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High-resolution reference ranges for estradiol, luteinizing hormone, and follicle-stimulating hormone in men and women using the AxSYM assay system

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Abstract

Objectives: High-resolution reference ranges are essential for reproductive hormones due to the significant day-to-day variation seen with these analytes.

Design and methods: The performance of AxSYM assays for estradiol, luteinizing hormone (LH), and follicle-stimulating hormone was evaluated.

Results: These studies validate the performance of each assay, permitting high-resolution reference ranges to be established.

Conclusions: The high-resolution reference range data provided herein for both normally cycling females and males should be applicable to a wide variety of clinical situations.
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Keywords: Estradiol; Luteinizing hormone; Follicle-stimulating hormone

Introduction

Determination of estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations in serum are important components of the evaluation and diagnosis of a wide range of conditions in both women and men [1]. The diversity of situations requiring measurement of estradiol, LH, and FSH necessitates assays for these analytes that are highly sensitive, precise, and accurate over wide analytical ranges.

Currently available random-access, fully automated, commercial systems have replaced radioimmunoassays for the measurement of FSH, LH, and estradiol. While performance characteristics distinguish the various systems, none are fully validated methods. Indeed, because of gonadotropin heterogeneity and steroid hormone matrix effects, it is arguable that these methods cannot be validated at the present time [2–6]. Furthermore, all of the current automated systems are poorly characterized with respect to clinical use with dependence almost exclusively on comparison to methods ultimately linking back to laboratory-specific radioimmunoassays. Thus, in order for these modern methods to be used rationally and related to one another, it is imperative that reference ranges be carefully established for each system. Unfortunately, this is often beyond the financial and technical capabilities of the typical clinical laboratory, which generally must rely upon simply verifying reference ranges established by instrument manufacturers using superficially characterized patient specimens.

The Abbott AxSYM is a fully automated, continuous, random-access immunoassay analyzer. In the present study, we assessed the performance of three Abbott AxSYM assays, estradiol, LH, and FSH. We then established high-resolution reference ranges for the three analytes using

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specimens from healthy men and women participating in investigational protocols.

Methods and materials

Apparatus and reagents

AxSYM (Assembly #37000-108, Abbott Diagnostics Division, Abbott Park, IL) preventative maintenance and troubleshooting was performed as recommended. Reagent kits and calibrators were purchased from Abbott Diagnostics. We utilized tri-level lyophilized human serum controls (AxSYM controls; Abbott Diagnostics) and tri-level human serum controls (Ligand Plus 1, 2, 3; Chiron Diagnostics).

Samples and volunteers

Permission to obtain serum specimens from human subjects was granted by the Human Studies Committee (an IRB) of the Massachusetts General Hospital prior to blood collection. Volunteers provided written informed consent prior to participation. Female subjects met the following criteria: age 18–35 years; BMI 18–35 kg/m^2; normal kidney and hepatic function; regular menstrual cycles (25–35 days with <4 days variation from cycle to cycle; off oral contraceptives for at least 3 months prior to study, no regular medications; normal prolactin and testosterone; negative pregnancy test and normal hematocrit). Male subjects met the following criteria: age 18–55 years; BMI <35 kg/m^2; normal kidney and hepatic function; normal prolactin and testosterone; no history of disorders of testicular function including orchitis, cryptorchidism, and radiation therapy; and no medications known to affect testicular function. In addition, specimens obtained for clinical diagnostic testing for various reproductive disorders including infertility being managed by assisted reproduction protocols (e.g., ovulation induction and in vitro fertilization) were utilized for method evaluation.

Results

Estradiol

Performance characteristics

Analytical sensitivity data were obtained from duplicate assays of 10 replicate zero-concentration samples. The minimum detectable concentrations in two replicate studies were 33.2 and 43.6 pmol/L. The manufacturer's stated analytical sensitivity for the estradiol assay is 73.4 pmol/L. Using replicate between run testing of patient serum specimens the functional sensitivity (e.g., the dose equivalent to a 20% between-run CV) of the AxSYM estradiol assay was approximately 110 pmol/L (Fig. 1).

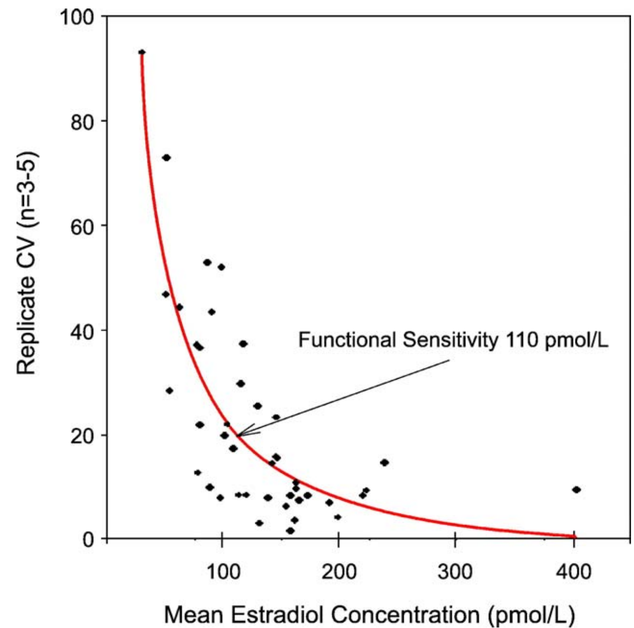

Fig. 1. Functional sensitivity of the AxSYM estradiol assay based upon a fitted curve (polynomial regression analysis).

These data are in close agreement with the manufacturer's reported mean functional sensitivity of 103 pmol/L. We found that only for estradiol concentrations above 147 pmol/L were all CVs less than 20%. This is an important factor when considering the clinical use of estradiol assays in applications for which low values have clinical significance [7–9].

The linearity of the AxSYM estradiol assay was evaluated using NCCLS protocol EP10. Three serum estradiol targets were evaluated in triplicate over five runs. Multiple regression analysis demonstrated linearity in this assessment (slope = 0.99). Intra-assay precision was determined in our laboratory using replicate measurements (n = 5) of three quality control sera containing estradiol at concentrations spanning the range typically encountered in women with regular menstrual cycles. The mean estradiol concentrations in these sera were 323, 1160, and 2588 pmol/L, and the intra-assay CVs were 4.50%, 2.12%, and 3.07%, respectively (data not shown), which are well within the limits described by the manufacturer for multiple instruments in multiple laboratories.

Reference ranges

Fig. 2A shows daily serum levels of estradiol in 51 healthy, normally cycling women across the menstrual cycle. (See also Table 2A.) Serum estradiol measurements and reference ranges for estradiol in 134 healthy men (<55 years of age) were also established (mean = 133.1 pmol/L, SD = 39.9, 13 of 134 measured <73.4 pmol/L and were excluded from statistical calculations). It is important to note that because the functional sensitivity (limit of quantitation) is approximately 147 pmol/L, the AxSYM

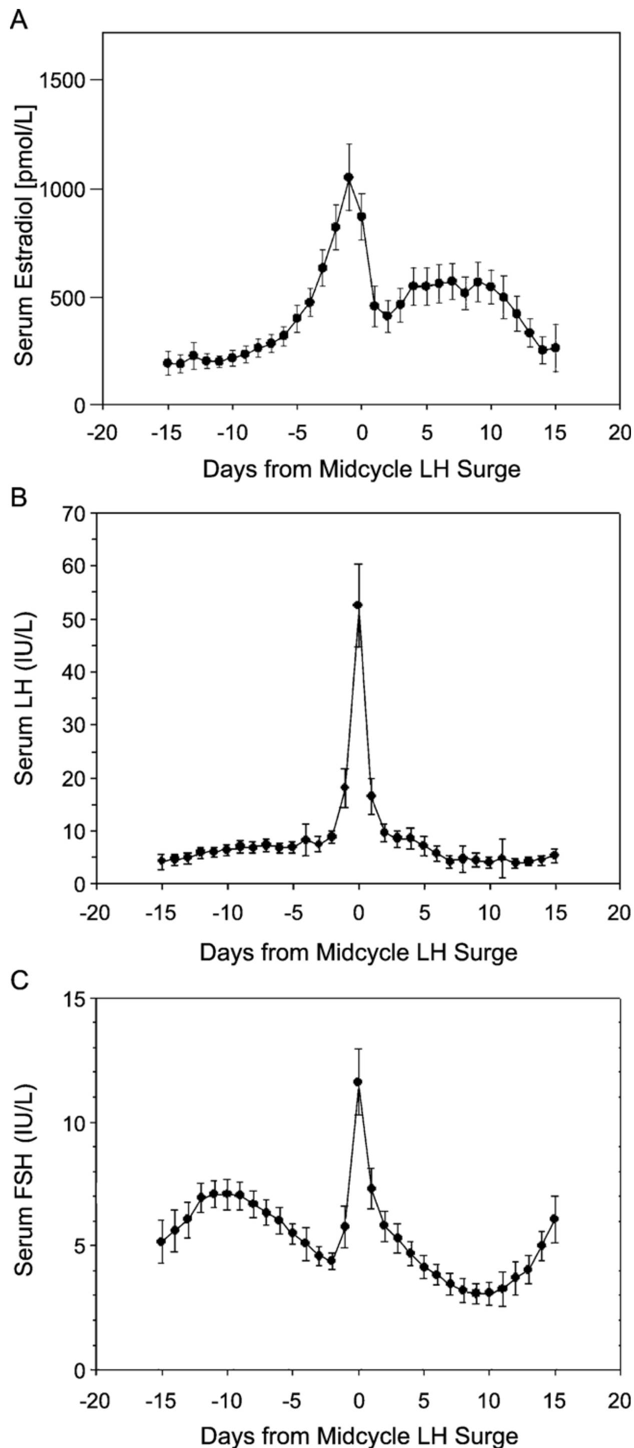

Fig. 2. (A) Estradiol levels measured by the AxSYM in daily serum specimens obtained across the menstrual cycle of reproductively normal, healthy women. Error bars represent 99% confidence intervals. (B) LH levels measured by the AxSYM in daily serum specimens obtained across the menstrual cycle of reproductively normal, healthy women. Error bars represent 99% confidence intervals. (C) FSH levels measured by the AxSYM in daily serum specimens obtained across the menstrual cycle of reproductively normal, healthy women. Error bars represent 99% confidence intervals.

Table 2A
Reference range data for serum estradiol in menstruating women

Cycle day	Mean	Standard deviation	Count	Lower 99% CI limit	Upper 99% CI limit
−15	202	121	19	128	275
−14	198	92	19	143	253
−13	239	158	24	154	319
−12	213	99	30	165	261
−11	209	81	35	176	246
−10	228	114	37	180	275
−9	246	125	37	191	301
−8	275	136	42	224	330
−7	297	143	43	242	352
−6	338	154	43	279	400
−5	422	213	45	341	503
−4	503	235	46	411	591
−3	672	308	48	554	786
−2	870	382	49	731	1013
−1	1116	562	48	907	1325
0	925	400	50	778	1068
1	481	352	49	352	613
2	433	268	48	334	532
3	492	275	46	385	595
4	580	312	46	463	697
5	580	312	45	459	701
6	595	312	44	474	716
7	606	290	44	496	720
8	547	261	42	444	650
9	602	316	42	477	727
10	580	264	42	474	683
11	525	319	38	393	661
12	444	253	36	338	554
13	352	198	35	264	437
14	264	173	28	184	349
15	275	264	22	132	422

estradiol method is of limited utility for measuring estradiol in serum in most men (98/134 measured less than or equal to 147 pmol/L).

Luteinizing hormone (LH)

Performance characteristics

The manufacturer's reported (Package Insert 69-0274/R4; May, 1998) limit of detection is greater than 0.4 IU/L (IU based on the second IRP 80/552, a pituitary gonadotropin reference standard). Data obtained using the mean of 10 replicate zero-concentration samples resulted in a minimum detectable concentration of 0.18 IU/L. Studies performed to confirm the linearity of the AxSYM LH assay compared the dilution of two patient serum specimens to assay calibrators. Because these specimens contained relatively high concentrations of LH, linearity could be assessed by visually comparing the response curves generated by assay calibrators to those generated by dilutions of the specimens. We found that measurement of LH was linear over the entire dynamic range of the assay (data not shown). Intra-assay test precision was confirmed in our laboratory based on the performance of assay calibrators and assessment of frequent sampling specimens from normal males (data not shown).

Table 2B
Reference range data for serum LH in menstruating women

Cycle day	Mean	Standard deviation	Count	Lower 99% CI limit	Upper 99% CI limit
−15	4.2	2.9	18	2.4	6.0
−14	4.6	2.4	21	3.2	6.0
−13	4.9	2.4	24	3.6	6.1
−12	5.9	2.7	30	4.7	7.2
−11	6.0	2.7	35	4.9	7.2
−10	6.3	3.1	37	5.0	7.6
−9	7.0	3.4	39	5.6	8.4
−8	6.8	3.4	41	5.4	8.1
−7	7.3	3.9	42	5.7	8.8
−6	6.8	3.0	43	5.6	7.9
−5	6.8	3.4	45	5.5	8.1
−4	8.2	10.1	45	4.4	12.1
−3	7.4	5.0	48	5.6	9.3
−2	8.8	4.0	49	7.3	10.3
−1	18.0	12.4	48	13.5	22.6
0	52.3	27.9	50	42.1	62.4
1	16.6	11.5	49	12.3	20.8
2	9.6	5.5	47	7.6	11.7
3	8.5	5.2	45	6.5	10.5
4	8.5	6.3	45	6.1	11.0
5	7.1	5.9	45	4.9	9.4
6	5.7	4.5	43	3.9	7.5
7	4.2	3.7	43	2.7	5.6
8	4.7	7.6	41	1.7	7.8
9	4.4	4.1	41	2.8	6.1
10	4.0	3.1	41	2.8	5.3
11	4.8	11.2	38	0.1	9.5
12	3.9	2.5	36	2.8	5.0
13	4.2	2.4	35	3.2	5.2
14	4.5	2.4	29	3.4	5.7
15	5.4	2.7	21	3.8	6.9

Reference ranges

Fig. 2B shows the profile of LH levels measured using the AxSYM system for daily serum specimens obtained across the menstrual cycle of reproductively healthy women. (See also Table 2B.) Reference ranges for serum LH measurements for 174 healthy men (<55 years of age) were also established (mean = 4.6 IU/L, SD = 2.3). The ±2 SD range is lower than that (based upon 19 men) reported by the manufacturer in the method package insert. Because the absolute ranges overlap, this difference likely represents the larger sample size in our study.

Follicle-stimulating hormone (FSH)

Performance characteristics

The minimum detectable concentration of FSH was verified using a mean of 10 zero-concentration determinations. The value associated with two standard deviations was 0.87 RLU (relative light units per unit time). This value corresponds to a minimum detectable concentration of 0.05 IU/L (IU based on the 2nd IRP 78/549, a pituitary gonadotropin reference standard), determined by extrapolation from the assay calibration curve. This result was considerably lower than the manufacturer's specified minimum detectable concentration of approximately 0.4 IU/L. Specimens drawn from two patients were serially diluted to generate response curves for the AxSYM FSH assay. The response curves for both individuals were visually linear and parallel relative to the assay calibrators over the dynamic range of the assay (from 4 to 80 IU/L, data not shown). These data support the specificity of the method reported by the manufacturer. The manufacturer's characterization of intra-assay precision predicts that the CV of repeated measurements would be <8%. Intra-assay precision (based on 25 replicate samples per specimen) ranged from 1.9% to 2.8% for FSH calibrators ranging from 1 to 150 IU/L (data not shown), confirming the manufacturer's data.

Reference ranges

Fig. 2C shows daily serum levels of FSH measured using the AxSYM FSH across the menstrual cycle of reproductively healthy women. (See also Table 2C.) Serum FSH measurements and reference ranges for 129 healthy men (<55 years of age) were established (mean = 4.38 IU/L, SD = 2.14). The ±2 SD range (0.1 to 8.66) is close to that reported by the manufacturer (n = 31 men) in the package insert (1 to 8 IU/L).

Table 2C
Reference range data for serum FSH in menstruating women

Cycle day	Mean	Standard deviation	Count	Lower 99% CI limit	Upper 99% CI limit
−15	5.2	1.8	19	4.1	6.2
−14	5.6	1.8	21	4.6	6.6
−13	6.0	1.7	25	5.2	6.9
−12	6.9	1.6	31	6.2	7.7
−11	7.1	1.6	36	6.4	7.8
−10	7.1	1.9	38	6.3	7.8
−9	7.0	1.8	39	6.3	7.7
−8	6.7	1.7	42	6.0	7.4
−7	6.3	1.7	43	5.7	7.0
−6	6.0	1.7	43	5.4	6.7
−5	5.5	1.4	46	4.9	6.0
−4	5.1	2.2	46	4.2	5.9
−3	4.6	1.3	48	4.1	5.1
−2	4.4	1.1	49	4.0	4.8
−1	5.8	2.9	49	4.7	6.8
0	11.6	4.7	51	9.9	13.3
1	7.3	2.9	49	6.3	8.4
2	5.8	2.2	48	5.0	6.6
3	5.3	2.0	46	4.5	6.0
4	4.7	1.6	46	4.1	5.3
5	4.1	1.5	45	3.6	4.7
6	3.8	1.3	44	3.3	4.3
7	3.5	1.5	44	2.9	4.0
8	3.2	1.6	42	2.6	3.8
9	3.1	1.3	43	2.5	3.6
10	3.1	1.4	42	2.5	3.7
11	3.2	2.1	39	2.4	4.1
12	3.7	2.0	36	2.8	4.6
13	4.0	1.7	35	3.3	4.8
14	5.0	1.6	29	4.2	5.7
15	6.1	2.1	21	4.9	7.2

Discussion

To ensure that the analytical performance of the instrument used to obtain the reference range data reported herein was within the manufacturer's specifications, we carefully evaluated the AxSYM assays for the measurement of estradiol, FSH, and LH in human serum. Sensitivity, patient linearity (a surrogate indicator of specificity), and precision were all well within the manufacturer's specifications for each of the assays. Thus, the reference range data we have obtained should be widely applicable.

Additionally, the functional sensitivity of the estradiol assay was studied in particular detail due to the importance of measurement of low levels of serum estradiol in numerous clinical situations [7–9]. Because a 20% CV is conservative limit, it is reasonable to define functional sensitivity based upon an absolute limit, requiring all specimens to be below that level, rather than basing functional sensitivity on a fitted curve. Thus, from this perspective, the functional sensitivity of the estradiol method was 147 pmol/L. This is substantially higher than the 110 pmol/L functional sensitivity specified by the manufacturer and confirmed in our laboratory based upon curve fitting. In either case, it is important to note that functional sensitivity limits the application of the method for precise measurements of estradiol in healthy men. We found that 98 out of 134 men had estradiol levels below 146.8 pmol/L (56 out of 134 had levels below or equal to 110 pmol/L). This limitation of the assay's use would apply to other groups where low-level estradiol measurements would be expected, including post menopausal females and follicular phase, normally cycling women.

The reference ranges established for healthy men and women are detailed in this report. As discussed above, these data should have wide applicability to a wide variety of contexts in which the AxSYM system is used to measure serum estradiol, LH, and FSH. The ranges that were established are in general similar to those reported by the manufacturer (using much smaller numbers of specimens and samples tested) in the method package inserts. Perhaps most useful to clinical laboratories will be the high-resolution data of the expected values for these assays across regular menstrual cycles reported herein. This high-resolution data permits appreciation of the striking changes that occur from day to day in the course of a regular menstrual cycle.

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