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Evaluation of the Analytical Performance of the Androstenedione Assay on the Atellica CI Analyzer

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Background

Androstenedione is a 19-carbon steroid that acts as a precursor to both testosterone and estrone (Figure 1). It is primarily produced from dehydroepiandrosterone (DHEA) through the action of 3β-hydroxysteroid dehydrogenase in the ovaries, testes, and adrenal glands.[1-3] Testing for androstenedione is often performed alongside other steroid assays to assess the function of the adrenal glands, ovaries, or testes, and to help identify the cause of symptoms related to androgen excess.[3-7] Additionally, androstenedione tests are used to monitor treatment for congenital adrenal hyperplasia.[8-9]

Previously, the Atellica IM Androstenedione (ANDRO) assay[14] was developed and commercialized for use on the Atellica IM Analyzer. Recently, the Atellica CI Analyzer (Figure 2) was added to the Atellica Solution portfolio, with a reduced footprint of 1.9 square meters.[15] The Atellica CI Analyzer is an automated, integrated chemistry and immunoassay analyzer employing both Atellica CH and Atellica IM assays, designed for low- to mid-volume laboratories and features the same reagents, consumables, and sophisticated user interface as the Atellica IM and CH Analyzers.[15]

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

Principles of the Procedure

The Atellica IM ANDRO assay is a competitive chemiluminescent assay. Androstenedione in the sample competes with acridinium ester-labeled hapten in the Lite Reagent for binding to the anti-androstenedione sheep monoclonal antibody in the Solid Phase reagent. An inverse relationship exists between the amount of androstenedione present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Figure 2. The Atellica CI Analyzer

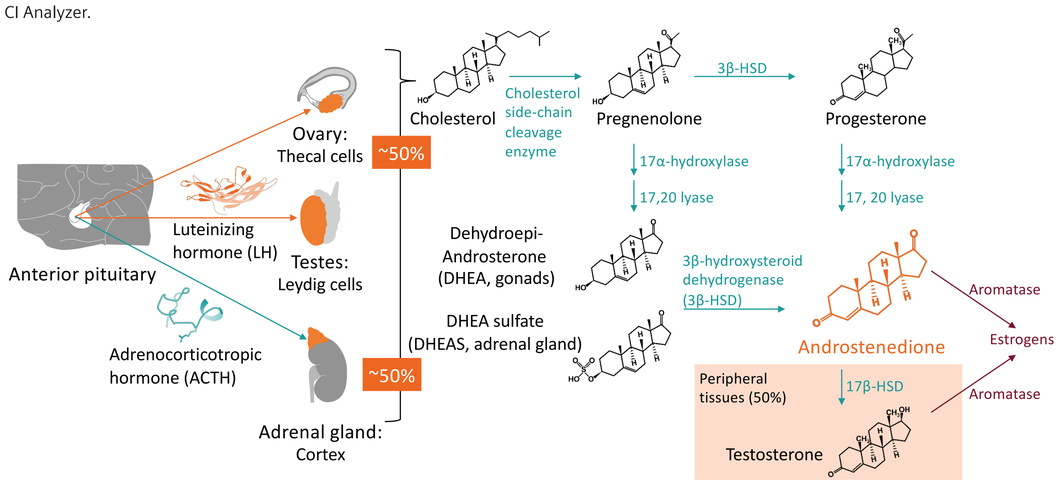

Figure 1. Androstenedione is derived from cholesterol and is a precursor of testosterone.[10-13]

Material and Methods

Precision (CLSI EP05-A3)

Repeatability and Within-Laboratory Precision

- Sample type: Native human serum and quality control samples.
- Repeatability and within-laboratory precision samples were tested in duplicate/run, two runs/day, 20 days (n=80/sample/analyzer), one reagent lot, two analyzers.

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

- n=5 replicates/sample, one run/day, 5 test days, three lots, three analyzers (n=225 measurements/sample).
- Data analysis: 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 variance 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 the ANDRO assay performance on the Atellica IM and the Atellica CI analyzers. Samples (n≥105 native human serum and contrived) were assayed in a singlicate with three reagent lots and one analyzer. Slopes were calculated by Weighted Deming regression. The system determines the result using the calculation scheme described in the system online help. The system reports results in ng/mL (common units) or nmol/L (SI units), depending on the units defined when setting up the assay.
  Conversion formula: 1.0 ng/mL = 3.4915 nmol/L.

Detection Capability (CLSI EP17-A2)

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

- Five blank samples, 6 replicates/run, two runs/day, 5 test days, three reagent lots, each on one of three analyzers (total n=300 measurements/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.

- Ten samples were assayed in 6 replicates/run, two runs/day for 5 test days, one analyzer and three reagent lots (n=60 measurements/sample/reagent lot).
- Due to the Levene and normality tests failing, a nonparametric method was used. For each lot, the 5th percentile value of the measurement results was calculated for each low-level sample. The lowest median of a sample where the 5th percentile was >LoB was taken as LoD for the lot. The largest LoD calculated among 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 ≤11%.

- Samples (native human serum x 11, contrived mixture of 11 unique human serum samples), n=6 replicates/run, two runs/day (minimum of 2 hours between runs) for 5 days, three reagent lots, one analyzer/lot (n=60 measurements/sample/reagent lot).
- Data analysis: The within-laboratory precision was plotted versus the measured analyte concentration in each sample for each lot. These data were 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)

- A dilution series with 11 levels was prepared by mixing high (contrived human serum/processed serum spiked with antigen) and low (native human serum) samples.
- Six replicates/level over 1 day/lot for three reagent lots.
- Expected values were calculated from the measurand 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 Weighted Least Squares regression model.

Results

The following results are representative of the performance of the assay.

Precision

Table 1. Precision for the Atellica IM ANDRO assay on the Atellica CI Analyzer.

Sample Type	n[a] = 80 Mean		Repeatability			Within-Lab		
	ng/mL	nmol/L	SD[b] (ng/mL)	SD (nmol/L)	CV[c] (%)	SD (ng/mL)	SD (nmol/L)	CV (%)
Serum A	0.41	1.43	0.015	0.052	3.7	0.03	0.105	7.3
Serum B	0.91	3.18	0.02	0.07	2.2	0.039	0.136	4.3
Serum C	2.16	7.54	0.04	0.14	1.9	0.08	0.279	3.7
Serum D	4.48	15.64	0.085	0.297	1.9	0.13	0.454	2.9
Serum E	6.77	23.64	0.193	0.674	2.9	0.243	0.848	3.6
Control 1	0.51	1.78	0.015	0.052	2.9	0.031	0.108	6.1
Control 2	1.03	3.6	0.02	0.07	1.9	0.038	0.133	3.7
Control 3	2.38	8.31	0.043	0.15	1.8	0.072	0.251	3.0

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

Across the sample interval, repeatability was ≤4.3 %CV and within-laboratory precision ≤5.0 %CV.

Results

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

Sample Type n=225	Mean (ng/mL)	Mean (nmol/L)	Repeatability			Between-Day			Between-System			Between-Lot			Reproducibility		
			SD (ng/mL)	SD (nmol/L)	CV %	SD (ng/mL)	SD (nmol/L)	CV %	SD (ng/mL)	SD (nmol/L)	CV %	SD (ng/mL)	SD (nmol/L)	CV %	SD (ng/mL)	SD (nmol/L)	CV %
Serum A	0.43	1.50	0.017	0.059	4.0	0.013	0.045	3.0	0.023	0.080	5.3	0.008	0.028	1.9	0.032	0.112	7.4
Serum B	0.93	3.25	0.022	0.077	2.4	0.020	0.070	2.2	0.008	0.028	0.9	0.018	0.063	1.9	0.036	0.126	3.9
Serum C	2.21	7.72	0.047	0.164	2.1	0.036	0.126	1.6	0.044	0.154	2.0	0.023	0.080	1.0	0.077	0.269	3.5
Serum D	4.61	16.10	0.104	0.363	2.3	0.094	0.328	2.0	0.142	0.496	3.1	0.000	0.000	0.0	0.199	0.695	4.3
Serum E	6.80	23.74	0.197	0.688	2.9	0.111	0.388	1.6	0.233	0.814	3.4	0.000	0.000	0.0	0.324	1.131	4.8
Serum F	0.57	1.99	0.018	0.063	3.2	0.014	0.049	2.5	0.026	0.091	4.6	0.005	0.017	0.9	0.035	0.122	6.1
Control 1	1.26	4.40	0.030	0.105	2.4	0.022	0.077	1.7	0.057	0.199	4.5	0.017	0.059	1.3	0.070	0.244	5.6
Control 2	1.84	6.42	0.039	0.136	2.1	0.029	0.101	1.6	0.108	0.377	5.9	0.030	0.105	1.6	0.122	0.426	6.6
Control 3	0.43	1.50	0.017	0.059	4.0	0.013	0.045	3.0	0.023	0.080	5.3	0.008	0.028	1.9	0.032	0.112	7.4

Across the sample interval, reproducibility was ≤7.4 %CV.

Method Comparison

Table 3. Performance of the Atellica IM ANDRO assay on the Atellica CI and Atellica IM Analyzers.

Sample Type	n	r	Regression Equation	Sample Range
Serum	110	0.998	y=1.01 + -0.10 nmol/L (y=1.01x + -0.03 ng/mL)	1.15 to 31.28 nmol/L (0.33 to 8.96 ng/mL)

n: number of samples; r: correlation coefficient.

The design requirements for method comparison were met for Atellica IM ANDRO assay on the Atellica CI Analyzer. When analyzed by Weighted Deming regression, the Atellica IM ANDRO assay on the Atellica CI Analyzer recovered samples spanning the measuring interval, with a slope of 1.00 ± 0.10 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 range indicated in Table 3 are shown for the ANDRO assay on Atellica CI vs Atellica IM Analyzers in Figure 3.

Atellica IM ANDRO assay

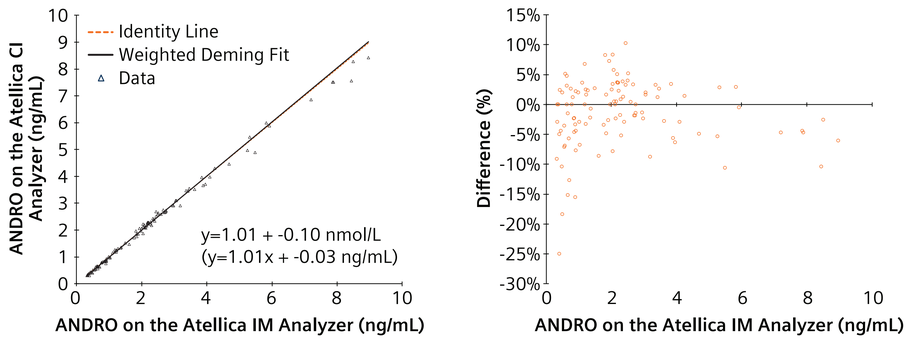

Figure 3. Weighted Deming regression and difference plot for the Atellica IM ANDRO assay and on the Atellica IM and Atellica CI Analyzers.

Detection Capability

Table 4. LoB, LoD, and LoQ for the Atellica IM ANDRO assay on the Atellica CI Analyzer.

Specimen Type	LoB and LoD Total Measurements per Reagent Lot	LoB Reported	LoD Reported	LoQ Total Replicates per Reagent Lot	LoQ Reported
Serum	LoB 300/lot (60/sample) LoD 600/lot (60/sample)	0.49 nmol/L (0.14 n/mL)	0.63 nmol/L (0.18 ng/mL)	640 (80/sample)	0.70 nmol/L (0.20 ng/mL)

Linearity

Table 5. Linearity interval for the Atellica IM ANDRO assay on the Atellica CI analyzer.

Specimen Type	# of Sample Combinations Tested	Linearity Interval Reported
Serum	11	1.05–31.42 nmol/L (0.30–9.00 ng/mL)

The Atellica IM ANDRO assay is linear on the Atellica CI Analyzer across the interval indicated.

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

Conclusions

The Atellica IM ANDRO assay on the Atellica CI Analyzer demonstrated acceptable precision and equivalent performance to the Atellica IM Analyzer.

References

1. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev. 2004 Dec;25(6):947–970.
2. Speiser PW, Azziz R, Baskin LS, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010 Sep;95(9):4133–4160.
3. Rodin A, Thakkar H, Taylor N, et al. Hyperandrogenism in polycystic ovarian syndrome. Evidence of dysregulation of 11ß-hydroxysteroid dehydrogenase. N Engl J Med. 1994 Feb;330(7):460–465.
4. Georgopoulos NA, Papadakis E, Armeni AK, et al. Elevated serum androstenedione is associated with a more severe phenotype in women with polycystic ovarian syndrome (PCOS). Hormones (Athens). 2014 Apr-Jun;13(2):213–221.
5. O'Reilly MW, Taylor AE, Crabtree NJ, et al. Hyperandrogenemia predicts metabolic phenotype in polycystic ovarian syndrome: the utility of serum androstenedione. J Clin Endocrinol Metab. 2014 Mar;99(3):1027–1036.
6. Fox R, Corrigan E, Thomas PA, et al. The diagnosis of polycystic ovaries in women with oligo-amenorrhea: predictive power of endocrine tests. Clin Endocrinol (Oxf). 1991 Feb; 34(2):127–131.
7. Summers RH, Herold DA, Seely BL. Hormonal and genetic analysis of a patient with congenital adrenal hyperplasia. Clin Chem. 1996 Sep;42(9):1433–1487.
8. Debono M, Mallappa A, Gounden V, et al. Hormonal circadian rhythms in patients with congenital adrenal hyperplasia: identifying optimal monitoring times and novel disease biomarkers. Eur J Endocrinol. 2015 Dec;173(6):727–737.
9. Otten BJ, Wellen JJ, Rijken JC, et al. Salivary and plasma androstenedione and 17-hydroxyprogesterone levels in congenital adrenal hyperplasia. J Clin Endocrinol Metab. 1983 Dec;57(6):1150–1154.
10. Turcu A, et al. Compr Physiol 2014;4:1369-81.
11. Rosenfield RL, et al. Endocr Rev 2016;37:467-520.
12. Lizneva D, et al. Best Pract Res Clin Obstet Gynaecol 2016;37:98-118.
13. http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/basics/steroidogenesis.html (Accessed Nov. 13, 2019).
14. Atellica IM Analyzer. 11646604_EN Rev. 03, 2023-11.
15. Technical specifications. Atellica CI Analyzer. CLS-23-3123-76. QR700003930. 12-2023.

The products/features mentioned here are not commercially available in all countries. Their future availability cannot be guaranteed.

Data/some data first presented at Worldlab IFCC 2025.

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