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Development of a New Sex-Hormone Binding Globulin (sHBG) Assay on the ADVIA Centaur System

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Abstract

Background: 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. SHBG measurements are most useful in the evaluation of androgen disorders including virilization, hirsutism and polycystic ovary syndrome.

Methods: The ADVIA Centaur® SHBG assay is a sandwich immunoassay that uses anti-SHBG bound magnetic microparticles as solid phase and an anti-SHBG acridinium labeled conjugate as tracer. The ADVIA Centaur assay allows for the determination of the free testosterone index (FTI) by calculating the ratio of testosterone to SHBG. Samples are pre-diluted onboard. The assay dynamic range is 0–200 nmol/L. The FTI is automatically calculated and reported by the ADVIA Centaur. Since SHBG greatly influences the bioavailable testosterone level, total testosterone may fluctuate with varying SHBG levels. The FTI is a sensitive measurement of androgen status. Assay imprecision was evaluated following the CLSI EP5-A2 protocol using one reagent lot and one instrument over 20 days.

Results: Preliminary data showed that total imprecision of the two assay controls with values at 10.5 and 50.4 nmol/L was 6.1% and 5.5%, respectively. Analytical sensitivity across two reagent lots and three instruments was <0.015 nmol/L (95% confidence). Dilutional linearity of multiple serum samples (n = 12) demonstrated mean recoveries of 91.7 – 98.3% (mean 94.1%). The assay showed no detectable cross-reactivity with a wide variety of compounds and was not significantly affected by a variety of potentially interfering substances. Comparison of the assay to another commercially available method gave the following data (linear regression): ADVIA Centaur SHBG = 0.94 (IMMULITE SHBG) + 6.4 (n = 168, r = 0.98).

Conclusion: The ADVIA Centaur SHBG assay provides reliable results across a wide range of clinically relevant concentrations.

Background

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 and androgens in the body, and SHBG concentration is a major factor regulating hormone distribution between free and protein-bound states. SHBG measurements are most useful in the evaluation of androgen disorders including virilization, hirsutism and polycystic ovary syndrome.

Materials and Methods

SHBG was assayed on an automated ADVIA Centaur immunoanalyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The analytical evaluation of these assays included precision, dilution recovery, and interference studies using patient samples, calibrators, and controls.

Samples: The ADVIA Centaur SHBG assay requires a total of 10 µL of serum for a single determination and samples are prediluted onboard. Patient serum samples were spiked with SHBG to prepare sample pools with low, medium and high concentrations for SHBG. These pools were used for the interference study. Random serum samples were used for the correlation study.

Precision: The precision of the ADVIA Centaur SHBG assay was estimated from calibrators, controls, and from medical decision pools at 10.5 and 50.4 nmol/L. These samples were assayed in 2 replicates in 20 runs, on one system over a period of 20 days. Imprecision estimates were collected and computed according to the CLSI protocol EP5-A2.[1]

Minimum detectable concentration (MDC): The MDC, i.e., analytical sensitivity, was determined using the "zero" level.

Dilutional linearity: Volumetric dilutions of a high sample were prepared and measured in triplicate on one analyzer (in 10-fold for the high and low sample).

Interference studies: Potential interferents including hemoglobin, triglycerides, protein (human serum albumin), and bilirubin were added to the 3 patient sample pools (with low, medium, and high concentrations of the SHBG analyte) and evaluated for potential interferences in each of the ADVIA Centaur SHBG assay. Several potential cross-reactants were spiked into SHBG negative serum and the apparent SHBG value was determined. The percent cross-reactivity is the apparent result compared to the amount added.

Results

Precision

The total % CVs had ranges of 5.5% to 6.1%. The within-run % CVs were under 4.3% for all samples. These data are summarized in Table 1.

Table 1. Precision of the ADVIA Centaur SHBG assay.

Mean Concentration (nmol/L)	Within-Run %^{CV}	Total %CV
10.5	4.3	5.5
18.6	3.7	5.7
50.4	3.9	6.1

Minimum detectable concentrations: MDC (analytical sensitivity) results are shown in Table 2. The MDCs are each considerably lower than any clinically expected concentrations.

Table 2. Minimum detectable concentrations (analytical sensitivity).

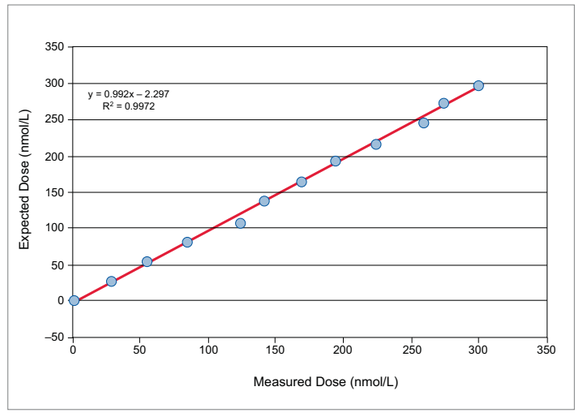
	Run 1	Run 2	Run 3	Mean
Number of replicates	20	20	20	
Limit of blank (nmol/L)	0.007	0.019	0.019	0.015

Dilution recovery: To determine the linearity across the reportable range of the SHBG assay, volumetric dilutions of an overrange sample were prepared and measured on two reagent lots according to the CSLI EP6-A guideline.[2] Data shown in Table 3 are the individual results obtained using lot 2. Graphical linearity is demonstrated in Figure 1.

Table 3. Dilution recovery.

	Sample	Measured nmol/L	Expected nmol/L	Recovery %
1	High	299	299	
2	10 High + 1 Low	274	274	100.0
3	9 High + 2 Low	258	248	104.0
4	8 High + 3 Low	224	216	103.7
5	7 High + 4 Low	194	195	99.5
6	6 High + 5 Low	169	166	101.8
7	5 High + 6 Low	141	138	102.2
8	4 High + 7 Low	123	108	113.9
9	3 High + 8 Low	84.1	80.8	104.1
10	2 High + 9 Low	54.4	55.1	98.7
11	1 High + 10 Low	28.0	27.3	102.6
12	Low	1.11	1.11	
Mean recovery				**103.0**

Figure 1. Dilutional linearity observed using SHBG assay reagent lot 2.



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 in the ADVIA Centaur SHBG assay (Table 4). The assay showed no cross-reactivity with a wide variety of compounds (Table 5).

Table 4. Interference study results.

Specimens that have:	Demonstrate <10% change in results up to:
Hemolysis	500 mg/dL of hemoglobin
Lipemia	1000 mg/dL of triglycerides
Bilirubin	40 mg/dL of conjugated bilirubin 40 mg/dL of unconjugated bilirubin
Total protein	5.0 g/dL of protein

Cross-reactivity: Table 5 shows that no or limited cross-reactivity was observed.

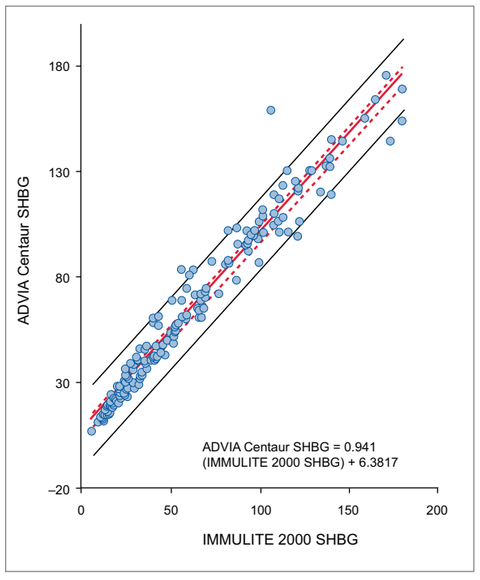
Table 5. Cross-reactivity study results.

Compound	Amount Added	% Cross-reactivity
AFP	400 IU/mL	ND
Thyroglobulin	300 ng/mL	ND
Thyroxin binding globulin	193 µg/mL	ND
Transferrin	4.0 mg/mL	ND
Cortisol	100 µg/mL	ND
11-Deoxycortisol	4.0 µg/mL	0.16
5α-Dihydroxytestosterone	20 µg/mL	0.04
Estradiol	3600 pg/mL	ND
Testosterone	20 µg/mL	ND

ND = Nondetectable

Method comparison: Comparison of the ADVIA Centaur SHBG assay with the Siemens IMMULITE® 2000 SHBG assay generated the data presented in Figure 2 (n = 168).

Figure 2. Comparison of the ADVIA Centaur SHBG assay to the IMMULITE SHBG assay.



Conclusions

- The ADVIA Centaur SHBG assay accurately measures the level of SHBG across the reportable range of the assay.
- The assay demonstrated repeatability in precision, accurate dilution recovery, and acceptable correlation with another commercially available method.
- No critical interference was observed with common serum components.

References:

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