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

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Thyroglobulin
- Testing System Change –

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Affected Tests:

Mnemonics:	**TG**	**THYRO QUAN**	**THY PNL**
Test Name:	THYROGLOBULIN	THYROGLOBULIN GROUP	THYROID PANEL
Test Number:	1750029	1750030	1750023
Specimen:	0.5 mL Serum, Refrigerated (2-8^{o}C) 48 hours; 30 days frozen (-20^{o}C)		

Effective Date: Testing will begin on August 11, 2014 with samples received on August 9, 2014.

Performed: Monday through Saturday
Reference Range: 1.6 – 50.0 ng/mL

Method Change:
Thyroglobulin will be moved from the Siemens Immulite to the Beckman-Coulter DxI, as the Immulite is being phased out.

Adjustment to Patient Baseline:
For the period of August 11 through October 31 2014, CPAL will offer concurrent testing with the old and new methods (at no additional charge) to allow for determining a new baseline for each patient with the new method. The test result reported will be that of the new method. The result on the same sample utilizing the old method will be added in a result comment field for comparative purposes. ***All laboratories should announce this to their respective physicians so their patients can be notified to be tested during this window.***

Background:
The thyroid is a small endocrine gland located in the base of the neck. It consists of two lateral lobes connected by an isthmus. The gland produces a variety of metabolic hormones in a negative biofeedback loop.

Thyroglobulin (Tg) is a large glycoprotein (MW = 660,000) that is stored in the follicular colloid of the thyroid gland. Thyroglobulin functions as a prohormone in the intrathyroid synthesis of T4 and T3. Lysosomes containing proteases cleave T4 and T3 from Tg, resulting in release of T4 and T3.

Thyroglobulin is present in the serum of normal healthy individuals and can be elevated in numerous disorders which disrupt thyroid tissue. Elevated circulating levels of Tg have been reported in a number of thyroid conditions including Hashimoto's disease, Graves' disease, thyroid adenoma, subacute thyroiditis and thyroid

carcinoma.

Thyroid cancer is a relatively common form of cancer. It is not generally highly malignant, and normal life span can be obtained with appropriate follow-up and treatment. Females are affected 2 to 3 times more frequently than males. Thyroglobulin has become a useful tool in the follow-up of patients with differentiated thyroid carcinoma (i.e. papillary-follicular or follicular carcinoma of the thyroid). The thyroid is the only source of Tg; therefore, the serum Tg level will drop to a very low or undetectable level after total or near-total thyroidectomy and successful radioiodine ablation of the residual thyroid tissue. A rise in the serum level of Tg points to the recurrence of the disease. Thyroglobulin levels in patients who have undergone only a partial thyroidectomy will retain measurable levels of Tg, depending on how much tissue is remaining after surgery. These patients can be monitored by Tg measurement, but the post-surgical Tg level must be taken into account.

An additional monitoring tool used in conjunction with Tg is whole body scan (WBS) following a dose of ^{131}I. Generally, both Tg and WBS can be used to follow newly diagnosed and treated patients.

A limiting factor in the use of serum Tg measurements is the presence of Tg autoantibodies found in some patients. These antibodies may interfere with the immunoassay used to measure Tg and can cause false high or false low values. It is important to determine the levels of Tg autoantibodies in patients requiring serum Tg measurements.

Principle of Test:
The thyroglobulin assay is a simultaneous one-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel, along with a biotinylated mixture of four monoclonal anti-Tg antibodies, streptavidin coated paramagnetic particles, and monoclonal anti-Tg antibody alkaline phosphatase conjugate. The biotinylated antibodies and the serum or plasma thyroglobulin binds to the solid phase, while the conjugate antibody reacts with a different antigenic site on the thyroglobulin molecule. After incubation in a reaction vessel, 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 in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Results Interpretation:
Old Reference range: 2.0 – 56.0 ng/mL
New Reference range: 1.6 – 50.0 ng/mL

In patients who have undergone total or near-total thyroidectomy, with or without ^{131}I radioablation, the Tg concentration should approach zero or the functional sensitivity of the assay.

Limitations:

A. Any changes in serum Tg concentrations should be interpreted in light of the total clinical presentation of the patient, including clinical history, data from additional testing and other appropriate information. Single measurements of thyroglobulin are of minimal value in assessing disease status. Serial determinations are required, and should be referenced to the post-surgical baseline Tg result.

B. 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.

C. The Access (DxI) Thyroglobulin 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.

D. Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triolein (triglycerides) and hemolyzed samples containing up to 1 g/dL (10 g/L) hemoglobin do not affect the concentration of thyroglobulin assayed. In addition, samples with 5 g/dL (50 g/L) human serum albumin added to the endogenous albumin in the samples do not affect the concentration of thyroglobulin assayed.

E. Samples containing up to 50 mg/dL Aspirin, 20 mg/dL Acetaminophen, 40 mg/dL Ibuprofen, and 218.5 µg/dL of Thyroxine do not affect the concentration of thyroglobulin assayed.

F. The lowest detectable level of Tg distinguishable from zero (Access (DxI) Thyroglobulin Calibrator S0) with 95% confidence is 0.1 ng/mL.

G. The Access (DxI) Thyroglobulin assay does not demonstrate any "hook" effect up to 40,000 ng/mL.

H. Samples containing thyroglobulin antibodies (TgAb) cannot be reliably measured. All samples should be screened for Tg antibodies, and samples which are TgAb antibody positive should be interpreted with caution as the true value may be higher than that obtained.

Validation Data:

Precision

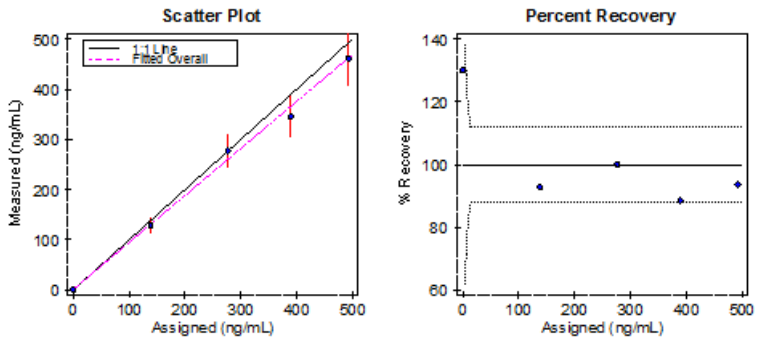
Precision was run using patient samples with 10 replicates in each run for two days. Manufacturer's claim for precision is CV <10% at >1 ng/mL. Criteria were met for both within run and between run precision (Table 1).

Table 1	**Thyroglobulin Precision**							
	Within Run Precision				**Between Run Precision**			
ng/mL	**%CV**	**ng/mL**	**%CV**		**ng/mL**	**%CV**	**ng/mL**	**%CV**
2.72	3.75%	38.09	4.70%		2.67	4.38%	38.45	3.76%

Linearity

To verify the analytical measurement range of the assay, a mixing experiment was conducting using patient samples and known standard materials to span the analytical measurement range 0-500 ng/mL. Linear regression analysis yielded a slope of 0.936 with an intercept of 0.894 ng/mL and error of 0.86 ng/mL or 6.1%. (Figure 1)

Figure 1: Thyroglobulin Linearity (ng/mL)



Linearity Summary

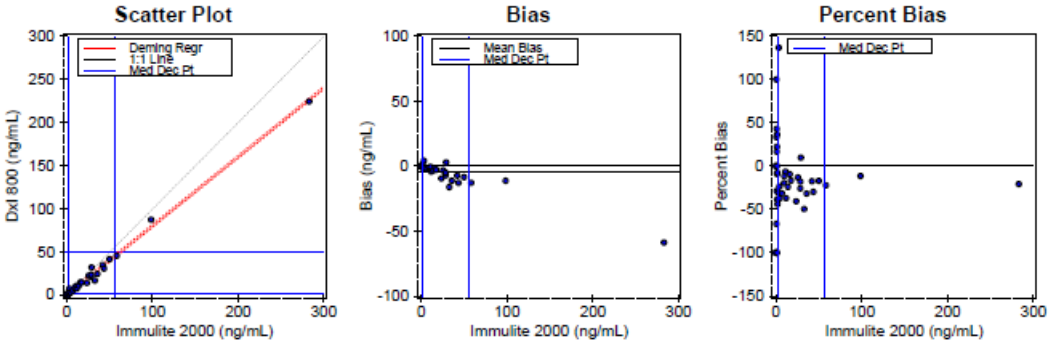
	N	Slope	Intercept	Error
Overall	5	0.936	0.894	0.86 ng/mL or 6.1%

LINEAR within Allowable Systematic Error of 1.7 ng/mL or 12.0%

Method Comparison

A total of 47 specimens were split and processed utilizing the Siemens Immulite 2000 assay and the Beckman-Coulter DxI 800 assay. Quantitative analysis yielded a correlation coefficient (r) of 0.997 with a slope of 0.80 and an intercept of 0.0 ng/mL. Overall bias for the DxI versus the Immulite was -19%, and bias within the reference range was -21%. Medical decision point analysis yielded values within 10% of manufacturer's stated range, with only 1 interpretive outlier of 47 specimens. Further, all 22 reference samples fell within the stated range of 1.6 – 50.0 ng/mL. (Figure 2)

Figure 2: Thyroglobulin Method Comparison (ng/mL)



Regression Analysis

	Deming	Regular
Slope:	0.800 (0.781 to 0.818)	0.798 (0.779 to 0.817)
Intercept:	0.00 (-0.91 to 0.91)	0.04 (-0.87 to 0.95)
Std Err Est:	2.81	2.80

95% Confidence Intervals are shown in parentheses

Medical Decision Point Analysis

Calculated by Deming Regression (R>=0.9)

X Method MDP	Y Method Pred. MDP	95% Conf. Limits Low	High
2.0	1.60	0.71	2.49
56.0	44.78	43.70	45.85

References:

1. Instructions for Use A34087D. Beckman-Coulter Inc., Brea CA. 2010.
2. Package Insert A34085D. Beckman-Coulter Inc., Brea CA. 2010.
3. Assay Summary Tables for use with the Access Family of Immunoassay Systems. Beckman-Coulter Inc., Brea CA. November 2013