

Multi-Center Evaluation of the Dehydroepiandrosterone Sulfate (DHEA-S) Assay on the Abbott ARCHITECT® i System

C. van Duren¹, H. Buddiger¹, M. Martens¹, T. Mart², J. Bertran², J. Dhein³, M. Eppinger³, M. Hausmann³, N. Jenne³, M. Stieler³ and E. Sickinger³ ¹ Future Diagnostics, Wijchen, The Netherlands; ² Biokit, Lliçà d'Amunt, Barcelona, Spain;

³ Abbott, Diagnostics Division, Delkenheim, Germany

American Association for Clinical Chemistry Annual Meeting • Chicago, Illinois • July 23 – 27, 2006

Abstract

Background and Objective

Dehydroepiandrosterone sulfate (DHEA-S) is the most abundant adrenal androgen. It exhibits weak androgenic activity, but can be metabolized to more active androgens. Measurement of DHEA-S is a useful marker for adrenal function, and for investigating a variety of other conditions. The objective of our study was to perform a multi-center evaluation of the Abbott ARCHITECT DHEA-S assay.

Methods and Results

The assay utilizes a chemiluminescent magnetic microparticle immunoassay (CMIA) format and has a calibration range of 0 – 1,500 µg/dL. Assay imprecision was evaluated following NCCLS protocol EP5-A using two reagent lots and four instruments over a period of 20 days. Total imprecision of the assay controls at 10.7 (Low), 104.8 (Medium) and 984.5 µg/dL (High) was 7.41%, 2.68% and 2.93%, respectively. Analytical sensitivity across two reagent lots and three instruments was ≤3.0 µg/dL (95% confidence). Mean recovery of DHEA-S spiked into multiple serum samples (n = 10) was 102%. Dilution linearity of multiple samples demonstrated mean recoveries from 91 – 108%. Assay results were not significantly affected by a variety of potentially cross-reacting or interfering substances. Expected values (median and 5th – 95th percentile) determined using serum samples from 246 women and 240 men were calculated for each 5 years of age from 11 to 70 years. Highest values were seen in women and men aged 20 – 24 years: 281.9 µg/dL (134.2 – 407.4 µg/dL) and 353.6 µg/dL (238.4 – 539.3 µg/dL), respectively. Expected values were also determined for children <10 years of age, and are shown in this poster. Comparison of the assay to another commercially available method gave the following data (Passing-Bablok regression): ARCHITECT DHEA-S = 1.08x + 1.77, n = 550, r = 0.98.

Conclusion

Our data demonstrate the ARCHITECT DHEA-S assay is sensitive and precise, and provides reliable results across a wide range of clinically relevant concentrations.

Introduction

Dehydroepiandrosterone sulfate (DHEA-S) is the most abundant adrenal androgen and also functions as a neurosteroid that is produced by the adrenal cortex.

DHEA-S is an excellent indicator of adrenal androgen production.

DHEA-S exhibits only weak androgenic activity but can be metabolized to more active androgens such as testosterone and androstenedione.

Serum concentrations decline with age and can serve as a prognostic factor in both critical illnesses and breast cancer progression.

Elevated levels of DHEA-S are found in the plasma of patients with adrenal tumors or congenital adrenal hyperplasia.

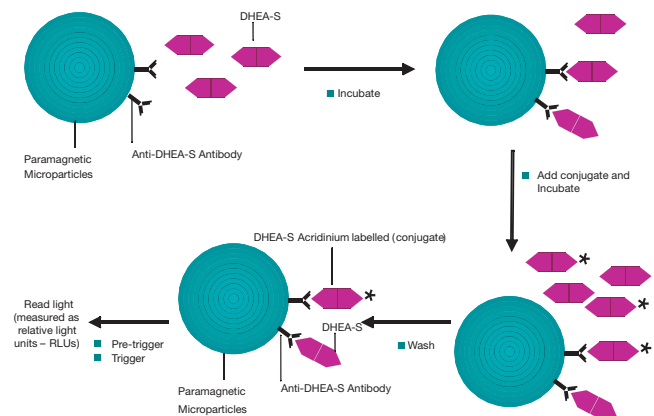
DHEA-S may also be slightly elevated in patients with polycystic ovaries.

Tumors in men that produce hCG may lead to increased levels of testicular DHEA-S.

The ARCHITECT DHEA-S assay is a delayed 1-step, competitive immunoassay using direct, chemiluminometric technology in combination with paramagnetic particles. The assay utilizes a monoclonal antibody specific for DHEA-S.

Methods

Assay Protocol

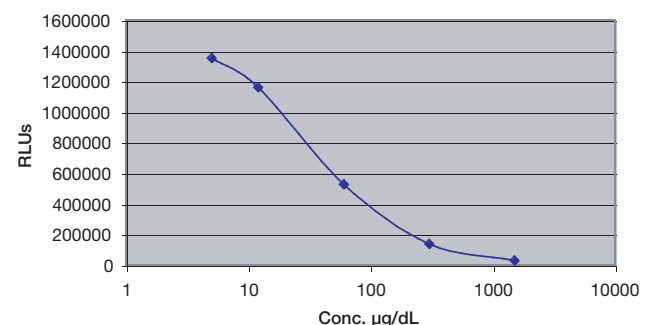


Assay Standardization and Calibration

Internal Reference Calibrators are manufactured gravimetrically using purified synthetic DHEA-S. The ARCHITECT DHEA-S Calibrators are matched to the Internal Reference Calibrators.

The assay is calibrated with six ready-to-use calibrators prepared in human serum.

A representative calibration curve, without the zero calibrator, is shown below.



Results

Expected Values

A total of 586 samples were analyzed from the following specimen categories:

- 240 sera from males and 246 from females from a population of adult blood donors that were classified by age
- Specimens of each of the following age groups of children:
 - <1 week
 - 1 to 4 weeks
 - 1 to 12 months
 - 1 to 4 years
 - 5 to 10 years

Results (cont.)

Age (years)	N	50th Percentile		5 – 95th Percentile	
		µmol/L	µg/dL	µmol/L	µg/dL
Females					
11 – 14	10	2.0	73.8	0.2 – 4.6	8.6 – 169.8
15 – 19	16	4.0	147.0	1.7 – 13.4	61.2 – 493.6
20 – 24	21	7.6	281.9	3.6 – 11.1	134.2 – 407.4
25 – 34	45	6.9	255.3	2.6 – 13.9	95.8 – 511.7
35 – 44	55	4.9	179.2	2.0 – 11.1	74.8 – 410.2
45 – 54	58	3.9	142.3	1.5 – 7.7	56.2 – 282.9
55 – 64	36	1.9	69.2	0.8 – 4.9	29.7 – 182.2
65 – 70	5	1.6	58.1	0.9 – 2.1	33.6 – 78.9
Males					
11 – 14	10	2.8	102.1	0.5 – 6.6	16.6 – 242.7
15 – 19	8	8.3	306.2	1.2 – 10.4	45.1 – 385.0
20 – 24	9	9.6	353.6	6.5 – 14.6	238.4 – 539.3
25 – 34	57	9.3	344.2	4.6 – 16.1	167.9 – 591.9
35 – 44	66	8.8	323.6	3.8 – 13.1	139.7 – 484.4
45 – 54	50	6.8	249.1	3.7 – 12.1	136.2 – 447.6
55 – 64	38	2.8	104.9	1.3 – 9.8	48.6 – 361.8
65 – 70	2	6.9	256.1	6.2 – 7.7	228.5 – 283.6
Children					
<1 week	20	2.3	86.2	0.7 – 8.2	24.6 – 302.8
1 – 4 weeks	20	2.4	87.2	0.2 – 8.6	8.5 – 317.3
1 – 12 months	20	1.8	65.3	0.9 – 5.8	31.6 – 214.1
1 – 4 years	20	2.0	75.4	0.9 – 7.5	32.7 – 276.0
5 – 10 years	20	2.9	108.5	0.7 – 5.7	24.4 – 209.7

Assay Imprecision

Imprecision testing was performed at the following sites:

- Future Diagnostics (The Netherlands)
- Biokit S.A. (Spain)
- Free University Medical Centre, Amsterdam, The Netherlands (Dr. Blankenstein)
- Hospital Universitario Del Rio Hortega, Valladolid, Spain (Dr. F.J. Martin)

Imprecision was evaluated, using 3 controls (human serum based), according to the EP-5A protocol from the NCCLS.

Testing was performed using 2 lots of assay reagents.

Overall Results, Based on Calibration With Each Run at Each Site

Member	N	µg/dL	Intra-Assay	Inter-Assay
			%CV	%CV
Low Control	2178	10.3	4.35	6.36
Medium Control	2178	103.9	1.39	2.24
High Control	2178	977.3	1.66	2.33

Overall Results, Based on Initial Calibration of Each Lot and Each Site

Member	N	µg/dL	Intra-Assay	Inter-Assay
			%CV	%CV
Low Control	2178	10.7	4.24	7.41
Medium Control	2178	104.8	1.38	2.68
High Control	2178	984.5	1.68	2.93

Method Comparison

Method comparison data were generated from a total of 550 samples at the following sites:

- Future Diagnostics, The Netherlands (111 non-categorized samples)
- Free University Medical Centre, Amsterdam, The Netherlands (Dr. Blankenstein)
- Hospital Universitario Del Rio Hortega, Valladolid, Spain (Dr. F.J. Martin)

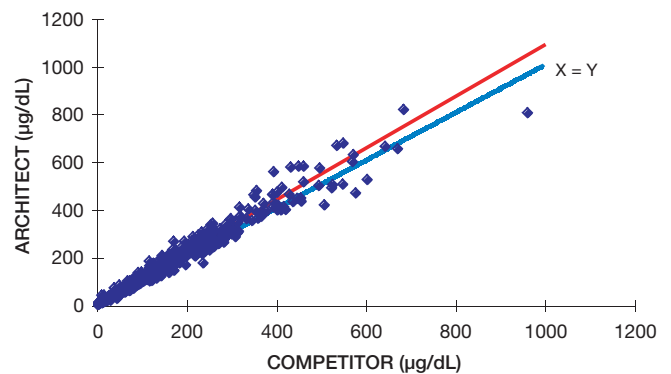
Samples Tested at the Free University Medical Centre, Amsterdam, The Netherlands

- 57 Normal men
- 16 Pre-menopausal women (non-pregnant without estrogen intake)
- 31 Post-menopausal women
- 28 Pregnant women
- 23 Women on oral contraceptives
- 36 Pre-pubertal women
- 50 Women with PCO

Samples Tested at the Hospital Universitario Del Rio Hortega, Valladolid, Spain

- 50 Normal men
- 34 Pre-menopausal women (non-pregnant without estrogen intake)
- 30 Post-menopausal women
- 25 Pregnant women
- 8 Women on oral contraceptives
- 28 Pre-pubertal women
- 17 Women with PCO
- 8 Unknown category

Passing-Bablok: ARCHITECT vs. Competitor



ARCHITECT vs. Competitor		95% CI
Correlation Coefficient (Pearson)	0.9793	0.9755 to 0.9824
Slope (Passing-Bablok)	1.0823	1.0648 to 1.0999
Slope (Linear Regression)	1.0425	1.0244 to 1.0606
Intercept (Passing-Bablok)	1.7676	0.2794 to 3.3137
Intercept (Linear Regression)	9.3414	5.5760 to 13.1068
N	550	
ARCHITECT concentration range	4.3 to 820.0	
Competitor concentration range	0.2 to 961.1	

Results (cont.)

Specificity

Analytical specificity was determined by testing either the zero calibrator (cross-reactivity) or 5 serum samples (interference) both supplemented with various compounds. Cross-reactivity and interference were calculated using the following formulas:

Cross-reactivity:

$$\% \text{ Cross-reactivity} = \frac{\text{Mean Value spiked} - \text{Mean Value nonspiked } (\mu\text{g/dL})}{\text{Concentration of Cross-reactant } (\mu\text{g/dL})} \times 100$$

Interference:

$$\% \text{ Recovery} = \frac{\text{Observed Value } (\mu\text{g/dL})}{\text{Expected Value } (\mu\text{g/dL})} \times 100$$

ARCHITECT DHEA-S has minimal cross-reactivity/interference with the following substances:

Cross-reactant	Concentration
DHEA	4000 µg/dL
Cortisol	10000 µg/dL
Aldosterone	5000 µg/dL
Estradiol	5000 µg/dL
Testosterone	2000 µg/dL
5-dihydrotestosterone	5000 µg/dL
Androstenedione	1000 µg/dL
Androsterone	2000 µg/dL
Andro-Glucuronide	2000 µg/dL
Estriol	5000 µg/dL
Estrone	5000 µg/dL
19-hydroxyandrostenedione	1000 µg/dL
Progesterone	5000 µg/dL
Androsterone Sulfate	5000 µg/dL
Estrone-3-Sulfate	5000 µg/dL
DHEA Glucuronide	5000 µg/dL

Interfering Substances

Human serum and EDTA plasma samples were supplemented with potentially interfering compounds as indicated below. Less than 10% interference was seen for all conditions tested.

Interfering Substance	Level(s) Tested	Mean of % Recovery of All Tested Samples
Triglycerides	5000 mg/dL	102
Hemoglobin	500 mg/dL	95
Bilirubin	20 mg/dL	100
Total Protein	40 and 120 g/L	94/100

Analytical Sensitivity (two lots of reagents, three instruments)

Reagent Lot 1	Mean Cal A RLU (20 replicates)	RLU Minus 2xSD	Analytical Sensitivity µg/dL
ARCHITECT i2000 _{SR} (instrument 1)	1437526	1407457	1.3
ARCHITECT i2000 _{SR} (instrument 2)	1430468	1410768	1.3
ARCHITECT i2000	1542612	1508583	1.5

Reagent Lot 2	Mean Cal A RLU (20 replicates)	RLU Minus 2xSD	Analytical Sensitivity µg/dL
ARCHITECT i2000 _{SR} (instrument 1)	1282001	1253104	1.4
ARCHITECT i2000 _{SR} (instrument 2)	1286079	1251591	1.8
ARCHITECT i2000	1374717	1350832	1.2

Spike Recovery

DHEA-S (20 to 1080 µg/dL) was spiked into serum samples with endogenous concentrations between 10.6 and 173.1 µg/dL. Spiking into Calibrator A was used to determine the actual spiking concentrations. Two ARCHITECT instruments were used.

Sample	Mean Recovery %	
	i2000	i2000 _{SR}
1	116	117
2	99	100
3	106	106
4	97	99
5	97	97
6	100	100
7	104	103
8	105	104
9	99	98
10	98	97
Mean	102	102
Grand Mean	102	
Range	97	117

Dilution Linearity

Ten serum samples with undiluted values ranging from 132.1 to 386.5 µg/dL of DHEA-S were diluted with normal human serum free of DHEA-S at 10 to 90% of the endogenous DHEA-S level.

Testing was performed using two lots of assay reagents.

Sample	Mean % Recovery of All Dilutions	
	Reagent Lot 1	Reagent Lot 2
A	103	104
B	105	103
C	105	104
D	104	107
E	101	101
F	106	108
G	91	103
H	108	107
K	104	101
L	97	98
Range	91	108

Conclusion

Based on our evaluation, we conclude that the ARCHITECT DHEA-S assay is sensitive and precise, and provides reliable results across a wide range of clinically relevant concentrations.

