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

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Thyroid Antibodies (Anti-TPO and Anti-TG) - Testing System Change –

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Affected Tests:

Mnemonics:	ATA	ATG	THYRO QUAN (includes TG and ATG)	Thyroid Ab (includes ATA and ATG)	THY PNL (includes ATA, ATG and TG)
Test Name:	Anti TPO Ab	Anti Thyroglobulin Ab	Thyroglobulin Group	Thyroid Ab Group	Thyroid Panel
Test Number:	1750025	1750027	1750030	1750024	1750023
Specimen:	1.0 mL Serum or Plasma (Li-Heparin or EDTA), refrigerated up to 48 hours (2-8^{o}C), freeze if longer storage is necessary (-20^{o}C)				

Effective Date: Testing will begin on January 26, 2015 with samples received in the afternoon on January 23, 2015.

Performed: Monday through Saturday

Reference Range:

Thyroglobulin Antibody	≤4 IU/mL
TPO Antibody	≤8 IU/mL

Method Change:
Testing for TPO Antibody and Thyroglobulin Antibody will be moved from the Siemens Immulite to the Beckman-Coulter Unicel DxI 800, as the Immulite is being phased out.

Background:
The measurement of thyroid autoantibodies may aid in the diagnosis of Hashimoto's disease, nontoxic goiter, and Graves' disease.

Thyroglobulin is produced by the thyroid gland. It is a water soluble glycoprotein of approximately 660,000 Daltons. It is a major component of the thyroid follicular colloid and is present in small amounts in serum. The principal role of thyroglobulin is the storage and synthesis of thyroid hormones. The thyroid hormones 3, 5, 3', 5', - tetraiodothyronine (thyroxine, T4) and 3, 5, 3', -triiodothyronine (T3) are synthesized from thyroglobulin.

Thyroglobulin autoantibodies (TgAb) are often present in patients with autoimmune thyroid disease.

Approximately 10% of healthy individuals have TgAb at measurable levels. TgAb can be detected in 30% of patients with Graves' disease and in 85% of patients with Hashimoto's thyroiditis. However, elevated levels of autoantibodies to thyroid peroxidase (TPO autoantibodies) occur more frequently than high TgAb levels in these diseases. Sensitive TgAb methods are needed to identify patient sera that contain thyroglobulin autoantibodies that may interfere with serum thyroglobulin measurements.

Disorders of the thyroid gland are frequently caused by autoimmune mechanisms with the production of autoantibodies. Thyroperoxidase (TPO) is a membrane-associated hemoglycoprotein expressed only in thyrocytes. This enzyme catalyzes the oxidation of iodide on tyrosine residues in thyroglobulin for the synthesis of T3 and T4 and is one of the most important thyroid gland antigens.

The determination of TPOAb levels is the most sensitive test for detecting autoimmune thyroid disease. The highest TPOAb levels are observed in patients suffering from Hashimoto's thyroiditis. In this disease, the prevalence of TPOAb is about 90% of cases confirming the autoimmune origin of the disease. These autoantibodies also frequently occur (60–80%) in the course of Graves' disease.

There is a good association between the presence of autoantibodies against TPO and histological thyroiditis. However, in view of the extensive regenerative capacity of the thyroid under the influence of TSH, chronic thyroid disease may be present for years before the clinical manifestation of hypothyroidism becomes evident, if ever.

The detection of TPOAb is an aid in the diagnosis of thyroid autoimmune disorders and enables the physician to differentiate thyroid autoimmune disorders from non-autoimmune goiter or hypothyroidism.

Principle of Test:
The Access Thyroglobulin Antibody II (TgAb) assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin antibody levels in human serum and plasma using the Access Immunoassay Systems. The Access Thyroglobulin Antibody II assay is a sequential two-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with paramagnetic particles coated with the thyroglobulin protein. The serum or plasma TgAb binds to the thyroglobulin. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. The thyroglobulin-alkaline phosphatase conjugate is added and binds to the TgAb. After the second incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of thyroglobulin antibody in the sample. The amount of analyte is determined from a stored, multi-point calibration curve.

The Access TPO Antibody assay is a sequential two-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with paramagnetic particles coated with thyroperoxidase protein. The serum or plasma TPOAb binds to the thyroperoxidase. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. The Protein A-alkaline phosphatase conjugate is added and binds to the TPOAb. After the second incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of TPOAb in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Results Interpretation:

Old Reference ranges:	Thyroglobulin Antibody	≤40 IU/mL
	TPO Antibody	≤34 IU/mL
New Reference ranges:	Thyroglobulin Antibody	≤4 IU/mL
	TPO Antibody	≤8 IU/mL

Limitations:

1. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies
2. The Access Thyroglobulin Antibody II and TPO Antibody results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
3. The Access Thyroglobulin Antibody II assay does not demonstrate any "hook" effect up to approximately 50,000 IU/mL.
4. The Access TPO Antibody assay does not demonstrate any "hook" effect up to 10,000 IU/mL.
5. The Access TPO Antibody test result in and of itself is not diagnostic for thyroid disease and should be considered in conjunction with iodine uptake and other standard thyroid tests and the clinical presentation of the patient.
6. Moderately increased levels of TPO Antibody may be found in patients with non-thyroid autoimmune disease such as pernicious anemia, type I diabetes mellitus, or other disorders which activate the immune system.

Validation Data:

Precision

Precision was analyzed using two levels of control material, with 10 replicates in each run for two days. The CVs fall within the Manufacturer's claims of <10% for Thyroglobulin Antibody concentrations ≥15 IU/mL, and <12% for TPO Antibody concentrations ≥0.6 IU/mL. Criteria were met for both within run and between run precision for both assays (Tables 1 and 2).

Table 1 Thyroglobulin Antibody Precision

Within Run Precision				Between Run Precision			
IU/mL	**%CV**	**IU/mL**	**%CV**	**IU/mL**	**%CV**	**IU/mL**	**%CV**
64.07	2.61%	406.21	3.87%	61.34	5.91%	403.46	5.51%

Table 2 TPO Antibody Precision

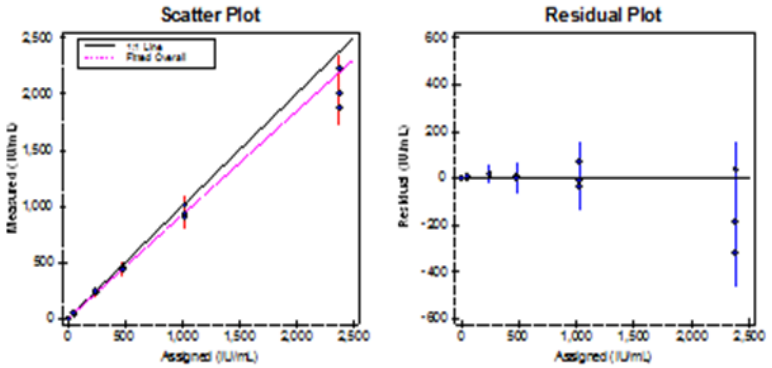
Within Run Precision				Between Run Precision			
IU/mL	**%CV**	**IU/mL**	**%CV**	**IU/mL**	**%CV**	**IU/mL**	**%CV**
18.03	5.06%	179.07	4.38%	18.37	5.04%	177.62	5.84%

Linearity

To verify the analytical measurement range of each assay, a minimum of five levels of known standard materials were used, spanning the analytical measurement range of 1-2500 IU/mL for Thyroglobulin Antibody and 1-1000 IU/mL for TPO Antibody.

For Thyroglobulin Antibody, linear regression analysis yielded a slope of 0.922 with an intercept of 0.04 and an error of 7.6%. The assay is linear within Allowable Systematic Error of 15.0%. (Figure 1)

Figure 1: Thyroglobulin Antibody Linearity



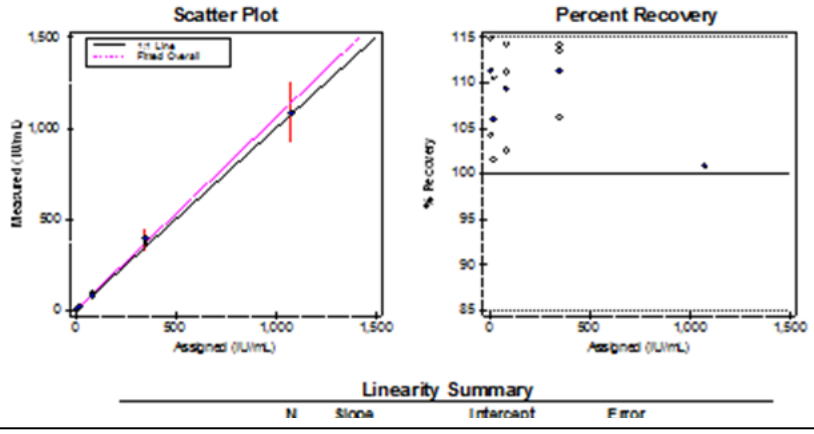
Linearity Summary

	N	Slope	Intercept	Error
Overall	6	0.922	0.04	7.6%

LINEAR within Allowable Systematic Error of 15.0%

For TPO Antibody, linear regression analysis yielded a slope of 1.058 with an intercept of 0.00 and an error of 5.0%. The assay is linear within Allowable Systematic Error of 15.0%. (Figure 2)

Figure 2: TPO Antibody Linearity



Linearity Summary

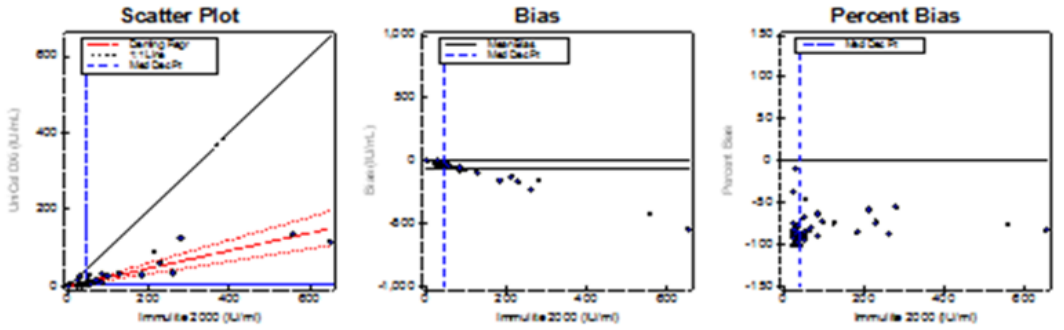
N	Slope	Intercept	Error

Method Comparison

For Thyroglobulin Antibody, a total of 58 specimens were split and processed utilizing the Siemens Immulite 2000 assay and the Beckman-Coulter DXi 800 assay. Four specimens were excluded from the calculations. Quantitative analysis yielded a correlation coefficient (R) of 0.9063 with slope of 0.231 and an intercept of -0.38. Overall bias for the DXi versus the Immulite was -56.20. Due to differences in antibody epitopes used by different manufacturers in immunoassays, large between-method variations in results for individual samples may be observed, even though the assays may both be traceable to standardized methods. Method comparisons in these cases may result in less than desirable correlation coefficients. The older Immulite assay is a less sensitive method, which may account for some of the variation. Overall performance of the assay is acceptable.

Figure 3: Thyroglobulin Antibody Method Comparison

X Method: Immulite 2000 Y Method: UniCel DXi



Regression Analysis

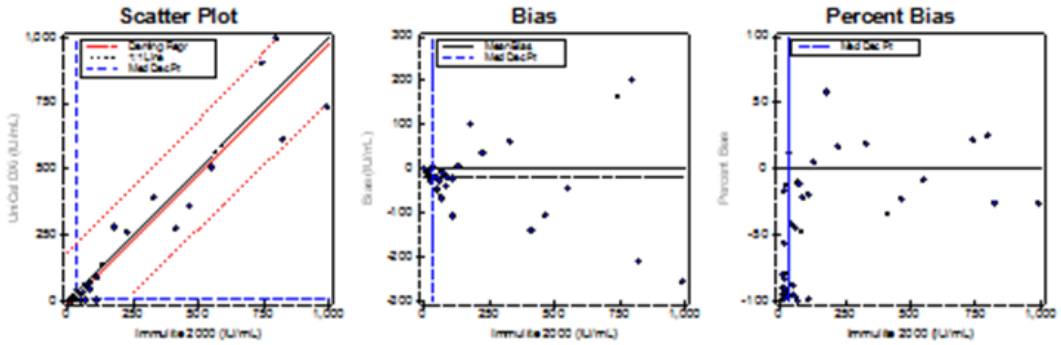
	Deming	Regular
Slope:	0.231 (0.201 to 0.261)	0.229 (0.199 to 0.258)
Intercept:	-0.38 (-4.60 to 3.84)	-0.20 (-4.41 to 4.02)
Std Err Est:	13.29	13.28

95% Confidence Intervals are shown in parentheses

For TPO Antibody, a total of 48 specimens were split and processed utilizing the Siemens Immulite 2000 assay and the Beckman-Coulter DxI 800 assay. Three specimens were excluded from the calculations. Quantitative analysis yielded a correlation coefficient (R) of 0.9571 with a slope of 0.996 and an intercept of -18.50. Overall bias for the DxI versus the Immulite was -19.04. (Figure 4)

Figure 4: TPO Antibody Method Comparison

X Method: Immulite 2000 Y Method: UniCel DXi



Regression Analysis

	Deming	Regular
Slope:	0.996 (0.907 to 1.086)	0.954 (0.865 to 1.043)
Intercept:	-18.50 (-44.58 to 7.58)	-12.05 (-37.86 to 13.75)
Std Err Est:	74.08	73.29

95% Confidence Intervals are shown in parentheses

Reference Range Verification

Manufacturer's published reference ranges were verified using separate studies of 20 normal samples and following CLSI EP28-A3C, section 11.2 guidelines, which describes transference "using small number of reference individuals."

References:

1. Beckman Coulter Thyroglobulin Antibody Assay Information Sheet, 2011.
2. Beckman Coulter TPO Antibody Assay Information Sheet, 2010.
3. CLSI EP28-A3C. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline