citrated plasma samples.
Within run and total (run to run and day to day) precision was assessed over multiple runs.

BIO-FLASH	Mean (CU)	CV% (Within run)	CV% (Total)
Low aCL IgG Control	16.4	6.8%	8.2%
High aCL IgG Control	158	6.1%	6.9%
aCL IgG plasma sample A	13.8	4.0%	4.4%
aCL IgG plasma sample B	19.1	3.7%	4.2%
aCL IgG plasma sample C	47.2	4.8%	7.2%
aCL IgG plasma sample D	515	3.7%	5.4%
aCL IgG plasma sample E	1029	3.5%	6.7%

Limits of Detection; Linear and Reportable Ranges

Lower Limit of Detection:

System	
BIO-FLASH	2.6 CU

Linearity:

System	
BIO-FLASH	2.6 - 2024 CU

When the rerun capability of the instrument is activated, the instrument makes an automatic dilution and corrects the final result for the dilution factor (20x), thereby expanding the test range to 40480 CU. The assay is not affected by prozone effect. The assay protocol has a washing step after the sample incubation that precludes the prozone effect. Samples above 2024 CU tested during the clinical performance study triggered the rerun.

References

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Symbols Used

IVD	*In Vitro* diagnostic medical device		Manufacturer
Rx Only	Prescription Only per US FDA	EC REP	Authorized representative
CE	European Conformity	Σ	Contains sufficient for < n > tests
	Consult instructions for use	CAL 1	Calibrator 1
	Temperature limitation	CAL 2	Calibrator 2
	Do not reuse	PB	Recycle paper box
	Biological risks		This end up
LOT	Batch code	Δ	Indicates revision change
REF	Catalog number		
	Use by		

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