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Siemens Global Headquarters

Siemens AG
Wittelsbacherplatz 2
80333 Muenchen
Germany

Global Siemens Healthcare Headquarters

Siemens AG
Healthcare Sector
Henkestrasse 127
91052 Erlangen
Germany
Telephone: +49 9131 84 - 0
www.siemens.com/healthcare

Global Division

Siemens Healthcare Diagnostics Inc.
1717 Deerfield Road
Deerfield, IL 60015-0778
USA
www.siemens.com/diagnostics

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Evaluation of a New Dehydroepiandrosterone Sulfate Assay on the ADVIA Centaur System

Lindhout E[1], Koeken H[1], Martens M[1], Readio J[2], Barbarakis M[2], Ku C[2], Jing Q[2], Anderson-Mauser L[2]

[1]Future Diagnostics, Wijchen, The Netherlands;
[2]Siemens Healthcare Diagnostics, Tarrytown, NY, US

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Abstract

Background: Dehydroepiandrosterone sulfate (DHEAS) is the most abundant adrenal androgen. It exhibits weak androgenic activity, but can be metabolized to more active androgens. Measurement of DHEAS is a useful marker for adrenal function and for investigating a variety of other conditions, such as hirsutism, alopecia, infertility, and precocious puberty.

Methods: The assay is a one-step, competitive immunoassay using direct, chemiluminescence technology in combination with paramagnetic particles. The time to first result is 18 minutes with subsequent results delivered every 15 seconds. The assay uses a monoclonal antibody specific for DHEAS and has a calibration range of 0–1,500 µg/dL.

Results: Preliminary data showed that total imprecision of the assay controls at 51.5 (low), 491 (medium), and 800 µg/dL (high) was 9.1%, 5.7%, and 5.9%, respectively. Analytical sensitivity across 2 reagent lots and instruments was ≤1.7 µg/dL (95% confidence interval). Dilution linearity of multiple samples (n = 12) demonstrated mean recoveries of 94.3–105.5% (mean of 99.1%). Assay results were not significantly affected by a variety of potentially cross-reacting or interfering substances. Comparison of the assay to another commercially available method gave the following data (Passing-Bablok regression): Centaur DHEAS = 0.99x – 10.1, n = 92, r = 0.94.

Conclusions: Our data demonstrate that the ADVIA Centaur® DHEAS provides reliable results across a wide range of clinically relevant concentrations.

Background

Measurement of circulating levels of DHEAS, an adrenal steroid, is important in investigations of abnormal hair growth (hirsutism) and balding (alopecia) in women.[1–3] It is also of value in the assessment of adrenarche and delayed puberty.[2,4] DHEAS in circulation originates almost entirely from the adrenals, though in men some may also derive from the testes.[5,6] Although DHEAS is weakly androgenic it can metabolize to more potent androgens such as androstenedione and testosterone, and thus increased levels can be an indirect cause of hirsutism or virilization.[1,7]

Plasma levels of DHEAS increase steadily from about the seventh year of life, then gradually decline after the third decade.[5–7] The upper limit of normal for young adults is approximately 300 µg/dL for women, and 500 µg/dL for men.8

DHEAS is secreted into the bloodstream at a rate that is only somewhat greater than DHEA, but has a much slower turnover and its half-life is nearly 24 hours. Because of its slower catabolism, plasma levels for DHEAS are almost one thousand–fold higher than DHEA.[2,7,9] Unlike cortisol, DHEAS does not exhibit significant diurnal variation.[2,10] Also, unlike testosterone, it does not circulate bound to sex hormone binding globulin (SHBG).[7] Its abundance, together with its within-day and day-to-day stability makes DHEAS an excellent indicator of adrenal androgen output.[1,2,8,11]

In women, DHEAS is often assayed in conjunction with free testosterone as an initial screen for hyperandrogenism when hirsutism is present.[12,13] High DHEAS levels are also often encountered in polycystic ovary syndrome (PCOS)11 while very high levels of DHEAS, i.e., greater than 700–800 µg/dL, may suggest the presence of a hormone-secreting adrenal tumor.[9]

Materials and Methods

Samples: The ADVIA Centaur DHEAS assay requires 25 µL of serum for a single determination. Patient serum samples were obtained from a commercial source.

Precision: The precision of the DHEAS assay was estimated from medical decision pools, calibrators and Biorad controls run as unknowns. The dose was calculated using 2-point data reduction from the day 0 calibration. Seven (7) samples were analyzed using two reagent lots and two analyzers. Imprecision estimates were collected and computed according to the CLSI EP5-A protocol.[14]

Analytical Sensitivity: The analytical sensitivity was determined using the 95th percentile of the “zero” level 1 calibrator.

Dilutional linearity: The dilutional linearity was determined using serial dilutions of low- and high concentration samples in accordance with the CSLI EP6-A guideline.[15]

Interference studies: Potential interferents including hemoglobin, triglycerides, protein (bovine serum albumin), and bilirubin were added to 3 patient sample pools (with low, medium, and high concentrations of DHEAS) and evaluated for potential interference in the DHEAS assay.

Results

Precision

The total CVs ranged from 5.7% to 9.1%. The within-run CVs were less than 6.8% for all samples. Results for each reagent lot and the RMS (root mean square) are shown in Table 1.

Table 1. Precision of the ADVIA Centaur DHEAS assay determined using medical decision pools (MDP), controls (BRctrl) and the calibrator (CalH).

Within-run precision (dose, µg/dL)

		MDP1	MDP2	CalH	MDP3	BRctrl1	BRctrl2	BRctrl3
Lab lot 1	Mean	50.2	381	484	789	111	129	453
	SD	3.00	17.2	23.2	34.5	5.63	4.70	18.3
	CV(%)	6.0	4.5	4.8	4.4	5.1	3.6	4.0
Lab lot 2	Mean	52.8	404	498	810	112	131	445
	SD	3.54	17.7	16.2	45.1	4.79	6.80	17.1
	CV(%)	6.8	4.4	3.3	5.6	4.3	5.2	3.8
	Mean	51.5	393	491	800	112	130	449
	Mean SD	3.27	17.4	19.7	39.8	5.21	5.75	17.72
	Mean CV(%)	6.4	4.5	4.1	5.0	4.7	4.4	3.9

Total precision (dose, µg/dL)

		MDP1	MDP2	CalH	MDP3	BRctrl1	BRctrl2	BRctrl3
Lab lot 1	Mean	50.2	381	484	789	111	129	453
	SD	4.29	21.8	29.4	44.8	7.79	7.08	28.1
	CV(%)	8.6	5.8	6.1	5.6	7.0	5.5	6.2
Lab lot 2	Mean	52.8	404	498	810	112	131	445
	SD	5.00	26.2	25.9	50.2	7.56	8.88	28.6
	CV(%)	9.5	6.5	5.2	6.2	6.8	6.8	6.4
	Mean	51.5	393	491	800	112	130	449
	Mean SD	4.64	24.0	27.7	47.5	7.68	7.98	28.3
	Mean CV(%)	9.1	6.2	5.7	5.9	6.9	6.2	6.3

Analytical Sensitivity (minimal detectable concentration)

Analytical sensitivity is defined as the concentration corresponding to the concentration (in µg/dL) at the 95th percentile of the expected calibrator level 1 rate distribution.

The analytical sensitivity was a calculated as the mean of 3 lots:
- Lot 1: 1.59 µg/dL
- Lot 2: 2.69 µg/dL
- Lot 3: 0.69 µg/dL
- Mean dose: 1.66 µg/dL

Cross-reactivity

Cross-reactivity was tested for 14 different steroids (Table 2).

Table 2. Cross-reactivity of similar hormones.

	Spike dose (µg/dL)	Mean dose (µg/dL)	Cross-reactivity
DHEA	4000	1.44	0.04%
Aldosterone	5000	0.00	0.00%
Androstenedione	1000	0.00	0.00%
Androsterone	2000	0.00	0.00%
Androsterone-glucuronide	5000	1.29	0.03%
Cortisol	10000	2.01	0.02%
5-Dihydrotestosterone	5000	0.00	0.00%
Estradiol	5000	2.31	0.05%
Estriol	5000	1.08	0.02%
Estrone	5000	0.00	0.00%
Testosterone	2000	0.00	0.00%
19-Hydroxyandrostenedione	5000	0.00	0.00%
Progesterone	5000	0.00	0.00%
β-Estradiol-3-sulfate-17-glucuronide	5000	0.95	0.02%

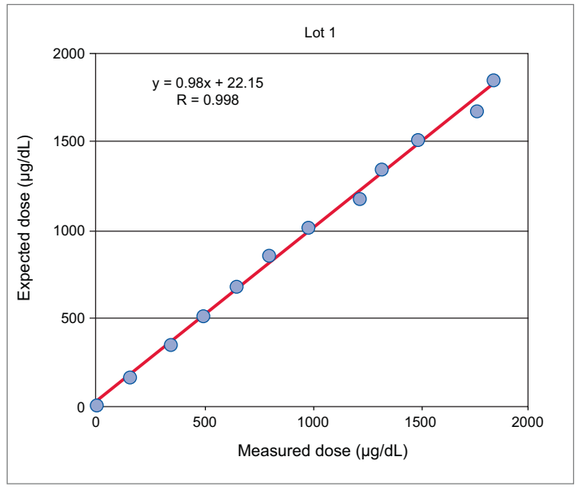
Dilution Recovery

Linearity was evaluated across the reportable range of the DHEAS assay. Volumetric dilutions of an overrange sample were prepared and measured using two reagent lots as per the CSLI EP6-A guideline. Table 3 and Figure 1 show the individual results obtained with lot 1.

Table 3. Dilution recovery results for lot 1.

	Sample	Measured IU/mL	Expected IU/mL	Recovery %
1	High	1844	1844	
2	10 High + 1 Low	1764	1672	105.5
3	9 High + 2 Low	1496	1505	99.4
4	8 High + 3 Low	1322	1343	98.4
5	7 High + 4 Low	1225	1182	103.6
6	6 High + 5 Low	986	1008	97.8
7	5 High + 6 Low	803	852	94.2
8	4 High + 7 Low	654	682	95.9
9	3 High + 8 Low	496	514	96.5
10	2 High + 9 Low	351	348	100.8
11	1 High + 10 Low	163	165	98.8
12	Low	5.29	5.29	
	Mean recovery			**99.1**

Figure 1. Dilution linearity of lot 1.



Interference

Endogenous interfering substances had no effect beyond the limits of random within-run variations. The results demonstrated <10% interference from each of the substances tested using the DHEAS assay (Table 4).

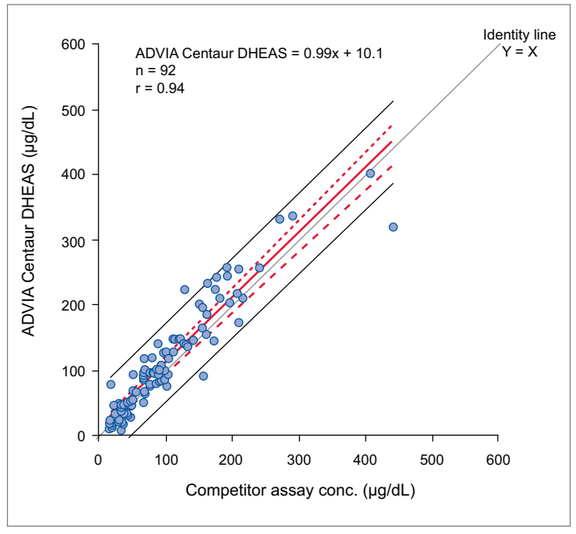
Table 4. Interference study results.

Substance	Sample (µg/dL)	Amount of substance added	Result (µg/dL)	Recovery %
Conjugated Bilirubin	Low	0 mg/dL	47.7	
		5 mg/dL	46.5	97.5
	Middle	0 mg/dL	364	
		5 mg/dL	362	99.5
	High	0 mg/dL	711	
		5 mg/dL	701	98.6
Unconjugated Bilirubin	Low	0 mg/dL	50.0	
		5 mg/dL	50.1	100.2
	Middle	0 mg/dL	384	
		5 mg/dL	383	99.7
	High	0 mg/dL	766	
		5 mg/dL	779	101.7
Intralipid 20%	Low	0 mg/dL	48.6	
		1000 mg/dL	48.3	99.4
	Middle	0 mg/dL	349	
		1000 mg/dL	355	101.7
	High	0 mg/dL	699	
		1000 mg/dL	724	103.6
Hemoglobin	Low	0 mg/dL	35.8	
		500 mg/dL	38.6	107.8
	Middle	0 mg/dL	292	
		500 mg/dL	299	102.4
	High	0 mg/dL	577	
		500 mg/dL	587	101.7
Protein (total)	Low	0 mg/dL	51.1	
		12g/dL	52.0	101.8
	Middle	0 mg/dL	351	
		12g/dL	349	99.4
	High	0 mg/dL	689	
		12g/dL	689	100.0
D-Biotin	Low	0 ng/dL	46.3	
		10 ng/mL	47.7	103.0
	Middle	0 ng/mL	349	
		10 ng/mL	366	104.9
	High	0 ng/mL	739	
		10 ng/mL	725	98.1
	Low	0 ng/mL	46.3	
		60 ng/mL	50.1	108.2
	Middle	0 ng/mL	349	
		60 ng/mL	375	107.4
	High	0 ng/mL	739	
		60 ng/mL	765	103.5
	Low	0 ng/mL	46.3	
		100 ng/mL	53.4	115.3
	Middle	0 ng/mL	349	
		100 ng/mL	391	112.0
	High	0 ng/mL	739	
		100 ng/mL	785	106.2
Silwet L720	Low	0 µg/mL	45.8	
		10 µg/mL	47.4	103.5
	Middle	0 µg/mL	344	
		10 µg/mL	344	100.0
	High	0 µg/mL	696	
		10 µg/mL	698	99.0
	Low	0 µg/mL	45.8	
		100 µg/mL	47.8	104.4
	Middle	0 ng/dL	344	
		100 µg/mL	360	104.7
	High	0 µg/mL	696	
		100 µg/mL	700	100.6
	Low	0 µg/mL	45.8	
		1000 µg/mL	49.4	107.9
	Middle	0 µg/mL	344	
		1000 µg/mL	357	103.8
	High	0 µg/mL	696	
		1000 µg/mL	721	103.6

Method Comparison

A total of 92 random donor samples were tested in the ADVIA Centaur and a commercially available DHEAS assay. Linear regression indicates excellent comparison between the two assays (Figure 2).

Figure 2. Comparison between the Advia Centaur DHEAS assay and a competitor's assay.



Conclusion

- The Siemens ADVIA Centaur DHEAS assay is a rapid competitive immunoassay that is sensitive and precise.
- The assay provides reliable results across a wide range of clinically relevant concentrations.

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