Analytical and Clinical Performance of the ADVIA Centaur Anti-thyroglobulin (aTgII) Assay*

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Clinical Brief

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Introduction

Thyroglobulin (Tg) is a large, heterogeneous glycoprotein found in the follicular cells of the thyroid. Thyroglobulin plays an important role in the biosynthesis of thyroid hormones triiodothyronine (T3) and thyroxine (T4). Within the thyroid gland, the enzyme thyroid peroxidase catalyzes the iodination of thyroglobulin's tyrosyl groups. The iodinated thyroglobulin is stored in the colloid of the follicle and serves as a storage reservoir for T3 and T4. When the thyroid gland is stimulated, thyroglobulin is degraded, and energy-regulating hormones T3 and T4 are released into the bloodstream.[1-3]

Production of autoantibodies against thyroglobulin (anti-Tg) is a result of the immune system targeting components of the thyroid gland. The measurement of autoantibodies against thyroglobulin (anti-Tg) is useful in differentiating patients with autoimmune thyroid disease.[4-13] In patients with thyroid carcinoma, measurement of Tg antigen must consider the likelihood of the presence of significant levels of anti-Tg since measurement and detection of Tg antigen may be influenced by the presence of anti-Tg antibodies.[14-15]

Assay Principle

The ADVIA Centaur® Anti-thyroglobulin II (aTgII) Assay* is a fully automated, one-step, analyte-bridging immunoassay using acridinium ester chemiluminescent technology for the detection of thyroglobulin autoantibodies. This assay uses human thyroglobulin in both the Lite Reagent and the Solid Phase. A direct relationship exists between the amount of anti-Tg present in the patient sample and the number of light units (RLUs) detected by the system.

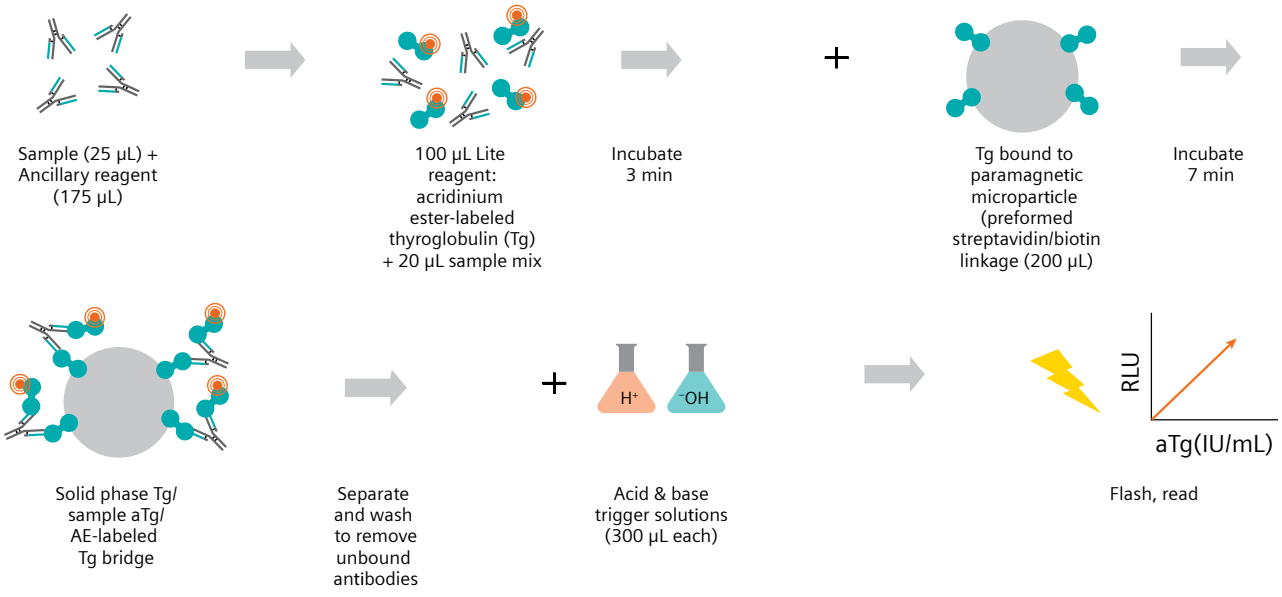

Figure 1. ADVIA Centaur aTgII Assay format.

Table 1. Assay attribute summary.

Sample Volume	25 µL
Time to Result	18 minutes
Specimen Types	Serum, plasma (lithium heparin and EDTA)
Measuring Interval	1.8–1000 IU/mL
Limit of Detection	1.3 IU/mL
Limit of Quantitation	1.8 IU/mL
Hook Effect	No hook effect up to 50,000 IU/mL

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Experimental Design

Analytical performance was studied in terms of precision, detection capability (LoB, LoD, and LoQ), interference, linearity, and hook effect. Clinical performance was established using a reference interval study and clinical concordance against a commercially available assay.

Limit of quantitation (LoQ)
- CLSI protocol EP17-A2[16]
- Nine serum pools, two runs/day for 5 days
- One reagent lot
- Within-laboratory precision calculated by analysis of variance (ANOVA)
- LoQ determined as the concentration at which the within-laboratory CV is ≤20% CV

Linearity
- CLSI protocol EP06-A[17]
- 11 samples, tested n = 3
- One reagent lot
- Analysis by percent bias from weighted linear regression

Precision
- CLSI protocol EP05-A3[18]
- Six specimens, two runs/day for 20 days
  – Six patient serum pools
- One reagent lot
- Data calculated by analysis of variance (ANOVA)
- Data reported as the upper 95% confidence limit

Interference
- CLSI protocol EP07-ed3[19]
- Three analyte test levels tested: 5, 50, and 500 IU/mL, tested n = 5
- Analysis by percent bias

Reference interval
- 198 serum specimens from apparently healthy males <30 years
- Tested in singlicate on one ADVIA Centaur XP system and one Beckman ACCESS 2 system
- One reagent lot on each system
- Analysis by receiver operating characteristic (ROC) curve

Assay clinical cutoff vs. predicate
- CLSI protocol EP09-ed3[20]
- 293 patients (Hashimoto's disease, Graves' disease, anemia, thyroid disease, non-thyroid autoimmune disease, and healthy patients)
- Tested in singlicate on one ADVIA Centaur XP system and one Beckman ACCESS 2 system (TgAb assay)
- One reagent lot on each system
- Analysis by receiver operating characteristic (ROC) curve

Results

Limit of quantitation

A limit of quantitation of 1.8 IU/mL was achieved for the assay.

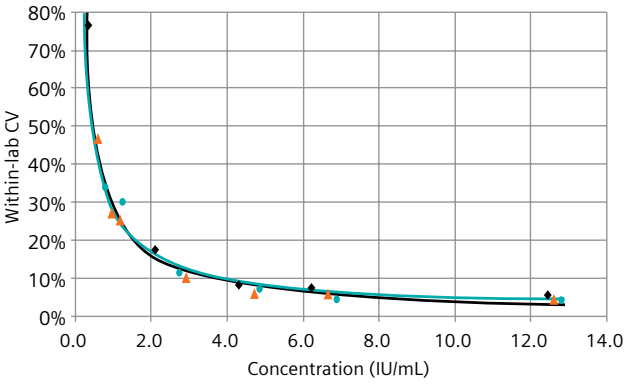

Figure 2. LoQ determination.

Assay linearity

Linearity of the assay was achieved within the measuring interval of 1.8–1000 IU/mL. The percent bias from the weighted linear fit was <10% across the assay range.

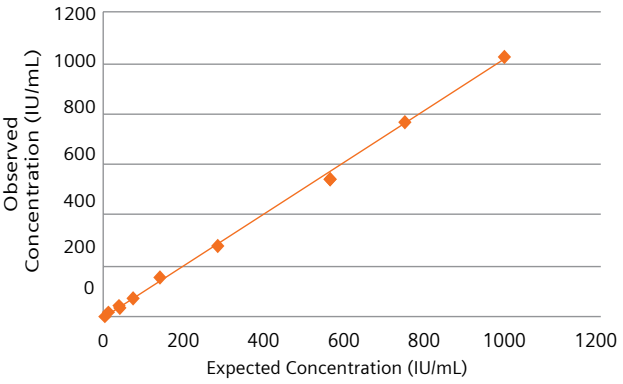

Figure 3. Assay linearity.

Precision

The precision of the assay met our design requirements. The 95% upper confidence limit for repeatability ranged from 1.8 to 6.0% CV. The 95% upper confidence limit for within-lab precision ranged from 4.2 to 7.7% CV.

Table 3. Precision.

Specimen	Dose (IU/mL)	Repeatability		Within Lab	
		SD	%CV	SD	%CV
1	6.73	0.4	5.9	0.5	7.3
2	13.0	0.8	6.0	1.0	7.7
3	18.3	0.7	4.0	1.0	5.6
4	50.4	1.6	3.1	2.4	4.8
5	496	8.9	1.8	20.7	4.2
6	872	27.9	3.2	49.3	5.7

Interferents

The ADVIA Centaur aTgII Assay was tested for interference from the substances listed in Table 4. No significant interference (<6%) was observed for these substances.

Table 4. Potential interferents.

Acetaminophen	Insulin
Aspirin	Iodine
Bilirubin (conjugated)	Lipemia using INTRALIPID
Bilirubin (unconjugated)	Low protein
Biotin at 3500 ng/mL	L-thyroxine (T4)
Hemoglobin	Methimazole
High protein	RF
Ibuprofen	T3 antibodies

High-dose hook effect

High anti-Tg concentrations can cause a paradoxical decrease in the RLUs (high-dose hook effect). In this assay, patient samples with anti-Tg concentrations above the measuring interval and as high as 50,000 IU/mL will report >1000 IU/mL.

Assay reference interval

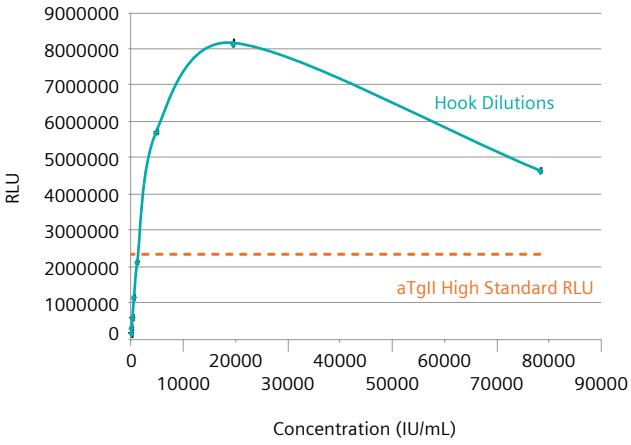

Figure 4. High-dose hook effect.

A reference interval for the presence of antithyroglobulin antibodies was established using 198 apparently healthy males <30 years of age who had no personal or family history of thyroid disease and no history of autoimmune disease. The 95% nonparametric upper reference limit was calculated to be <1.3 IU/mL. A cutoff value for autoimmune thyroid disease was determined using a receiver operating characteristic (ROC) curve to optimize clinical agreement with the Beckman anti-Tg II assay.

Clinical cutoff vs. predicate

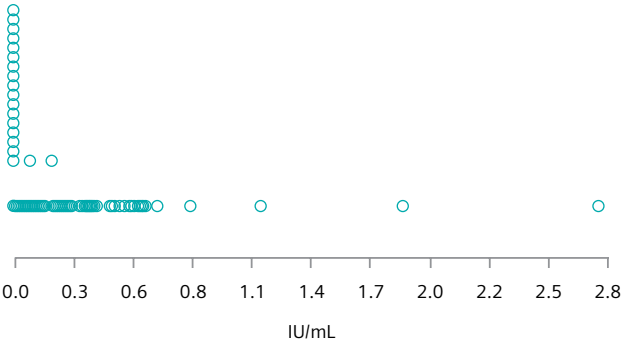

Figure 5. Nonparametric results (reference level 95%) for male patients <30 years old.

A total of 293 individuals were included in the study. With a cutoff value of 4.5 IU/mL, the positive percent agreement was 98.6% (71/72), with a 95% confidence interval (CI) of 92.5–100.0%. The negative percent agreement was 94.6% (209/221), with a 95% CI of 90.7–97.2%. The overall percent agreement was 95.6% (280/293), with a 95% CI of 92.5–97.6%.

Table 5. Method comparison.

ADVIA Centaur aTgII Assay	Beckman ACCESS 2 Tg-ab assay		
	Positive (≥4.0 IU/mL)	Negative (<4.0 IU/mL)	Total
Positive (≥4.5 IU/mL)	71	12	83
Negative (<4.5 IU/mL)	1	209	210
Total	72	221	293

Conclusion

The ADVIA Centaur aTgII Assay combines strong analytical performance with the workflow advantages of the automated ADVIA Centaur Immunoassay Systems.

- Fully automated assay on the ADVIA Centaur system with results in 18 minutes for serum and plasma samples
- Cutoff for autoimmune disease of 4.5 IU/mL
- LoQ of 1.8 IU/mL
- Repeatability ≤6.0%; within-lab precision ≤7.7%
- No interference to 3500 ng/mL biotin
- High positive (98.6%), negative (94.6%), and overall 95.6%) agreement with the Beckman ACCESS Thyroglobulin Antibody II assay

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