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Evaluation of the Analytical Performance of the Thyroglobulin and Anti-Thyroglobulin II Assays on the Atellica CI Analyzer

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Background

Thyroglobulin (Tg) is a homodimeric 660 kDa glycoprotein produced by the follicular cells of the thyroid gland where it serves as a substrate for the synthesis of thyroxine (T4) and triiodothyronine (T3). Upon stimulation, T3 and T4 are released into the circulation. Tg is present in the serum of healthy individuals and Tg concentrations can be elevated in various thyroid disorders such as subacute thyroiditis, autonomous adenoma, amiodarone induced thyrotoxicosis and thyroid carcinoma,[1] as well as in autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves' disease. Quantitative Tg measurements are used as an aid in monitoring differentiated thyroid cancer in patients after thyroidectomy, with or without radioiodine ablation.[1] Detectable or rising concentrations of serum Tg after total thyroidectomy can reflect persistent or recurrent disease.[2]

Elevated levels of autoantibodies against thyroid-specific proteins (Tg) and thyroid peroxidase (TPO) – anti-Tg antibodies along with anti-TPO antibodies are commonly seen in more than 90% of patients with Hashimoto's thyroiditis and Graves' disease, and anti-Tg antibodies are detected in 30%–60% of thyroid carcinoma cases. These autoantibodies play a key role in the diagnosis, monitoring, and management of these conditions. Moreover, because the presence of anti-Tg antibodies may interfere with serum Tg measurement with possible inaccurate results and diagnosis, anti-Tg testing should accompany every serum Tg measurement in the clinical setting.[3,4] Anti-Tg antibodies have been detected in other autoimmune diseases, clinically normal euthyroid patients, elderly patients, and various other conditions, including type 1 diabetes.

Previously, two quantitative assays were developed and commercialized for use on the Atellica IM Analyzer: Atellica IM Thyroglobulin (Tg)[5] and Anti-Thyroglobulin II (aTgII)[6] assays.

Recently, the Atellica CI Analyzer (Figure 1) was added to the Atellica Solution portfolio, with a reduced footprint of 1.9 square meters. The Atellica CI Analyzer is an integrated clinical chemistry and immunoassay analyzer designed for low- to mid-volume laboratories and features the same reagents, consumables, and sophisticated user interface as the Atellica IM and CH Analyzers.[7]

To evaluate the analytical performance of the Atellica IM Tg and aTgII assays using this new analyzer, precision, method comparison, detection capability, and linearity studies were assessed as performance indicators for the Atellica IM Tg and aTgII assays on the Atellica CI Analyzer.

Figure 1. The Atellica CI Analyzer

Principles of the Procedures

The Atellica IM Tg assay is a fully automated 1-step sandwich immunoassay using acridinium ester (AE) chemiluminescent technology and constant amounts of 2 monoclonal antibodies (mAbs)—one in the Lite Reagent (anti-human Tg mouse mAb labeled with AE) and the other in the Solid Phase (biotinylated anti-human Tg mouse mAb bound to streptavidin-coated paramagnetic latex particles). The Atellica IM aTgII assay is a fully automated 1-step analyte-bridging immunoassay using AE chemiluminescent technology for detection of Tg auto-Abs and uses human Tg in both the Lite Reagent (Tg labeled with AE) and the Solid Phase (Tg biotinylated and preformed to streptavidin-coated paramagnetic particles). Anti-Tg in the patient sample binds to Tg in the Lite Reagent and Solid Phase, forming a bridge. For both assays, a direct relationship exists between the amount of analyte in the sample and relative light units detected by the system.

Material and Methods

Precision (CLSI EP05-A3)

Repeatability and Within-Laboratory Precision

- Sample type:
  - Tg: Native human, contrived serum (mix of two unique native serum or mix of two unique native human serum spiked with Tg from thyroid tissue), and quality control samples (Bio-Rad Tumor Marker Control levels 1 to 3).
  - aTgII: One human stripped serum and five unique human serum pools spiked with aTg mAb.
- Samples were tested in duplicate/run, two runs/day, 20 days (n=80/sample), one reagent lot, two analyzers.
- Data were analyzed using a nested, two factor (days and runs nested within days) ANOVA model. Each system was analyzed separately.

Reproducibility: Composite estimate of the root sum of squares of standard deviation (SD) estimates of the following: repeatability, between-run, -day, -site and -lot variance components.

- n=5 replicates/sample, one run/day, 5 days, three lots, three analyzers (n=225 measurements/sample).
- Data analysis used a balanced crossed and nested ANOVA design. Instrument (Site) and Reagent Lot are crossed factors. Days is nested within both Instrument (Site) and Reagent Lot and Replicates are nested with Days. The following components of precision were calculated: repeatability, between-day, between-lot, between-instrument, and reproducibility (total).

Method Comparison (CLSI EP09C-ED3)

- Method comparison (MC) studies compared assay performance on Atellica IM and Atellica CI analyzers using singlicate native human serum and contrived samples (n>100). Testing was completed in 4 days on three reagent lots. Samples with a result outside the measuring interval of the assay were excluded from the analysis. Slopes were calculated by weighted Deming regression.

Detection Capability (CLSI EP17-A2)

LoB: Highest measurement result that is likely to be observed on a blank sample.

- Tg: Four blank samples (BSA buffer [BSAB]); BSAB with stripped human serum; BSAB with horse serum; stripped human serum), n=3 replicates/sample, one run/day, 5 test days, one analyzer, three reagent lots (total n=60/reagent lot).
- aTgII: Five blank (human native serum) samples, n=5/sample, two runs/day, 5 test days, three reagent lots, one analyzer (total n=250/reagent lot).
- LoB was calculated non-parametrically at the 95th percentile. For each lot, the rank position at the 95th percentile was determined as: Rank position = 0.5 + (n x 0.95), where n is the total number of replicates. The largest LoB calculated among the lots was the assay's LoB.

LoD: Smallest amount reliably detected for presence or absence of an analyte. The LoD = lowest concentration detectable with 95% probability.

- Tg: Six samples (BSAB x 2, stripped human serum, horse serum x 2, and buffered goat serum—each mixed with pooled human serum) were assayed in two replicates/run, two runs/day for 20 test days, one analyzer and three reagent lots (n=80/sample/reagent lot).
- aTgII: Eight samples (human native serum, human stripped serum x 3, human native serum spiked with aTg mAb x 4). Five replicates/run, two runs/day, 5 test days, three reagent lots, one analyzer (n=50/sample/reagent lot).
- The within-laboratory standard deviation was plotted versus the measured analyte concentration in each sample for each lot. These data were fitted with a power function to give a precision profile. The lowest analyte concentration that could be differentiated from the LoB with 95% confidence was the LoD for that lot. The largest LoD among the lots was the assay's LoD.

LoQ: Here, LoQ is defined by functional sensitivity—analyte level defined by modeling with a within-laboratory CV of 20%.

- Tg: Six samples (BSAB x 2, stripped human serum, horse serum x 2, buffered goat serum mixed with pooled human serum), n=2 replicates/sample, two runs/day for 20 days, three reagent lots, one analyzer/lot (n=80/sample/reagent lot).
- aTgII: Eight samples (human native serum, human stripped serum x 3, human native serum spiked with aTg mAb x 4), n=5 replicates/sample, two runs/day, 5 test days, three reagent lots, one analyzer (total n=50/sample/reagent lot).
- Data analysis: The within-laboratory precision was plotted versus the measured analyte concentration in each sample for each lot, then fitted using a power function to give a precision profile. Functional sensitivity for each reagent lot was determined as the analyte concentration corresponding to 20% within-laboratory CV or the LoD, whichever is greater. The largest calculated functional sensitivity among the lots was the assay's functional sensitivity (LoQ).

Linearity (CLSI EP06-ED2)

- Tg: A dilution series with eleven levels was prepared by mixing high (stripped human serum spiked with human Tg from thyroid tissue) and low (stripped human serum) samples.
- aTgII: A dilution series with eleven levels was prepared by mixing high (stripped human serum spiked with aTg positive human serum) and low (pool of native low human serum) samples.
- n=5 replicates/level, one test day/lot, three reagent lot (n=5/level/lot).
- Expected values were calculated from the measured concentrations of the low and high samples. Bias was calculated for each sample as the difference between the mean observed value and the value predicted by the linear regression model.

Results

Precision

Table 1. Precision for the Atellica IM Tg assay on the Atellica CI Analyzer. Results presented are representative of one instrument/lot combination.

Specimen Type	Mean (n=80)		Repeatability			Within-laboratory Precision		
	(ng/mL)	(pmol/L)	SD (ng/mL)	SD (pmol/L)	CV (%)	SD (ng/mL)	SD (pmol/L)	CV (%)
Serum A	0.088	0.133	0.0041	0.0062	4.7	0.0095	0.0144	10.8
Serum B	0.439	0.665	0.0073	0.0111	1.7	0.0142	0.0215	3.2
Serum C	1.798	2.724	0.0262	0.0397	1.5	0.0504	0.0764	2.8
Serum D	6.230	9.438	0.1431	0.2168	2.3	0.2543	0.3853	4.1
Serum E	25.737	38.992	0.2446	0.3706	1.0	0.8424	1.2762	3.3
Serum F	67.511	102.279	0.6054	0.9172	0.9	2.4320	3.6845	3.6
Serum G	128.784	195.108	1.9440	2.9452	1.5	5.8018	8.7897	4.5
Control 1	4.197	6.358	0.0890	0.1348	2.1	0.1441	0.2183	3.4
Control 2	45.894	69.529	0.7298	1.1056	1.6	2.0852	3.1591	4.5
Control 3	115.990	175.725	1.8699	2.8329	1.6	5.8618	8.8806	5.1

[a]n: number of measurements; [b]SD: standard deviation; [c]CV: coefficient of variation.

Across the sample interval, repeatability CV was ≤4.7% and within-laboratory CV was ≤10.8%. Repeatability and within-laboratory precision fulfilled requirements at <0.439 ng/mL (0.665 pmol/L).

Table 2. Reproducibility for the Atellica IM Tg assay on the Atellica CI Analyzer.

Specimen Type	Mean (n=225) ng/mL (pmol/L)	Repeatability		Between Day		Between Lot		Between Instrument		Reproducibility	
		SD ng/mL (pmol/L)	CV (%)	SD ng/mL (pmol/L)	CV (%)	SD ng/mL (pmol/L)	CV (%)	SD ng/mL (pmol/L)	CV (%)	SD ng/mL (pmol/L)	CV (%)
Serum A	0.089 (0.135)	0.0033 (0.0050)	3.7	0.0053 (0.0080)	6.0	0.0122 (0.0185)	13.7	0.0099 (0.0150)	11.1	0.0169 (0.0256)	19.0
Serum B	0.451 (0.683)	0.0084 (0.0127)	1.9	0.0085 (0.0129)	1.9	0.0143 (0.0217)	3.2	0.0045 (0.0068)	1.0	0.0192 (0.0291)	4.3
Serum C	1.954 (2.960)	0.0246 (0.0373)	1.3	0.0389 (0.0589)	2.0	0.0705 (0.1068)	3.6	0.0511 0.0774)	2.6	0.0985 (0.1492)	5.0
Serum D	6.567 (9.949)	0.1137 (0.1723)	1.7	0.0984 (0.1491)	1.5	0.3307 (0.5010)	5.0	0.1304 (0.1976)	2.0	0.3859 (0.5846)	5.9
Serum E	26.638 (40.357)	0.3616 (0.5478)	1.4	0.4946 (0.7493)	1.9	0.9224 (1.3974)	3.5	0.3166 (0.4796)	1.2	1.1517 (1.7448)	4.3
Serum F	70.086 (106.180)	0.9751 (1.4773)	1.4	1.5071 (2.2833)	2.2	1.293 (1.9589)	1.8	1.1913 (1.8048)	1.7	2.5126 (3.8066)	3.6
Serum G	135.162 (204.770)	2.0246 (3.0673)	1.5	2.8774 (4.3593)	2.1	2.8341 (4.2937)	2.1	3.0228 (4.5795)	2.2	5.4358 (8.2352)	4.0
Control 1	4.121 (6.243)	0.0867 (0.1314)	2.1	0.0453 (0.0686)	1.1	0.2159 (0.3271)	5.2	0.0408 (0.0618)	1.0	0.2405 (0.3644)	5.8
Control 2	45.804 (69.393)	0.9773 (1.4806)	2.1	0.9989 (1.5133)	2.2	1.1834 (1.7929)	2.6	0.2158 (0.3269)	0.5	1.8438 (2.7934)	4.0
Control 3	118.461 (179.468)	2.1682 (3.2848)	1.8	2.9207 (4.4249)	2.5	1.9636 (2.9749)	1.7	1.0914 (1.6535)	0.9	4.2753 (6.4771)	3.6

Tg reproducibility was ≤5.9%CV at ≥6.567 ng/mL (9.949 pmol/L); and SD was 0.0169 ng/mL (0.0256 pmol/L) at <0.451 ng/mL (0.683 pmol/L).

Table 3. Precision for the Atellica IM aTgII assay on the Atellica CI Analyzer. Results presented are representative of one instrument/lot combination.

Specimen Type	n = 225 Mean (IU/mL)	Repeatability		Within-laboratory	
		SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)
Serum A	8.1	0.43	5.3	0.50	6.2
Serum B	13.0	0.36	2.8	0.50	3.8
Serum C	18.4	0.64	3.5	0.71	3.9
Serum D	48.5	1.23	2.5	1.56	3.2
Serum E	486.5	15.02	3.1	15.49	3.2
Serum F	840.3	24.96	3.0	28.82	3.4
Control 1	52.4	1.98	3.8	2.08	4.0
Control 2	489.8	11.25	2.3	14.95	3.1

Across the sample interval, repeatability CV was ≤5.3% and within-laboratory precision CV was ≤6.2%.

Table 4. Reproducibility for the Atellica IM aTgII assay on the Atellica CI Analyzer.

Specimen Type	Mean (n=225) ng/mL (pmol/L)	Repeatability		Between Day		Between Lot		Between Instrument		Reproducibility	
		SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)
Serum A	2.4	0.20	8.3	0.16	6.7	0.20	8.3	0.07	2.9	0.33	13.8
Serum B	5.0	0.39	7.8	0.10	2.0	0.14	2.8	0.13	2.6	0.44	8.8
Serum C	15.1	0.48	3.2	0.34	2.3	0.80	5.3	0.02	0.1	0.99	6.6
Serum D	43.6	1.23	2.8	0.38	0.9	2.36	5.4	0.39	0.9	2.71	6.2
Serum E	440.5	11.88	2.7	9.66	2.2	26.88	6.1	3.84	0.9	31.17	7.1
Serum F	728.7	16.93	2.3	16.37	2.2	44.90	6.2	8.13	1.1	51.35	7.0
Control 1	46.4	1.33	2.9	0.81	1.7	2.30	5.0	0.48	1.0	2.82	6.1
Control 2	443.2	13.50	3.0	6.35	1.4	23.10	5.2	5.64	1.3	28.07	6.3

Total reproducibility CV was ≤8.8% at ≥5.0 IU/mL; SD was <0.33 IU/mL at <5.0 IU/mL.

Method Comparison

Table 5. Performance of the Atellica IM Tg and aTgII assays on the Atellica CI Analyzer compared with the performance of the comparison assay on the indicated system (x). The following results are representative of the performance of the assay using one reagent lot.

Specimen Type	Assay	Comparison Analyzer/system (x)	n	r	Regression Equation	Sample Range
Serum	Atellica IM Tg	Atellica IM	102	0.998	y=1.02x-0.033 ng/mL (y=1.02x-0.050 pmol/L)	0.074–148.115 ng/mL (0.112–224.394 pmol/L)
Serum	Atellica IM aTgII	Atellica IM	100	1.00	y=1.01x-0.1 IU/mL	2.1–892.1 IU/mL

n: number of samples; r: correlation coefficient.

The design requirements for method comparison were met for Atellica IM Tg and aTgII assays on the Atellica CI Analyzer. When analyzed by regression, each Atellica IM assay on the Atellica CI Analyzer recovered samples spanning the measuring interval, with a slope of 1.0 ± 0.1 and a correlation coefficient ≥ 0.95 (r) compared to the Atellica IM Analyzer.

Weighted Deming fit and percent difference plots on the Atellica CI Analyzer for sample ranges indicated in Table 5 are shown for the two assays on Atellica CI vs Atellica IM Analyzers in Figure 2.

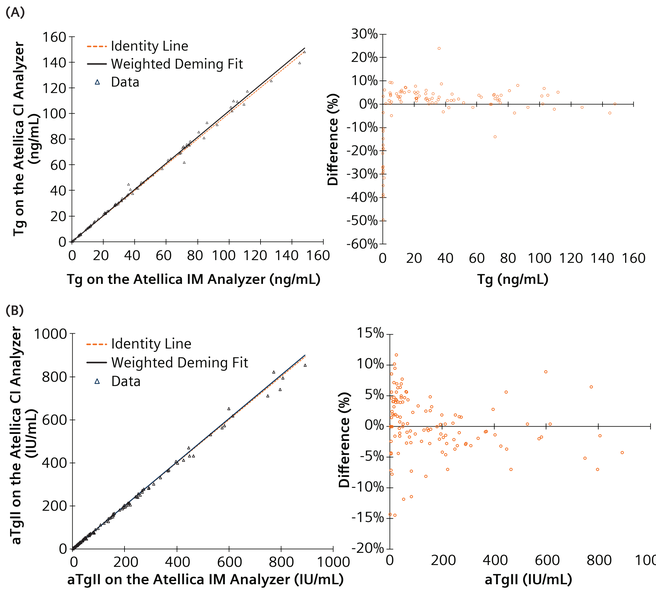

Figure 2. Weighted Deming regression and difference plots for the Atellica IM Tg assay (A) and Atellica IM aTgII (B) assay on the Atellica IM and Atellica CI Analyzers.

Detection Capability

Table 6. LoB, LoD, and LoQ for the Atellica IM Tg and aTgII assays on the Atellica CI Analyzer.

Specimen Type	Assay	LoB and Total LoD Replicates per Reagent Lot	LoB Reported	LoD Reported	LoQ Total Replicates per Reagent Lot	LoQ Reported
Serum	Atellica IM Tg	LoB 60 LoD 480 (80/sample)	0.036 ng/mL (0.055 pmol/L)	0.046 ng/mL (0.070 pmol/L)	480 (80/sample)	0.050 ng/mL (0.076 pmol/L)
Serum	Atellica IM aTgII	LoB 250 LoD 400 (50/sample)	0.6 IU/mL	1.0 IU/mL	400 (50/sample)	1.3 IU/mL

Linearity

Table 7. Linearity interval for the Atellica IM Tg and aTgII assays on the Atellica CI Analyzer. The following results are representative of the performance of the assay.

Specimen Type	Assay	# of Sample Combinations Tested	Linearity Interval Reported
Serum	Atellica IM Tg	11	0.050–150.000 ng/mL (0.076–227.250 pmol/L)
Serum	Atellica IM aTgII	11	1.3–1000 IU/mL

The Atellica IM Tg and aTgII assays are linear on the Atellica CI Analyzer across the intervals indicated.

Analytical study results on the Atellica CI Analyzer demonstrated similar performance to claims for the Atellica IM Analyzer.

Conclusion

Evaluation of the Atellica IM Tg and aTgII assays using the Atellica CI Analyzer demonstrated acceptable analytical performance and acceptable concordance compared to the Atellica IM Analyzer.

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