CPAL
Central **P**ennsylvania **A**lliance
Laboratory

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Anti-CCP
- Testing System Change –

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Affected Tests:

Mnemonics:	**CCP**	**RA GRP**
Test Name:	Anti-CCP	RA Group
Test Number:	3000317	3000314
Specimen:	0.5 mL Serum, frozen	RF: 0.5 mL Serum, Refrig (2-8ºC)7 days (do not freeze) CCP: 0.5 mL Serum, Frozen

Effective Date: Testing will begin on May 19, 2014 with samples received on May 15, 2014.

Performed: Monday, Wednesday, Friday
Reference Range: Negative; <7 U/mL

Method Change:
Anti-CCP will be moved from the Diasorin EtiMax to the Phadia 250, as the EtiMax is being phased out. The Phadia 250 is a fully automated platform that is primarily used for allergy and autoimmune testing. Anti-CCP will be performed using the EliA CCP method.

Background:
Rheumatoid Arthritis (RA) is one of the most common systemic autoimmune diseases (prevalence 1-2%). It is characterized by chronic inflammation of the joints and may lead to progressive erosions and cartilage destruction. Until recently, the early diagnosis of RA relied chiefly on clinical manifestations and on rheumatoid factors (RF) as serological marker. Determination of RF is rather sensitive for RA (50-90%), but only of limited specificity (70-90%). Patients with various other diseases (e.g. SLE, Sjögren's syndrome, systemic sclerosis, polymyositis/dermatomyositis) and some healthy individuals were reported to be positive for RF as well. In 1998, highly RA specific antibodies were described that were directed against citrullinated peptides. An ELISA made with the originally described CCP sequence was not broadly marketed. EliA CCP contains a mixture of synthetic peptides selected on the basis of approved performance in the detection of RA autoantibodies. In the literature, this antigen preparation is usually referred to as CCP2 or second generation. Assays using this preparation showed a sensitivity of 68% and a specificity of at least 96%. Thus, anti-CCP testing is a tool to aid in the diagnosis of RA. Additionally, anti-CCP antibodies may be of prognostic value with respect to the development of radiographic joint damage.

Principle of Test:
The EliA CCP Wells are coated with citrullinated synthetic peptides (second generation antigen). If present in the patient's specimen, antibodies to CCP bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The assay directly measures the amount of antibody of interest bound to the antigen coating the EliA well, therefore the higher the value of fluorescent signal detected by the instrument, the higher the amount of antibody bound and detected in the sample tested. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

Results Interpretation:
The ranges are as follows:

Negative	<7	U/ml
Equivocal	7 – 10	U/ml
Positive	>10	U/ml

In case of equivocal results, it is recommended to retest the patient after 6-8 weeks.

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. The measuring range (detection limit, upper limit) for EliA CCP is from 0.4 to ≥ 340 U/ml. No hook effects could be observed for concentrations up to 10 fold above the measuring range.
3. Only values above the Detection Limit can be regarded as valid results. The upper limit of the reported results can vary due to a lot-specific conversion from ug/L to U/ml. Results above the upper limit are reported as >340 U/mL. All values less than 0.4 will be reported as <0.4.
4. Please note that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the measuring range.
5. The following substances in concentrations corresponding to those indicated in undiluted samples were analyzed for interference with EliA CCP: Bilirubin F/C (18.8/20 mg/dl), Chyle (236,000 Units/dl). Hemoglobin (453 mg/dl) and Rheumatoid factor IgM (550 IU/ml). No interference could be observed.

Validation Data:
Precision
Manufacturer's criteria for precision are within run CV <10% and between run CV<12% for results within the equivocal and positive ranges. No claims are made for results in the negative range. Laboratory evaluation criteria for negative results was CV <20% for both within run and between run precision. All precision criteria were met (Tables 1 and 2).

Table 2 Anti-CCP Within Run Precision

U/ml	%CV	U/mL	%CV	U/mL	%CV	U/ml	%CV
202.0	5.868	33.2	1.347	10.1	3.8	0.6	14.8

Table 3 Anti-CCP Between Run Precision

U/ml	%CV	U/mL	%CV	U/ml	%CV
200.1	10.733	34.2	6.286	0.6	14.7

Linearity
To verify the analytical measurement range of the assay, a high patient sample was diluted with a low patient sample to cover the reportable range of 0.4 to 340 U/mL. Linear regression analysis yielded a slope of 0.989 with an intercept of 0.03 U/mL and error of 0.18 U/mL or 5.3%.

Method Comparison
A total of 40 specimens were split and processed utilizing the Phadia 250 EliA CCP assay and the DiaSorin EtiMax CCP assay.

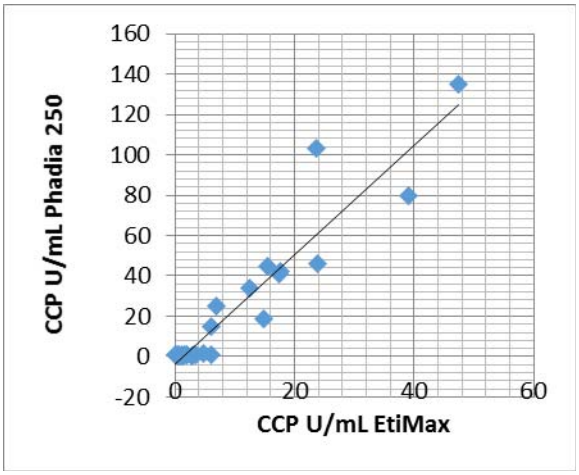
Qualitative analysis was performed using recommended decision cutoff values of:

EtiMax: negative: ≤ 5 U/mL, positive: >5 U/mL
Phadia 250: negative: <7 U/mL, equivocal: 7-10 U/mL, positive: >10 U/mL

There was one discordant interpretation between the two assays. The specimen was repeated on the Phadia and produced the same result. The % positive agreement between the two methods is 95.8 and the % negative agreement is 100.

Quantitative analysis, excluding results above the linear ranges of the methods, yielded a correlation coefficient (r) of 0.981 with a slope of 2.39 and an intercept of -1.14. (Figure 1)

Figure 1: Anti-CCP Quantitative Method Comparison Phadia 250 vs EtiMax



References:

1. EliA CCP package insert, September 10, 2013.