CPAL
Central Pennsylvania Alliance Laboratory

Technical Bulletin
No. 149

January 25, 2016

Beta 2-Glycoprotein IgA, IgG, and IgM
- New Assays -

Contact: Steph Frey, 717.812.4768
Operations Manager, Clinical Pathology, CPAL

Dr. Jennifer Thebo, 717.851.3210
Director of Technical Operations and Scientific Affairs, CPAL

Ordering Information:

Test Name	Beta 2 Glycoprotein I IgG	Beta 2 Glycoprotein I IgA	Beta 2 Glycoprotein I IgM	Beta 2 Glycoprotein I GAM
	B2 IgG	B2 IgA	B2 IgM	B2 Grp
PDM Number	3000846	3000848	3000850	3000844
LOINC Codes	44448-9	44447-1	44449-7	
CPT Codes	86146	86146	86146	86146 x3
Sample	Serum	Serum	Serum	Serum
Stability (Temp)	Refrigerate	Refrigerate	Refrigerate	Refrigerate
Stability(Time)	48 hours	48 hours	48 hours	48 hours
Alternate Spec Type	Plasma (Li Hep, EDTA, Citrate)	Plasma (Li Hep, EDTA, Citrate)	Plasma (Li Hep, EDTA, Citrate)	Plasma (Li Hep, EDTA, Citrate)
Test Method:	Fluoroenzyme Immunoassay	Fluoroenzyme Immunoassay	Fluoroenzyme Immunoassay	Fluoroenzyme Immunoassay

Effective Date: Testing offered beginning on Monday, February 29, 2016.

Performed: Monday, Wednesday, and Friday dayshift

Reference Range:

Test	Negative	Equivocal	Positive
EliA β2-Glycoprotein IgA	<7	7 – 10	>10
EliA β2-Glycoprotein IgG	<7	7 – 10	>10
EliA β2-Glycoprotein IgM	<7	7 – 10	>10

(In case of equivocal results, it is recommended that the patient be retested in 8 – 12 weeks)

Background:

The antiphospholipid syndrome (APS), also known as "Hughes syndrome", is characterized by typical clinical features such as arterial/venous thromboses or recurrent miscarriages together with persistently positive tests for antiphospholipid antibodies. The criteria for classification of the APS have been revised in 2004 in Sydney. Besides the clinical criteria, three different laboratory tests are listed: lupus anticoagulant, anticardiolipin antibodies (IgG and IgM), and anti-β2 Glycoprotein I antibodies (IgG and IgM). The latter was not included in

the former Sapporo criteria. However, by majority, the Sydney committee agreed that they are an independent risk factor for thrombosis and pregnancy complications. The agreement achieved in Sydney was that so far it is premature to implement IgA Anti-β2-Glycoprotein I antibodies into the new criteria. Anti-β2-Glycoprotein I antibodies of the IgA isotype are not part of the classification criteria as data did not reach sufficient evidence level to support the presence as independent risk factor.

For APS diagnosis, β2-Glycoprotein I antibody tests show higher specificity than anticardiolipin assays. In 3-10% of APS patients, β2-Glycoprotein I antibodies may be the only positive test. The association of β2-Glycoprotein I antibodies with pre-eclampsia and/or eclampsia in unselected pregnant women who tested negative for anticardiolipin antibodies implies that the inclusion of β2-Glycoprotein I antibodies may also help clarify this type of pregnancy morbidity. Outside the context of clinical studies, testing for β2-Glycoprotein I antibodies can be helpful for APS diagnosis, particularly when anticardiolipin antibodies and lupus anticoagulant are negative and APS is strongly suspected.

Principle of Test:

The EliA β 2-Glycoprotein IgA, IgG, and IgM assays are performed on the Phadia 250 immunoassay system. The assays utilize human or human recombinant protein and enzyme-labeled antibodies against human IgA, IgG, or IgM antibodies. Results are directly related to the signal produced.

Validation Data:

Precison:

For within run precision, two levels of control were run ten times each, within the same run. For between run precision, two levels of control were run five times each on two different days. The %CVs fall within the Manufacturer's claim of ≤12% for the positive control. The negative control was negative for all replicates.

Table 1: Precision

Test	Within Run Precision (N1778)				Within Run Precision (N1926)				Accept?
	Mean (Neg)	CV	Mean (Pos)	CV	Mean (Neg)	CV	Mean (Pos)	CV	
Ab2	0.10	0.0%	56.1	2.1%	0.09	35.1%	54.2	2.6%	Yes
Gb2	0.60	0.0%	51.9	2.1%	0.60	0.0%	51.3	3.3%	Yes
Mb2	0.90	0.0%	58.3	2.6%	0.90	0.0%	57.9	3.7%	Yes

Table 2: Between Run Precision

Test	Between Run Precision (N1778)				Between Run Precision (N1926)				Accept?
	Mean (Neg)	CV	Mean (Pos)	CV	Mean (Neg)	CV	Mean (Pos)	CV	
Ab2	0.12	34.5%	57.0	3.1%	0.09	27.7%	54.2	2.7%	Yes
Gb2	0.60	0.0%	52.4	2.3%	0.60	0.0%	51.5	3.0%	Yes
Mb2	0.90	0.0%	58.3	2.3%	0.90	0.0%	58.7	4.3%	Yes

Method Comparison:
Specimens were split and processed utilizing the Phadia 250 EliA β2-Glycoprotein assays versus results from Quest Diagnostics. (Figure 1).

Figure 1

Assay	Phadia 250				
	Positive Agreement vs Quest	Negative Agreement vs Quest	Overall Agreement	N	Acceptable?
Ab2	95.0%	80.0%	87.5%	40	Yes
Gb2	95.7%	100.0%	97.0%	30	Yes
Mb2	81.8%	95.0%	88.1%	42	Yes

Clinica Chimica Acta 428 (2014) evaluated the performance of different ELISAs and other immunoassays for the detection of anti-β2 glycoprotein I antibodies in a wet workshop at the 13th International Congress on Antiphospholipid Antibodies in Galveston, TX. The journal article summarizing the results is titled 'Evaluation of different immunoassays for the detection of antiphospholipid antibodies: Report of a wet workshop during the 13th International Congress on Antiphospholipid Antibodies.' The results demonstrated that the Phadia 250 EliA assays, when using the manufacturer's stated reference ranges, showed a clinical specificity of 95% and a clinical sensitivity of 100%.

Reference Range:
Manufacturer's stated reference ranges were verified using medical decision point analysis.

Limitations:
1. A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Rheumatoid Factor (RF) can interfere with the determination of IgM anti-β2-Glycoprotein I antibodies.
3. The expected value in the normal population is negative, however, up to 3% of apparently healthy, asymptomatic individuals may have increased levels of β2-Glycoprotein I IgG and IgM antibodies.
4. Antibody prevalence in autoimmune patients varies widely depending on disease area. The proportion of sera from normal population found positive for the β2-Glycoprotein I IgA covered by the EliA β2-Glycoprotein I test is below 3% increasing with age. Men tend to show higher values.
5. Anti-β2-Glycoprotein I antibodies may be present in the so-called antiphospholipid syndrome, often appearing in conjunction with SLE, and sometimes occurring without symptoms of SLE. Clinical manifestations can be thrombosis, recurrent pregnancy loss, thrombocytopenia, hemolytic anemia, and other features such as livedo reticularis, migraines, epilepsy, and others.

References:

1. EliA β2-Glycoprotein I IgA package insert, January 2015.
2. EliA β2-Glycoprotein I IgG package insert, January 2015.
3. EliA β2-Glycoprotein I IgM package insert, January 2015.