CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Cardiolipin IgA, IgG, and IgM
- New Assays -

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Ordering Information:

Test Name	Cardiolipin IgG	Cardiolipin IgA	Cardiolipin IgM	Cardio GAM	Cardio GM
	Card IgG	Card IgA	Card IgM	Cardio GAM	Cardio GM
PDM Number	3000836	3000838	3000840	3000832	3000834
LOINC Codes	3181-5	5076-5	3182-3	45057-7	24319-6
CPT Codes	86147	86147	86147	86147 x3	86147 x2
Specimen Type	Serum	Serum	Serum	Serum	Serum
Stability (Temp)	Refrigerate	Refrigerate	Refrigerate	Refrigerate	Refrigerate
Stability(Time)	48 hours	48 hours	48 hours	48 hours	48 hours
Alternate Spec Type	Plasma(Li Heparin, EDTA or Citrate)	Plasma(Li Heparin, EDTA or Citrate)	Plasma(Li Heparin, EDTA or Citrate)	Plasma(Li Heparin, EDTA or Citrate)	Plasma(Li Heparin, EDTA or Citrate)
Method:	Fluoroenzyme Immunoassay	Fluoroenzyme Immunoassay	Fluoroenzyme Immunoassay	Fluoroenzyme Immunoassay	Fluoroenzyme Immunoassay

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

Performed: Monday/Wednesday/Friday dayshift

Reference Range:

Test	**Negative**	**Equivocal**	**Positive**
Cardiolipin IgA	<14	14-20	>20

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

	Negative	**Weak Positive**	**Positive**
Cardiolipin IgG	<10	10 – 40	>40
Cardiolipin IgM	<10	10 – 40	>40

(In case of weak positive results, it is recommended that the patient be retested after 12 weeks.)

Background:

Anti-cardiolipin antibodies (ACA) belong to the group of anti-phospholipid antibodies (aPL). Their occurrence was first demonstrated in sera of syphilis patients, but later they have also been described frequently in SLE (systemic lupus erythematosus) patients (prevalence 30-40%) and in patients with other rheumatic diseases. 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

aPL. In contrast to "secondary APS" which occurs in association with SLE or other rheumatic disorders, there is no evidence for another relevant underlying disease in primary APS. New criteria for classification of the antiphospholipid syndrome have been defined recently. Although ACA of the IgA isotype are not part of the classification criteria, they are considered as to be associated with APS. IgA ACA are in particular associated with thrombocytopenia, skin ulcers, and vasculitis in connective tissue disease patients. They are of particular interest in patients with African genetic background, where the prevalence is increased.

Anti-cardiolipin antibodies in infectious diseases and in APS can be distinguished with respect to their dependence on cofactors: whereas ACA from patients with infectious diseases recognize the pure phospholipid as antigen, binding of ACA from patients with APS requires ß2-glycoprotein I as a cofactor. For this reason, ACA ELISAs need ß2-glycoprotein I to be incorporated into the assay. The so-called, lupus anticoagulant' (LA) describes a phenomenon that is related to the presence of antiphospholipid antibodies. It is defined by the measurement of antibody dependent coagulation inhibition in vitro.

ACA/LA are considered to be of significant diagnostic relevance, as a correlation has been found between these antibodies and a tendency towards thromboses. This results in an increased incidence of venous/arterial thromboses, thrombocytopenia, habitual abortion, and neurological manifestations in ACA/LA positive patients. Elevated levels of ACA/LA may also be found in patients with cerebrovascular insufficiency or myocardial infarction. aPL are discussed to play a direct role in the pathogenesis of APS.

Principle of Test:

The EliA Cardiolipin 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	
Acl	0.30	15.7%	51.2	3.0%	0.28	15.1%	51.2	4.8%	Yes
Gcl	0.50	0.0%	48.5	3.1%	0.50	0.0%	46.8	3.5%	Yes
Mcl	0.80	0.0%	48.9	1.5%	0.80	0.0%	50.8	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	
Acl	0.31	14.9%	52.1	3.6%	0.29	12.3%	50.9	4.0%	Yes
Gcl	0.50	0.0%	49.9	5.1%	0.50	0.0%	47.6	4.0%	Yes
Mcl	0.80	0.0%	49.1	3.1%	0.80	0.0%	50.3	3.4%	Yes

Method Comparison:
Specimens were split and processed utilizing the Phadia 250 EliA Cardiolipin 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?
Acl	85.0%	100.0%	92.5%	40	Yes
Gcl	75.0%	100.0%	87.5%	40	Yes
Mcl	80.0%	100.0%	88.9%	45	Yes

Clinica Chimica Acta 428 (2014) evaluated the performance of different ELISAs and other immunoassays for the detection of aCL 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 100% and a clinical sensitivity of 100%.

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. Antibody prevalence in autoimmune patients varies widely depending on disease area. The proportion of sera from a normal population found positive for the Cardiolipin IgA covered by the EliA Cardiolipin IgA test is below 5 %, increasing with age, and men tend to show higher values. Expected values may vary depending on the populations tested.
3. The expected value for Cardiolipin IgG and IgM in the normal population is negative. However, low percentages (up to 5%) of apparently healthy, asymptomatic individuals have been reported to be positive for ACA. Their proportion may increase with age. Anti-cardiolipin antibodies are characteristic for the antiphospholipid syndrome (primary or secondary) and occur with a prevalence of 30-40% in SLE patients. Clinical manifestations can be thrombosis, recurrent abortions, and other features, such as thrombocytopenia, livedo reticularis or neurological disorders. Cardiolipin antibodies show the following prevalences in APS:
   Primary APS: Cardiolipin IgG 81%, IgM 40%, IgA 24%
   Secondary APS: Cardiolipin IgG 85%, IgM 65%, IgA 10%
   Expected values may vary depending on the population tested.
4. No hook effects could be observed for IgA concentrations up to 15 fold above the measuring ranges. No hook effects could be observed for IgG and IgM concentrations up to 10 fold above the measuring ranges.

References:

1. EliA Cardiolipin IgA package insert, January 2015.
2. EliA Cardiolipin IgG package insert, January 2015.
3. EliA Cardiolipin IgM package insert, January 2015.