

# Performance Characteristics of Five Automated Immunoassays for anti-Tg and anti-TPO

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## Abstract

Measurement of thyroglobulin (anti-Tg) and thyroid peroxidase (anti-TPO) autoantibodies are important in the diagnosis of thyroid disease. This study evaluated five automated immunoassays (Bayer ADVIA Centaur, Abbott ARCHITECT *i*2000, Abbott AxSYM, DPC IMMULITE 2000 and Beckman Coulter UniCel Dxl 800) for anti-Tg and five (ADVIA Centaur, ARCHITECT *i*2000, AxSYM, IMMULITE 2000 and Roche Modular E170) for anti-TPO for the following parameters: limit of detection, imprecision and concordance. The limit of detection for both anti-Tg and anti-TPO assays were below the manufacturers' claim for each method. Imprecision studies were conducted with three levels of quality control materials: low, medium and high. In the anti-Tg assay, total imprecision ranged from a low of 2.6% for the ARCHITECT *i*2000 (medium concentration) to a high of 14.9% for the UniCel Dxl 800 (low concentration). In the anti-TPO assay, total imprecision ranged from a low of 2.1% for the ADVIA Centaur (medium concentration) to a high of 15.8% for the Modular E170 (low concentration). Concordance was determined first by method comparison with the Nichols Advantage for both anti-Tg and anti-TPO assays using the manufacturers' recommended instrument cutoffs (see **Table 4**). The ARCHITECT *i*2000 demonstrated the best concordance with the Advantage methods for both assays. Reference interval studies were then conducted using 69 samples. These data were used to adjust the assay cutoffs and concordance with the Advantage method was recalculated based on the new cutoffs. Recalculated concordance for anti-Tg ranged from 68.5% (ADVIA Centaur) to 84.7% (ARCHITECT *i*2000). For the anti-TPO assays recalculated concordance with the Advantage method ranged from 77.6% (IMMULITE 2000) to 84.3% (Modular E170). Adjusted cutoffs improved concordance for all methods for both assays. Overall, all anti-Tg and anti-TPO assays performed well. However, the results of the concordance studies for the anti-Tg and anti-TPO assays may have important implications clinically.

## Introduction

The measurement of autoantibodies to thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) are useful in identifying patients with autoimmune thyroid disease.<sup>1</sup> In iodine sufficient areas it is usually not necessary or cost-effective to measure both TgAb and TPOAb, since TPOAb-negative patients with detectable TgAb rarely display thyroid dysfunction.<sup>2</sup> TPOAb measurements may also be useful as a risk factor for thyroid dysfunction during therapy with certain drugs, hypothyroidism in Down's syndrome, thyroid dysfunction during pregnancy and post-partum thyroiditis.<sup>2</sup> TgAb measurements are recommended for all samples prior to Tg analysis because even low TgAb titers may have unpredictable effects on Tg results.<sup>2</sup> TgAb measurements may also be useful in patients with nodular goiter and in monitoring iodine therapy for endemic goiter (in iodine deficient areas).<sup>2</sup> Several quantitative, automated immunoassays for the detection of TgAb and TPOAb in patient sera have been developed. Due to the known variability and poor standardization among methods, the National Academy of Clinical Biochemistry (NACB) has noted the importance of assessing the analytical and clinical performance of commercially available thyroid autoantibody assays.<sup>2</sup> As part of this assessment, the NACB has recommended criteria for establishing reference intervals for thyroid autoantibody assays comprised of young, biochemically euthyroid male subjects, with no goiter and no family history of autoimmune thyroid disease. A review of the literature found a limited group of method comparison studies, yet failed to identify a comprehensive study evaluating the performance characteristics of each method. Therefore, we conducted experiments evaluating the performance characteristics of five automated TgAb and TPOAb assays.

## Methods

TgAb and TPOAb assays were evaluated on the following automated instruments: ADVIA Centaur (Bayer Healthcare Diagnostics), ARCHITECT *i*2000 (Abbott Diagnostics), AxSYM (Abbott Diagnostics), IMMULITE 2000 (Diagnostic Products Corporation), Modular E170 (Roche Diagnostics, TPO only), and the UniCel Dxl 800 (Beckman Coulter, TgAb only). An Advantage analyzer (Nichols Diagnostics) was used as the comparison method for both assays.

The limit of detection was determined by performing four separate runs and averaging the mean. In each run, there were 10 replicates of the "0" material and four replicates of the "non-zero" calibrator specific for each instrument.

Imprecision was performed using commercially available quality control material (BIORAD<sup>®</sup> Liquichek<sup>™</sup> Specialty Immunoassay Control Levels 1, 2, and 3). Since BIORAD Control Level 1 was below the reportable range for IMMULITE 2000 TgAb, UniCel Dxl 800 TgAb, and the ADVIA Centaur TPOAb, different commercially available control materials were used for these three methods (IMMULITE Thyroid Autoantibody Control 1, ARCHITECT Calibrator B, and ADVIA Centaur a TPO 1 Control, respectively).

Method comparison studies were performed using serum specimens submitted for TgAb and TPOAb testing. Specimens were obtained and de-identified after completion of clinical testing. The Advantage, which was used in our clinical laboratory for both assays was arbitrarily chosen as the comparison method for this study.

To evaluate reference intervals, serum specimens from 69 apparently healthy male individuals, 15 – 33 years of age, TSH values between 0.3 – 4.0 mIU/L, who were not taking any prescription drugs, were used.

# Results

**Table 1. Limit of Detection**

Method	TgAb (kIU/L)		TPOAb (kIU/L)	
	Average	Claim	Average	Claim
ADVIA Centaur*	7.75	10	11.3	15
ARCHITECT $\geq$ 2000	0.02	1.0	0.07	1.0
AxSYM	1.36	2.0	0.55	1.0
IMMULITE 2000	0.34	2.2	0.63	5.0
Modular E170	NA	NA	1.52	5.0
UniCel Dxl 800	0.30	2.2	NA	NA

**Table 2. Summary of Imprecision Data for the Tg-Ab Assay**

Method	Mean Tg-Ab (kIU/L)	Within Run CV (%)	Between Run CV (%)	Between Day CV (%)	Total CV (%)
<b>ADVIA Centaur</b>					
Level 1	42.5	8.6	8.3	0.0	12.0
Level 2	206	4.3	0.0	0.0	4.3
Level 3	482	3.8	2.8	0.0	4.7
<b>ARCHITECT <math>\geq</math>2000</b>					
Level 1	8.33	7.4	10.4	0.0	12.8
Level 2	69.2	2.2	0.0	1.3	2.6
Level 3	146	1.6	0.5	2.2	2.8
<b>AxSYM</b>					
Level 1	34.3	4.3	2.6	4.3	6.6
Level 2	117	2.6	3.1	0.4	4.1
Level 3	262	3.2	3.1	0.0	4.5
<b>IMMULITE 2000</b>					
Level 1*	44.1	5.0	0.0	2.5	5.5
Level 2	115	3.4	3.0	3.3	5.6
Level 3	262	2.0	2.8	1.9	3.9
<b>UniCel Dxl 800</b>					
Level 1*	25.2	7.3	7.4	10.7	14.9
Level 2	217	3.3	1.0	7.7	8.5
Level 3	435	2.2	4.1	4.3	6.3

\*Used manufacturers' control material instead of BIORAD Liquichek Level 1.

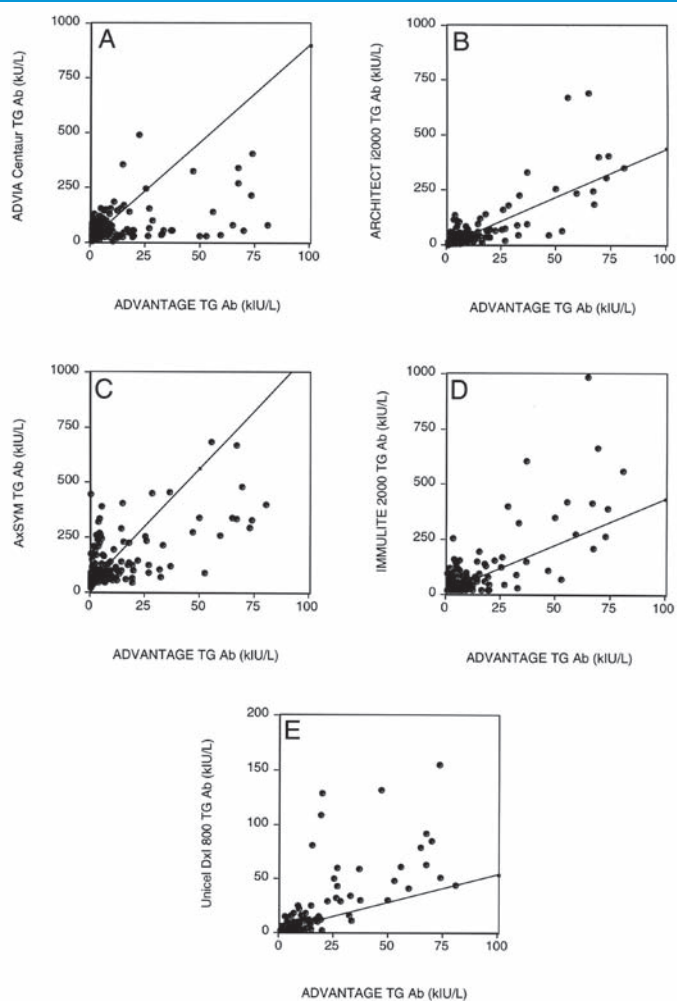
**Table 3. Summary of Imprecision Data for the TPO-Ab Assay**

Method	Mean TPOAb (kIU/L)	Within Run CV (%)	Between Run CV (%)	Between Day CV (%)	Total CV (%)
<b>ADVIA Centaur</b>					
Level 1*	76.0	12.7	0.0	6.6	14.3
Level 2	396	1.4	1.4	0.8	2.1
Level 3	1213	6.2	0.0	1.2	6.3
<b>ARCHITECT <math>\geq</math>2000</b>					
Level 1	3.16	11.2	5.7	0.0	12.6
Level 2	102	2.4	0.7	0.1	2.5
Level 3	245	3.3	0.0	1.0	3.4
<b>AxSYM</b>					
Level 1	13.1	3.6	3.2	2.7	5.5
Level 2	84.9	6.5	0.0	0.0	6.5
Level 3	214	4.5	1.0	1.2	4.7
<b>IMMULITE 2000</b>					
Level 1	24.5	3.9	2.2	5.3	7.0
Level 2	113	3.4	3.8	0.0	5.1
Level 3	263	2.9	2.6	0.0	3.9
<b>Modular E170</b>					
Level 1	26.8	9.4	0.0	12.6	15.8
Level 2	152	5.1	0.0	4.6	6.9
Level 3	318	5.5	0.0	2.9	6.2

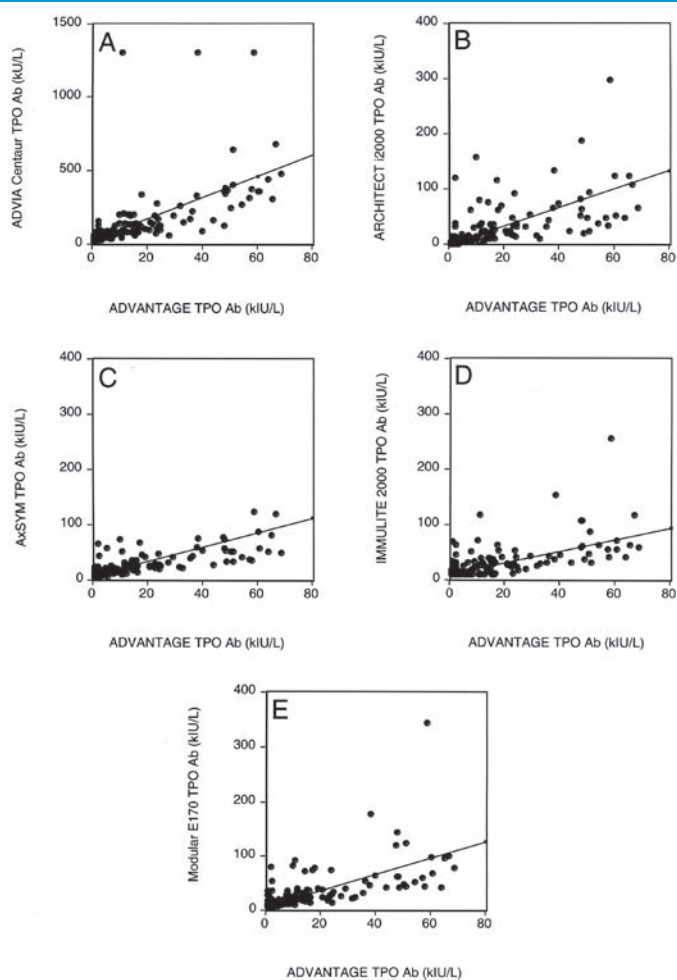
\*Used manufacturer's control material instead of BIORAD Liquichek Level 1

**Table 4. Summary of Diagnostic Concordance Data**

Instrument (Manufacturers' Recommended Cutoffs)	Anti-Tg		Anti-TPO				
	Concordance With Advantage (Manufacturers' Recommended Cutoffs)	Instrument (Reference Interval Cutoffs)	Concordance With Advantage (Reference Interval Adjusted Cutoffs)	Instrument (Manufacturers' Recommended Cutoffs)	Concordance With Advantage (Manufacturers' Recommended Cutoffs)	Instrument (Reference Interval Cutoffs)	Concordance With Advantage (Reference Interval Adjusted Cutoffs)
ADVIA Centaur Neg (<60.0) Pos (>60.0)	135/203 66.5%	ADVIA Centaur Neg (<71.2) Pos (>71.2)	139/203 68.5%	ADVIA Centaur Neg (<60.0) Pos (>60.0)	171/210 81.4%	ADVIA Centaur Neg (<52.2) Pos (>52.2)	171/210 81.4%
ARCHITECT $\geq$ 2000 Neg (< 4.11) Pos (>4.11)	165/203 81.3%	ARCHITECT $\geq$ 2000 Neg (<14.4) Pos (>14.4)	172/203 84.7%	ARCHITECT $\geq$ 2000 Neg (<5.61) Pos (>5.61)	178/210 84.8%	ARCHITECT $\geq$ 2000 Neg (<3.91) Pos (>3.91)	178/210 84.8%
AxSYM Neg (< 34.0) Pos (>34.0)	110/203 54.2%	AxSYM Neg (<71.2) Pos (>71.2)	148/203 72.9%	AxSYM Neg (<12.0) Pos (>12.0)	161/210 76.7%	AxSYM Neg (<26.2) Pos (>26.2)	164/210 78.1%
IMMULITE 2000 Neg (<40.0) Pos (>40.0)	161/203 79.3%	IMMULITE 2000 Neg (<20.0) Pos (>20.0)	167/203 82.3%	IMMULITE 2000 Neg (<35.0) Pos (>35.0)	132/210 62.9%	IMMULITE 2000 Neg (<30.8) Pos (>30.8)	163/210 77.6%
Modular E170	Assay not Available	Modular E170	Assay not Available	Modular E170 Neg (<34.0) Pos (>34.0)	136/210 64.8%	Modular E170 Neg (<20.1) Pos (>20.1)	177/210 84.3%
UniCel Dxl 800 Neg (<4.9) Pos (>4.9)	160/203 78.8%	UniCel Dxl 800 Neg (<3.8) Pos (>3.8)	163/203 80.3%	UniCel Dxl 800	Assay not Available	UniCel Dxl 800	Assay not Available



**Figure 1.** Method comparison of TgAb assays. **A**, Evaluation of the ADVIA Centaur TgAb method. Passing-Bablok regression analysis gave a slope of 8.82, an intercept of 14.69 kU/L and  $r = 0.48$ . **B**, Evaluation of the ARCHITECT *i*2000 TgAb method. Passing-Bablok regression analysis gave a slope of 4.36, an intercept of -0.16 kU/L and  $r = 0.82$ . **C**, Evaluation of the AxSYM TgAb method. Passing-Bablok regression analysis gave a slope of 10.45, an intercept of 40.62 kU/L and  $r = 0.67$ . **D**, Evaluation of the IMMULITE 2000 TgAb method. Passing-Bablok regression analysis gave a slope of 4.17, an intercept of 18.33 kU/L and  $r = 0.78$ . **E**, Evaluation of the UniCel Dxl 800 TgAb method. Passing-Bablok regression analysis gave a slope of 0.51, an intercept of 1.98 kU/L and  $r = 0.77$ .



**Figure 2.** Method comparison of TPOAb assays. **A**, Evaluation of the ADVIA Centaur TPOAb method. Passing-Bablok regression analysis gave a slope of 7.12, an intercept of 28.80 kU/L and  $r = 0.66$ . **B**, Evaluation of the ARCHITECT *i*2000 TPOAb method. Passing-Bablok regression analysis gave a slope of 1.65, an intercept of -0.40 kU/L and  $r = 0.66$ . **C**, Evaluation of the AxSYM TPOAb method. Passing-Bablok regression analysis gave a slope of 1.29, an intercept of 8.07 kU/L and  $r = 0.78$ . **D**, Evaluation of the IMMULITE 2000 TPOAb method. Passing-Bablok regression analysis gave a slope of 1.05, an intercept of 9.37 kU/L and  $r = 0.66$ . **E**, Evaluation of the Modular E170 TPOAb method. Passing-Bablok regression analysis gave a slope of 1.47, an intercept of 6.03 kU/L and  $r = 0.67$ .

## Conclusions

For all methods, the limit of detection was below the manufacturers' claimed value (see **Table 1**).

Imprecision for both TgAb and TPOAb assays was method-dependent, with total CVs as high as 15% and 16%, respectively. However, the AxSYM and IMMULITE 2000 had the best precision with total CVs being less than 7%, which is consistent with previous studies. For TgAb assays, the imprecision for Level 1 ranged from 5.5% (AxSYM) to 14.9% (UniCel Dxl 800) (see **Table 2**). For TPOAb assays, the imprecision for Level 1 ranged from 5.5% (AxSYM) to 15.8% (Modular E170) (see **Table 3**). As expected, methods were least precise at low antibody concentrations. However, in the case of UniCel Dxl 800 TgAb and Modular E170 TPOAb assays, the imprecision is around 15% at analyte concentrations near or above the upper reference limit.

The NACB guidelines state that TgAb and TPOAb assays should be standardized against the International Reference Preparation 65/93 and 66/387, respectively. Even though all assays claim to be referenced to the corresponding WHO International Reference Preparation, this standardization doesn't ensure cutoffs and/or results that are identical. Our data, which is consistent with previously published studies demonstrate that results vary widely depending on the method used.<sup>3,4</sup> The correlation coefficient for TgAb assays ranged from 0.48 (ADVIA Centaur) to 0.82 (ARCHITECT *i*2000) and Passing-Bablok slopes from 0.51 (UniCel Dxl 800) to 10.45 (AxSYM) (see **Figure 1**). For TPOAb assays, the correlation coefficient ranged from 0.66 (ADVIA Centaur, ARCHITECT *i*2000, and IMMULITE 2000) to 0.78 (AxSYM) and Passing-Bablok slopes from 1.05 (IMMULITE 2000) to 7.12 (ADVIA Centaur) (see **Figure 2**). Clearly, assay standardization with the WHO International Reference Preparation does not guarantee method agreement. This is demonstrated by both the varying slopes (not approaching unity) determined from the regression analysis and the discordant number of patient samples classified as positive or negative by the different methods. The method variability for both TgAb and TPOAb assays likely reflects antibody heterogeneity that is inherent in the patients and is independent of standardization efforts.

When we used our reference interval data to calculate new cutoffs, three of the five methods had decreased cutoffs for TgAb and four out of five had decreased cutoffs for TPOAb (see **Table 4**). Using these recalculated cutoffs generally improved diagnostic concordance for all methods. The differences we observed between the manufacturers' reference intervals and our in-house reference intervals supports concerns regarding inter-method variation.<sup>5</sup> They also highlight the need for individual laboratories to confirm that reference intervals are appropriate for the methods they use and the patient population they serve.

In conclusion, all TgAb and TPOAb assays showed acceptable analytical performance. However, the results of the concordance studies may have important implications clinically. Improved precision would be useful at lower concentrations at the upper reference limit. Also, continuing standardization efforts are needed for both assays. Additional studies are required for other performance characteristics such as functional sensitivity and antibody specificity. One area where this may be very important is the requirement to measure TgAb in all samples for which Tg testing is performed for thyroid cancer patients. The presence of TgAb may interfere with Tg measurements and their presence should be noted. The cutoff for detection of TgAb in this situation must be appropriate or misleading Tg results may be generated.

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