YALE-NEW HAVEN HOSPITAL	TITLE: **Beta 2 Glycoprotein IgM & IgG on the BioFlash** **Soft Code: B2GPM, B2GPG**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular diagnostcs Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 208** Page 1 of 10
WRITTEN BY: Katelyn Marreiros	**EFFECTIVE DATE:** May 17, 2013	**REVISION:** New	**SUPERCEDES:** **Beta 2 Glycoprotein IgG, DSX (IMM122)** **Beta 2 Glycoprotein IgM, DSX (IMM123)**

I. Intended Use

In-vitro diagnostic reagents are used for the semi-quantitative measurement of anti-β2 glycoprotein-1 IgM & IgG antibodies in human serum on the BIO-FLASH to aid in the diagnosis of thrombotic disorders related to antiphospholipid syndrome (APS) when used in addition to other laboratory and clinical findings.

II. Introduction

Anti- β2 glycoprotein-1 antibodies belong to a heterogeneous family of antiphospholipid (aPL) antibodies. These autoantibodies are directed against anionic phospholipids or protein-phospholipid complexes. Elevated levels of aPL antibodies are associated with an increased risk for antiphospholipid syndrome (APS) including vascular thrombosis and obstetrical complications. When a β2 glycoprotein screen is requested, IgA, IgG and IgM measurements will be performed. In addition, these antibodies can also be ordered individually.

III. Principle of the Assay

This assay is based on the principle of chemiluminescence. Magnetic particles coated with human purified β2GP1 capture anti β2GP1 antiphospholipid antibodies, if present in the patient sample. After incubation, magnetic separation and a wash step, a tracer consisiting of isoluminol-labeled anti-human IgM/IgG antibody is added and may bind with the captured anti β2GP1 IgM/IgG on the particles. Following a second incubation, magnetic separation and a wash step, reagents that trigger the luminescent reaction are added. The amount of light measured from the reaction is directly proportional to the amount of anti β2GP1 IgM/IgG in the sample. The BIO-FLASH utiltizes a 4 Parameter Logistic Curve (4PLC) fit data reduction method to generate a Master Curve. The Master Curve is pre-defined and lot dependent. The data is stored in the instrument through the barcoded reagent cartridge. With each calibration, the Master Curve is transformed to a 4PLC Working Curve. The concentration values depend upon the lot of calibrator and the data is stored on the barcodes of the tubes.

IV. **Specimen Collection**:

The test should be performed on serum only (from red top tube). Separate serum by centrifugation, 3000 rpm for 15 minutes. Serum aliquots can be stored at 2-8°C for up to 2 days or at below -20°C for up to three months. Repeated freeze-thaw cycles should be avoided. Do not perform the test on grossly hemolyzed samples. Lipemic serum should be spun at 10,000 rpm for 20 minutes to remove contaminating lipids. Icteric specimens should be taken to Clinical Chemistry and run on the analyzer to assess icteremia.

Standard Aliquot volume = 500 uL
Minimum Aliquot volume = 250 uL
Grossly Hemolyzed: Reject
Grossly Lipemic: Spin at 10,000 rpm for 20 min.
Grossly Icteric: Reject up to 18 mg/dL
Stability: 2 days refrigerated, 3 months frozen (-20°C)

V. **Materials:**

A. Reagents

1. Bio-Flash β2GP1 IgM reagent cartridge

   Composition: Each component is stored in a phosphate or borate buffer of bovine serum albumin, human β2GP1, mouse monoclonal IgM, stabilizers and a preservative

   a. 1 vial magnetic particle suspension coated with purified human β2GP1

   b. 1 vial assay buffer

   c. 1 vial tracer consisting of anti-human IgM antibody labeled with isoluminol

   d. 1 vial of sample diluent used for predilutions and automatic dilutions on a rerun

2. Bio-Flash β2GP1 IgG reagent cartridge

   Composition: Each component is stored in a phosphate or borate buffer of bovine serum albumin, human β2GP1, mouse monoclonal IgG, stabilizers and a preservative

   a. 1 vial magnetic particle suspension coated with purified human β2GP1

b. 1 vial assay buffer

c. 1 vial tracer consisting of anti-human IgG antibody labeled with isoluminol

d. 1 vial of sample diluent used for predilutions and automatic dilutions on a rerun

Preparation for IgM & IgG cartridges

e. Gently invert cartridge 30 times. Check for re-suspension of microparticles.
f. Place reagent cartridge on a solid surface and remove red pull-tab.
g. Press the two tabs on the side of the piercing cap (grey portion) and apply downward pressure to top of cartridge until it snaps into a locked position. The tabs should no longer be visible
h. Push the thick wedged end portion towards the thin end of the cartridge to ensure the vials have been pierced. DO NOT INVERT THE OPEN CARTRIDGE.
i. Carefully place the reagent cartridge into any open slot on the reagent carousel. Once the cartridge is onboard, the instrument performs additional periodic mixings of the microparticles.

Storage:

Stability at 2 to 8 °C: See expiration date on label. DO NOT FREEZE.

On-board stability: 6 weeks/42 days.

New Reagent Lots:

All new reagent lots are verified by testing previously tested patient or CAP samples. Refer to the Immunology Policy for Pretesting of test kits and reagents (Doc# IMM 174) for procedure and acceptability limits.

Precautions:

a. The human derived material in this product was tested by FDA approved methods and found nonreactive Hepatitis B Surface Antigen and anti-HCV and HIV 1/2 antibodies. Handle as if potentially infectious
b. All reagents contain less than 0.1% sodium azide that may form explosive azides in metal plumbing. Use proper disposal procedures
c. Avoid contact with skin and eyes. Harmful by ingestion.
d. Intended for *in vitro* diagnostic use only

3. Wash solution – see BioFlash Instrument Manual (Doc# IMM 207)

4. Triggers – see BioFlash Instrument Manual (Doc# IMM 207)

B. Calibrators

β2GP1 IgM Calibrator 1 & 2
β2GP1 IgG Calibrator 1 & 2

Composition and Standardization

1 mL barcoded tube of a solution with anti-β2GP1 IgM/IgG in a phosphate buffer containing bovine serum albumin, stabilizers and a preservative.

Preparation of the Calibrators

β2GP1 IgM/IgG Calibrator 1 & 2 are supplied ready-for-use. Invert gently to mix. Avoid vigorous shaking and foam formation.

Standard Storage and Stability:

Stability at 2 to 8 °C: The expiration date is given on the label. Do not freeze.

Stability once opened: For optimal stability, remove calibrators from the system immediately after calibration and store at 2 to 8 °C capped and in the original vial. Once opened, the calibrators are only good for **8 hours**.

C. Controls

BioFlash β2GP1 IgM Low Control
BioFlash β2GP1 IgM High Control
BioFlash β2GP1 IgG Low Control
BioFlash β2GP1 IgG High Control

Composition

QUANTA Flash β2GP1 IgM/IgG Low and High Controls are barcoded tubes with a solution of anti β2GP1 IgM/IgG in a phosphate buffer containing bovine and serum albumin, stabilizers and preservative.

Preparation of the Reagents

QUANTA Flash β2GP1 IgM/IgG Low and High Controls are supplied ready-for-use. Invert gently to mix. Avoid vigorous shaking and foam formation.

Control Storage and Stability:

Shelf life at 2 to 8 °C: The expiration date is given on the label. Do not freeze.

Stability once opened: Good for 15 uses. For optimal stability remove controls from the system immediately after control sampling and store them at 2 to 8 °C.

D. Consumables

1. BioFlash Cuvettes

VI. Assay Procedure

A. Before Starting

1. Call a Soft pending list by **Workstation**. Refer to the Soft Immunology Procedure (Doc# IMM 120).
2. Perform maintenance according to the maintenance charts (DOC#IMM 207 M1 and M2)
3. Inspect all samples for sufficient volume (250 uL), bubbles and the presence of interfering substances such as hemolysis, icteremia and lipemia.

B. Assay Protocol for the BioFlash System

1. The assay protocol is given in the Instruction Manual and software of the instrument. All steps are performed automatically by the system. Consult the BioFlash Instrument Manual (Doc# IMM 207) for details regarding operation of the instrument.
2. The reagents must not be used beyond the expiration date.

C. Assay of Specimens

1. Routine Samples

   - Samples are automatically diluted using the sample diluents from the reagent cartridge

2. Short Samples

   - Samples volumes at 250uL can be run in sample cups and programmed manually. Refer to the BioFlash Instrument Manual (Doc# IMM 207).

   - Volumes less than 250 uL cannot be tested.

VII. Calibration

A. Establishment of the Master Curve

A five point Master Curve is produced for each new lot of BioFlash β2GP1 IgM/IgG reagent. This is derived from the 4PLC encoded in the barcode of each reagent cartridge. Upon calibration, a machine specific working curve will be used to convert the relative light units (RLU) to chemiluminescent units (CU).

B. When to Calibrate (Working Curve):

1. If a new lot of reagent is loaded onto the instrument.
2. If the controls are out of range or the Westgard rules stated in the Quality Control procedure (Doc# IMM 37) are violated.
3. If a different lot of anti-serum is used, a new reference curve must be generated.
4. Major instrument maintenance has been performed.

C. How to calibrate

1. Use β2GP1 IgM/IgG Calibrator 1 & 2.
2. Refer to the BioFlash Instrument Manual (Doc# IMM 207) for instructions on programming a calibration.
3. The acceptability limit for β2GP1 IgM is a CV 8%. For β2GP1 IgG, the acceptability limit is 10% CV. The curve must be validated by the operator.
4. Always run quality control after calibration.

VIII. Quality Control

A. Quality control Material

BioFlash Controls:

- BioFlash β2GP1 IgM Low Control
- BioFlash β2GP1 IgM High Control
- BioFlash β2GP1 IgG Low Control
- BioFlash β2GP1 IgG High Control

B. Frequency

1. Both levels of controls are to be run at the beginning of each shift or every 8 hours.
2. Both levels are to be run following calibration.

C. Quality Control Guidelines

1. The 10X, 2-2S and 1-3S Westgard rules will be used for QC monitoring. For more information on quality control monitoring refer to Immunology Laboratory Guidelines for Quality Control (Doc# Imm 38).
2. Record QC as stated in the BioFlash Operational Procedure (IMM 207).

D. New lots of Quality Control

1. New lots of control material are pre-tested until at least 15 data points are collected to determine an in-house control range of +/- 3 standard deviations.

2. If a new lot of control is put into use before 15 points are collected the manufacturer's range will be used. Once 15 data points are collected, a new range will be established.

IX. Interpretation of Results

A. Reporting Results

1. The instrument automatically calculates and prints the concentration of BioFlash β2GP1 IgM/IgG in chemiluminescent units (CU).
2. If the results obtained are above the measuring range, the SOFT LIS will report the result as:
    a. >841 CU for β2GP1 IgM
    b. > 6,100 CU for β2GP1 IgG.
3. If the result is lower than the AMR, the the SOFT LIS will report the result as:
    a. < 10 CU for β2GP1 IgM
    b. < 10 CU for β2GP1 IgG
    c. Results should only be reported as less than the AMR after the sample has been evaluated for the presence of bubbles or fibrin.

B. Verification of Results:

1. Results are transmitted from the BioFlash to QuantaLink by approving each result. Refer to the QuantaLink 3.0 Operating Procedure (Doc# IMM) for more information on the QuantaLink.

    a. To approve results, select the appropriate result from the Results icon on the menu bar. Select approve.

2. From QuantaLink, the results are transferred to the SOFT LIS system and will be autoverified. Refer to the Soft Immunology Procedure (Doc# IMM 120).

X. Analytical Measuring Range (AMR)

The AMR for β2GP1 IgM and IgG are listed below:

AMR for β2GP1 IgM: 1.1-841 CU

AMR for β2GP1 IgG: 6.4-6,100 CU

AMR verification does not need to be performed every 6 months because the assay is semi-quantitative.

XI. Reference Range

For both β2GP1 IgM & IgG

≤20 CU = Negative

>20 CU = Positive

XII. Limitations

A. Interferences

1. According to guidelines set by INOVA no interference with the determinations in serum were detected for concentrations of triglycerides up to 1250 mg/dL, bilirubin up to 18 mg/dL, hemoglobin up to 500mg/dL, heparin (LMW and unfractionated) up to 2 IU/mL and rheumatoid factor (RF) up to 500 IU/mL.
2. Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

XIII. **YNHH Method Validation** Summary

a. β2GP1 IgM

Correlation:

Correlation was performed by comparing results of 41 patient samples tested on the BioFlash (YNHH) to results tested on the BioFlash (INOVA) and comparing results obtained on each instrument. One hundred percent agreement was achieved, for both positive (8) and negative (33) samples.

Precision:

Intrarun Precision: Intra-assay performance was evaluated by running a positive and a negative sample four times within the run. The results show a CV% of approximately 1.7%

Interrun Precision: Inter-assay performance was evaluated by testing 2 specimens, one negative and one positive, over the course of several days. The results show a CV% of approximately 4.6% and 1.7%, respectively.

Reference Range Verification:

The reference range in use was established by Inova Diagnostics by testing 250 samples. The upper limit normal range was established as 20.0 CU. This was verified by testing 20 randomly selected samples representing YNHH's testing population. All YNHH samples were negative

Carry-Over:

Carry-over was validated by running a sequence of 21 high and low values for β2 IgM in a particular order. The High-Low mean was 1.10 and the Low-Low mean was 1.10. The error limit was 0.10 and passed the carry-over qualifications.

CAP Proficiency Results:

Proficiency samples from the ACL -B 2012 were tested and all results were acceptable when compared to other BioFlash users.

b. β2GP1 IgG

Correlation

Correlation was performed by comparing results of 41 patient samples tested on the BioFlash (YNHH) to results tested on the BioFlash (INOVA) and comparing results obtained on each instrument. One hundred percent agreement was achieved, for both positive (11) and negative (30) samples.

Precision:

Intrarun Precision: Intra-assay performance was evaluated by by running a positive and a negative sample four times within the run. The results show a CV% of approximately 0.9% and 3.3%, respectively.

Interrun Precision: Inter-assay performance was evaluated by testing 2 specimens, one negative and one positive, over the course of several days. The results show a CV% of approximately 3.4% and 2.2%, respectively.

Reference Range Verification:

The reference range in use was established by Inova Diagnostics by testing 250 samples. The upper limit normal range was established as 20.0 CU. This was verified by testing 20 randomly selected samples representing YNHH's testing population. All YNHH samples were negative

CAP Proficiency Results:

Proficiency samples from the ACL -B 2012 were tested and all results were acceptable when compared to other BioFlash users.

XIV. References:

1. QUANTA Flash™ β2GP1 IgM [package insert]. San Diego, CA: INOVA Diagnostics, Inc.; Revision 1, April 2011.

2. QUANTA Flash™ β2GP1 IgM Controls [package insert] San Diego, CA: INOVA Diagnostics, Inc.; Revision 1, April 2011.

3. QUANTA Flash™ β2GP1 IgG [package insert] San Diego, CA: INOVA Diagnostics, Inc.; Revision 1, April 2011.

2. QUANTA Flash™ β2GP1 IgG Controls[package insert] San Diego, CA: INOVA Diagnostics, Inc.; Revision 1, April 2011.

Document Author
Katelyn Marreiros
May 17,2013

Signature Approval for Annual Review
Name: Beta 2 Glycoprotein IgM & IgG on the BioFlash

Document #: IMM 208

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
TEODORICO LEE	LAB MANAGER	Teodorico Lee	5/20/13	NEW		5/17/2013
BRIAN SMITH	LAB DIRECTOR		5/16/13	NEW		5/17/2013