

Development and Multi-Site Evaluation of an Assay for Sex-Hormone Binding Globulin (SHBG) on the Abbott ARCHITECT® Analyzer*

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Abstract

Background and Objective

Sex-hormone binding globulin (SHBG) is a glycoprotein with high affinity for steroid hormones such as estradiol and testosterone. It is the most important transport protein for estrogens/androgens in the body, and SHBG concentration is a major factor regulating hormone distribution between free and protein-bound states. The purpose of our study was to conduct a multi-center evaluation of the Abbott ARCHITECT SHBG assay.

Methods and Results

The assay is a two-step chemiluminescent magnetic microparticle immunoassay (CMIA) with a calibration range of 0 – 250 nmol/L. Assay imprecision was evaluated following NCCLS protocol EP5-A using two reagent lots and four instruments over 20 days. Total imprecision ranged from 5.6 – 9.5% (for control concentrations ranging from 8.8 to 154.1 nmol/L). Analytical sensitivity across two reagent lots and three instruments was <0.02 nmol/L (95% confidence). Mean recovery of SHBG spiked into multiple serum samples (n = 10) was 99%. Dilution linearity of multiple serum samples (n = 10) demonstrated mean recoveries from 93 – 108%. The assay showed no detectable cross-reactivity with a wide variety of potentially interfering substances. Expected values (median and 2.5th – 97.5th percentile) were: women (61.3, 19.8 – 155.2 nmol/L, n = 200) and men (34.8, 13.5 – 71.4 nmol/L, n = 224). Several sample categories were also tested with SHBG and testosterone (Abbott ARCHITECT) to determine the free androgen index. Comparison of the assay to another commercially available method gave the following data (Passing-Bablok regression): ARCHITECT SHBG = 1.11x + 0.09, n = 626, r = 0.98.

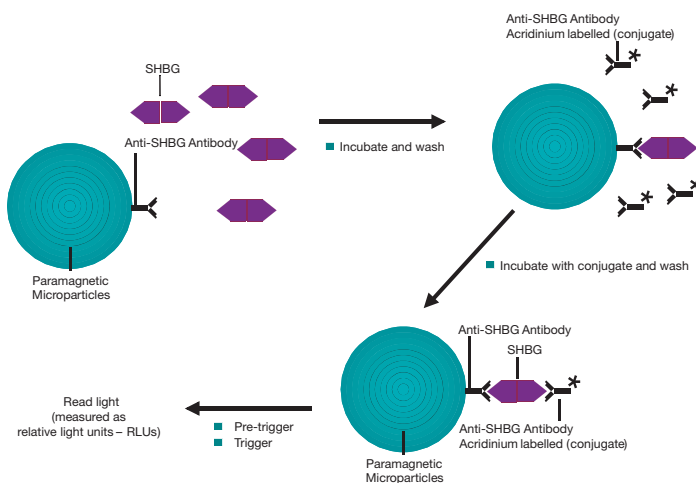
Conclusions

Based on our evaluation, we conclude the ARCHITECT SHBG assay is sensitive and precise, and will provide reliable results to aid in the evaluation of a variety of androgen disorders.

Introduction

- Sex hormone binding globulin (SHBG) is a glycoprotein of about 80-100 kDa; it has a high affinity for 17 β -hydroxysteroid hormones such as testosterone and estradiol.
- SHBG concentration in plasma is regulated by, amongst other things, androgen/estrogen balance, thyroid hormones, insulin and dietary factors.
- SHBG is the most important transport protein for estrogens and androgens in peripheral blood. SHBG concentration is a major factor regulating their distribution between the protein-bound and free states.
- Plasma SHBG concentrations are affected by a number of different diseases, high values being found in hyperthyroidism, hypogonadism, androgen insensitivity and hepatic cirrhosis in men. Low concentrations are found in myxoedema, hyperprolactinaemia and syndromes of excessive androgen activity.
- Measurement of SHBG is useful in the evaluation of mild disorders of androgen metabolism and enables identification of those women with hirsutism who are more likely to respond to estrogen therapy.
- The ARCHITECT SHBG assay is a two-step immunoassay that uses direct chemiluminometric technology in combination with paramagnetic particles. The assay utilizes two monoclonal antibodies specific for SHBG.

Assay Protocol

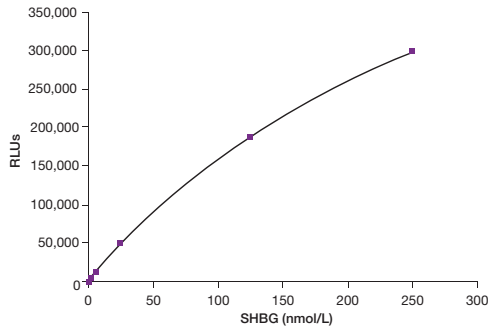


* Assay not available in the U.S.

Assay Standardization and Calibration

The assay is standardized against the WHO standard 95/560 for SHBG. The assay is calibrated with six ready-to-use (frozen) calibrators prepared in a phosphate buffered saline with a protein (goat) stabilizer. A representative calibration curve is shown below.

ARCHITECT SHBG Representative Calibration Curve



Results

Assay Imprecision

- Precision 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 three controls and three serum samples according to the EP-5A protocol from the NCCLS.

Testing was performed using two lots of assay reagents.

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

Member	N	nmol/L	Intra-Assay	Inter-Assay
			%CV	%CV
Low Control	1640	8.9	4.78	5.60
Medium Control	1640	24.6	4.79	5.80
High Control	1640	154.1	5.23	6.14
Human Serum high	760	147.4	5.03	6.82
Human Serum low	760	16.9	5.05	6.51
Human Serum medium	760	47.4	4.74	6.58

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

Member	N	nmol/L	Intra-Assay	Inter-Assay
			%CV	%CV
Low Control	1640	8.8	4.78	9.54
Medium Control	1640	24.5	4.80	5.65
High Control	1640	152.8	5.24	7.55
Human Serum high	760	146.2	5.11	8.47
Human Serum low	760	16.8	5.11	6.63
Human Serum medium	760	47.3	4.78	6.29

Results (cont.)

Analytical Sensitivity (Two Lots of Reagents, Three Instruments)

Reagent Lot 1	Mean Cal A RLU (20 Replicates)	RLU Plus 2xSD	Analytical Sensitivity nmol/L
ARCHITECT i2000 _{SR} ® (instrument 1)	123	147	0.012
ARCHITECT i2000 _{SR} ® (instrument 2)	130	151	0.011
ARCHITECT i2000®	139	163	0.014

Reagent Lot 2	Mean Cal A RLU (20 Replicates)	RLU Plus 2xSD	Analytical Sensitivity nmol/L
ARCHITECT i2000 _{SR} (instrument 1)	219	254	0.017
ARCHITECT i2000 _{SR} (instrument 2)	205	249	0.024
ARCHITECT i2000	238	289	0.024

Spike Recovery

SHBG (12.5 to 200 nmol/L) was spiked into serum samples with endogenous concentrations between 9.4 to 46.6 nmol/L. Spiking into Calibrator A was used to determine the actual spiking concentrations. Two ARCHITECT instruments were used.

Sample	Mean Recovery %	
	i2000	i2000 _{SR}
1	96	100
2	99	102
3	100	100
4	97	96
5	99	98
6	100	103
7	100	98
8	100	103
9	94	93
10	98	97
Mean	98	99
Grand Mean	99	
Range	93	103

Dilution Linearity

Ten serum samples with undiluted values ranging from 30.0 to 158.2 nmol/L of SHBG were diluted with ARCHITECT Multi-Assay Manual Diluent LN 7D82 at 10 to 80 % of the endogenous wSGBG level.

Testing was performed using two lots of assay reagents.

Sample	Mean % Recovery of All Dilutions	
	Reagent Lot 1	Reagent Lot 2
A	97	106
B	104	93
C	96	98
D	97	102
E	95	100
F	96	106
G	100	99
H	101	108
I	106	100
J	104	97
Range	93	108

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:

$$\text{Cross-reactivity: \% Cross-reactivity} = \frac{\text{Mean Value Spiked} - \text{Mean Value Nonspiked (nmol/L)}}{\text{Concentration of Cross-reactant (nmol/L)}} \times 100$$

$$\text{Interference: \% Recovery} = \frac{\text{Observed Value (nmol/L)}}{\text{Expected Value (nmol/L)}} \times 100$$

ARCHITECT SHBG has no cross-reactivity/interference with the following substances:

Cross-Reactant	Concentration
AFP	400 ng/mL
Cortisol	100000 ng/mL
11-Desoxycortisol	4000 ng/mL
Estradiol	3600 pg/mL
Testosterone	20000 ng/mL
5-Dihydrotestosterone	20000 ng/mL
TG	300 ng/mL
Transferrin	4 mg/mL
TBG	200 µg/mL

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	4000 mg/dL	103
Hemoglobin	500 mg/dL	99
Bilirubin	20 mg/dL	99
Total Protein	40 and 120* g/L	104/95* (serum)

* There is a significant interference with plasma samples at 120 g/L of protein, whereas serum did not show interference.

Expected Values (I)

224 sera from males and 200 from females from a population of adult blood donors were analyzed.

Member	N	SHBG (nmol/L)		
		Median nmol/L	2.5th Percentile	97.5th Percentile
Males	224	34.8	13.5	71.4
Females	200	61.3	19.8	155.2

Expected Values (II)

The following results summarize expected values for ARCHITECT SHBG, Testosterone and Free Testosterone Index for different populations (specimen category).

Free testosterone index (%FTI) or free androgen index (FAI) is calculated as following:

$$[\text{Testosterone (nmol/L)}] / [\text{SHBG (nmol/L)}] \times 100$$

This index correlates well with both measured and calculated values of free testosterone and helps to discriminate subjects with excessive androgen activity from normal individuals.

Specimen Category	ARCHITECT SHBG				ARCHITECT Testosterone			Free Testosterone Index		
	N	Median (nmol/L)	5th Percentile (nmol/L)	95th Percentile (nmol/L)	Median (ng/mL)	5th Percentile (ng/mL)	95th Percentile (ng/mL)	Median (%)	5th Percentile (%)	95th Percentile (%)
Normal Men	111	39.7	17.1	77.6	4.86	2.54	8.53	41.7	20.4	81.2
Post-Menopausal Women	54	57.2	26.4	118.0	0.45	0.16	1.00	2.5	0.6	8.0
Pre-Menopausal Women	59	88.9	34.3	147.7	0.58	0.16	1.17	2.5	0.5	7.3

Method Comparison

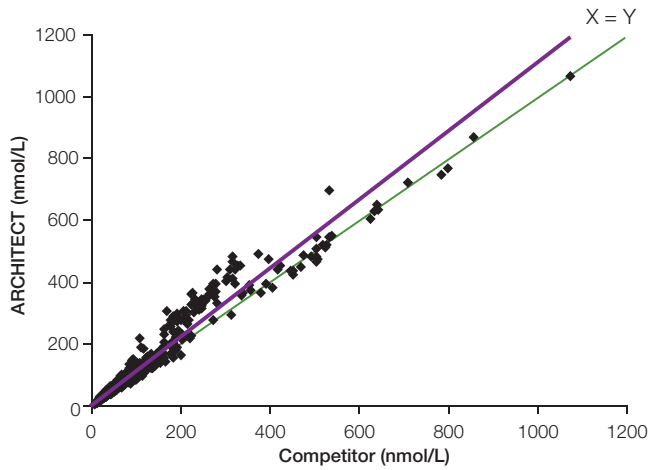
Method comparison data were generated from a total of 626 samples at the following sites:

- Future Diagnostics, The Netherlands (126 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.	Samples tested at the Hospital Universitario Del Rio Hortega, Valladolid, Spain.
56 Normal Men	60 Normal Men
16 Pre-menopausal women (non-pregnant without estrogen intake)	29 Pre-menopausal women (non-pregnant without estrogen intake)
31 Post-menopausal women	30 Post-menopausal women
29 Pregnant women first trimester	16 Pregnant women first trimester
28 Pregnant women second trimester	27 Pregnant women second trimester
23 Women on oral contraceptives	3 Women on oral contraceptives
18 Post-menopausal women on hormone replacement therapy	29 Hyperthyroidism Male/Female
17 Hyperthyroidism Male/Female	28 Hypothyroidism Male/Female
28 Hypothyroidism Male/Female	32 Obesity Male/Female

Results (cont.)

Passing-Bablok: ARCHITECT vs. Competitor



ARCHITECT vs. Competitor		95% CI
Correlation Coefficient (Pearson)	0.9779	0.9742 to 0.9811
Slope difference (Passing-Bablok)	1.1071	1.0806 to 1.1344
Slope difference (Linear Regression)	1.0672	1.0493 to 1.0851
Intercept (Passing-Bablok)	0.0860	-1.1815 to 1.1132
Intercept (Linear Regression)	7.6645	4.6246 to 10.7043
N	626	
ARCHITECT concentration range	5.7 to 1067.6	
Competitor concentration range	6.5 to 1072.0	

Conclusions

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