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The ADVIA Centaur Enhanced Estradiol Assay: Performance and Standardization

White Paper

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Answers for life.



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Introduction

Estradiol (E2, Figure 1) is an 18-carbon steroid hormone essential for regulating male and female reproductive processes and sexual maturation, and also serves multiple other physiologic functions. In females, it is primarily produced by the ovarian follicular granulosa cells and the follicular theca interna cells.¹

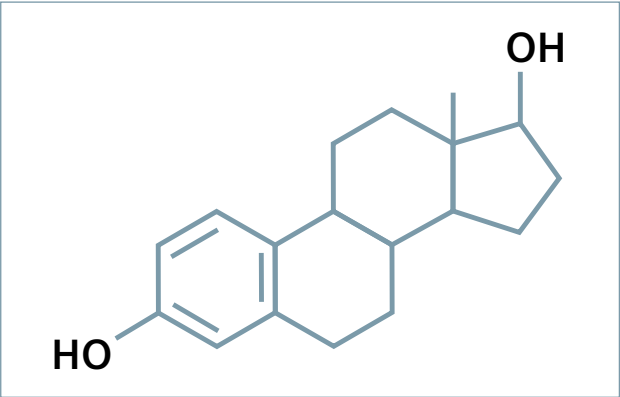


Figure 1. 17β—Estradiol

Levels vary considerably depending on menstrual cycle (Figure 2), and are also dependent on sex, sexual maturity or senescence, and pregnancy.^{2–4} Medians and ranges for the Siemens ADVIA Centaur® Enhanced Estradiol Assay are displayed in Table 1.

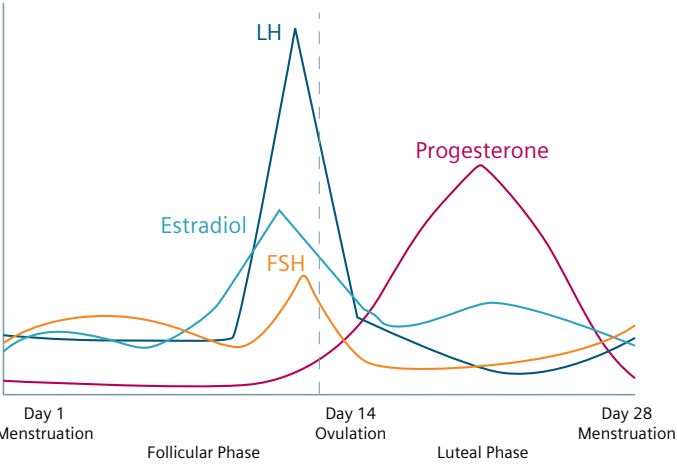


Figure 2. Hormone profiles throughout the menstrual cycle

Table 1. Medians and ranges for the Siemens ADVIA Centaur Enhanced Estradiol Assay

	N	Median (pg/mL)	Range (pg/mL)	Median (pmol/L)	Range (pmol/L)
Males	100	24.8	ND*–39.8	91.1	ND–146.1
Menstruating Females (by day in cycle relative to LH peak)					
Follicular Phase (–12 to –4 days)	196	51.8	19.5–144.2	190.1	71.6–529.2
Midcycle (–3 to +2 days)	117	153.3	63.9–356.7	562.2	234.5–1309.1
Luteal Phase (+4 to +12 days)	186	87.6	55.8–214.2	321.4	204.8–786.1
Post-menopausal female (untreated)	60	ND	ND–32.2	ND	ND–118.2

* Not detected

Why Test for Estradiol?

Because of the role E2 plays in follicle maturation and ovulation, measurement plays an important role in reproductive medicine and can serve as an indirect indicator of decreased follicular reserve.⁵ Additionally, E2 levels are associated with estrogen-sensitive breast cancer risk and a wide range of reproductive and endocrinological pathologies. Depending on the pathology, levels may either be elevated or depressed.²

Measuring Estradiol

Estradiol is found in serum predominantly bound to carrier proteins, principally sex-hormone binding globulin (SHBG), and albumin. Only ~1–3% circulates unbound and biologically active, thus most methods require extraction of binding proteins before quantitation.⁶ Reference ranges (Table 1) are based on total E2 levels. Because E2 levels can range from very low to very high concentrations, methods must be highly sensitive, but also highly accurate over a wide range. High specificity is also essential to differentiate E2 from related hormones.

Standardization of Estradiol assays using highly accurate reference methods

Estradiol performance diverges widely among commercial E2 immunoassays, and overestimation resulting from cross-reactivity and matrix effects can be particularly problematic.^{7,8} Thus, the need for good assay standardization and standardized reference materials is widely recognized.^{9–12}

Several mass spectrophotometry methods deliver very high sensitivity, specificity, and accuracy, but are not well adapted for use in most clinical laboratories. Sample preparation is time and labor intensive, and equipment use requires highly trained and skilled personnel. Because automated immunoassays are more accessible to most clinical laboratories, many have suggested that isotope dilution coupled with gas chromatography/mass spectrophotometry (ID-GC/MS) be used as the reference method for developing E2 standards and calibrating E2 immunoassays.^{11,13}

ID-GC/MS is widely recognized as a highly accurate and reliable reference method for assessing immunoassay performance.^{10,14,15} It is the preferred reference method for most organic analytes, including E2, of the Bureau International des Poids et Mesures (Joint Committee for Traceability in Laboratory Medicine) and UK NEQAS.^{6,9,11,16} Thienpont et al demonstrated its high precision for E2 (%CV = 0.77–2.1),^{10,12} and Wu et al. described an ID-GC/MS method for E2 with a limit of detection (LoD)

of 55 pmol/L (15 pg/mL), limit of quantitation (LoQ) of 110.13 pmol/L (30 pg/mL), and %CV averaging 6.1 across the range of the assay (determined by two curves: one at the low end of the assay, and the other at the high end).¹⁷ Using the Wu et al. assay, Tai et al. found that all E2 reference standards from the Community Bureau of Reference of the European Commission were within 4.2% of the stated values (31.0–364.5 pg/mL), demonstrating the high specificity of ID-GC/MS.^{17–20} The internal isotopic standard and the ability to accurately distinguish compounds by differentiation of isobars contribute to ID-GC/MS specificity. Similar performance has been demonstrated using isotope dilution coupled with liquid chromatography/mass spectrometry (ID-LC/MS).¹⁸

The new ADVIA Centaur Enhanced Estradiol Assay

Previous to 2010, two E2 assays were available for the Siemens ADVIA Centaur (Estradiol-6 [E2-6] and Estradiol-6 III [E2-6 III]) and ADVIA Centaur CP systems (E2-6 III). Both of these assays demonstrated good precision at higher concentrations of E2 than at lower concentrations. Both assays over-estimated E2 in comparison to ID-GC/MS, although overestimation was low in comparison to most other commercial assays.

The new ADVIA Centaur Enhanced Estradiol Assay (eE2) is a fully automated, monoclonal, competitive, chemiluminescent immunoassay; turn-around time is 18 minutes. The new eE2 assay has improved sensitivity, specificity, and precision (especially low-end precision), as well as an extended range. Because the new eE2 assay was standardized using real patient samples, thus minimizing matrix effects, it is substantially better aligned with ID-GC/MS than the earlier assays. In contrast, the E2-6 III and E2-6 assays were made traceable to ID-GC/MS indirectly by assigning calibrators with ID-GC/MS values.

The eE2 assay improvements and standardization scheme have resulted in reduced correlation with earlier Siemens E2 assays, leading to concerns over alignment to pre-existing results. To address these concerns, Siemens and independent investigators rigorously compared the analytical characteristics of the ADVIA Centaur Enhanced Estradiol, Estradiol-6, and Estradiol-6 III assays. These characteristics included limit of blank (LoB), LoD, and functional sensitivity (FS), as well as sensitivity, specificity, and assay imprecision. Better agreement of the new eE2 assay with ID-GC/MS was confirmed. Additionally, the eE2 assay was compared to another commercial assay calibrated to ID-GC/MS (Roche Elecsys® Estradiol Method). The results of these studies follow.

Methods

Performance Evaluation

Two independent precision studies were performed at Siemens Healthcare Diagnostics (Tarrytown, NY, USA) and the University of Maryland School of Medicine (Baltimore, MD, USA).

Assay Precision

The Siemens study followed CLSI protocol EP5-A2,²⁰ using eight samples with E2 concentrations ranging from 32 to 2870 pg/mL in two runs per day for 20 days on a single ADVIA Centaur system. A single reagent lot was tested.

The University of Maryland (UM) evaluated seven samples ranging from 83–2937 pg/mL using a 10-day CLSI EP5-A2 protocol. Two ADVIA Centaur eE2 reagent lots were tested in two runs per day in quadruplicate, two sample cups per concentration. Samples included three commercial controls and four patient serum pooled samples. Within-run and total imprecision was assessed by ANOVA.

Limit of Blank and Limit of Detection

The LoB and LoD were determined by Siemens as described in the CLSI EP17-A²¹ guideline. To determine the LoB, four lots of charcoal-stripped human sera were tested in duplicate twice a day for three days. To determine the LoD, four samples were prepared by diluting samples approximately 1 to 4 times the LoB. Each sample was assayed in duplicate twice a day for 3 days. Testing was done on two ADVIA Centaur systems with two lots of reagents. The LoD was set as the concentration at which 95% of the measurements would be greater than the LoB.

Functional Sensitivity

The functional sensitivity (FS) is typically defined as the lowest concentration with a total coefficient of variation of 20%²² and was determined by Siemens using two different approaches. In the first approach, FS was determined from a panel made from pooled samples, while in the second approach, individual samples were used. Testing was performed using two lots of reagents over the course of five days, two runs per day. All sample concentrations were below 150 pg/mL. UM also calculated FS using a panel of nine pooled serum samples ranging from 5 to 45 pg/mL. The UM samples were run over five consecutive days, eight replicates per day.

FS is calculated by plotting the Total CV (y axis) as a function of the analyte concentration (x axis), thus FS is the concentration at which the Total CV is 20%. It is defined by the power equation:

Total CV = a*(Conc)^b, where ‘a’ is a constant and ‘b’ is the power, both determined from the best fit curve to the data. Thus:

(20/a) = (Conc)^b

log (20/a) = b*log (Conc)

10^{(log (20/a)/b)} = Conc = FS

Correlation Studies

Correlation between two lots of the Siemens eE2 assay was determined at UM. Lot-to-lot variability was considered acceptable if it did not exceed 5%.²³ UM also compared these two lots of eE2, in addition to single lots of the E2-6 and E2-6 III assays, to another ID-GC/MS calibrated commercial assay (Roche Elecsys 2010; reported range 5–4300 pg/mL). Assays were run over five days on 188 serum specimens ranging from LoD (eE2, 11.8 pg/mL) or analytical sensitivity (AS: E2-6, 10 pg/mL; E2-6 III, 7 pg/mL) to 3000 pg/mL. Correlation was assessed over three analyte concentration ranges: AS or LoD–350 pg/mL (low range), AS–1000 pg/mL (full range for E2-6 and E2-6 III), and LoD–3000 pg/mL (full range for eE2).

In addition, Siemens eE2 assay results were compared directly to ID-GC/MS measurements. Forty-two samples with known ID-GC/MS values were tested on two systems using two different lots of reagents. The average dose of each sample was compared with the reference values.

Results and Discussion

Performance Evaluation

The results of the Siemens performance evaluation are presented in Table 2.

The precision profile demonstrates total CVs of 10.6% at 42 pg/mL and 3.7% at 2870 pg/mL.

Table 2. ADVIA Centaur eE2 assay precision data

Sample	Mean (pg/mL)	Within-Run SD (pg/mL)	Within-Run CV (%)	Total SD (pg/mL)	Total CV (%)
1	42.24	3.18	7.5	4.49	10.6
2	65.20	4.87	7.5	5.65	8.7
3	240.40	7.22	3.0	10.73	4.5
4	470.19	10.20	2.2	13.74	2.9
5	597.58	12.59	2.1	17.51	2.9
6	1543.26	53.85	3.5	57.62	3.7
7	2284.00	82.80	3.6	83.07	3.6
8	2870.45	86.92	3.0	106.44	3.7

The UM precision study results were comparable to those of the Siemens results, and are displayed in Table 3. A separate set of samples was used by UM: samples K1–K3 are commercial controls, while samples MDP1–MDP4 were created from human serum pools. Total %CV varied slightly between lots.

Table 3. UM precision study results

Sample	Mean (pg/mL)		Within-Run CV (%)		Total CV (%)	
	eE2 Lot 98	eE2 Lot 99	eE2 Lot 98	eE2 Lot 99	eE2 Lot 98	eE2 Lot 99
K1	83.7	84.2	5.8	12.7	9.4	14.5
K2	257.9	255.6	6.3	9.8	6.9	10.4
K3	565.0	578.3	3.2	6.8	5.1	7.3
MDP1	71.5	71.6	6.2	8.9	8.2	10.7
MDP2	493.6	493.1	3.0	3.5	4.9	4.8
MDP3	1490.4	1536.4	3.0	3.8	4.0	4.9
MDP4	2892.1	2936.8	1.8	3.7	2.4	4.0

Limit of Blank and Limit of Detection

The LoB is determined from the mean value of matrix samples containing no analyte in question. The LoD is defined in terms of the LoB, and is the lowest concentration that can be distinguished from the LoB within a 1% confidence limit, which is approximately 3 SD above the blank (Figure 3).

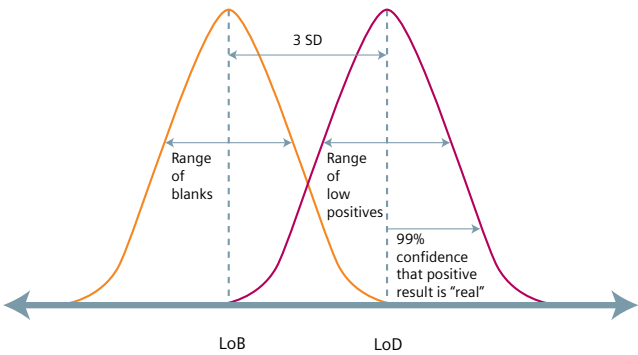


Figure 3. LoB vs. LoD

Additional data confirmed the LoB at 7.4 pg/mL (27.2 pmol/L); LoD was 11.8 pg/mL (43.6 pmol/L). As shown in Figure 3, values returned between the LoB and the LoD can be either falsely positive or falsely negative, and therefore measurements below the LoD have no analytical or clinical relevance. For this reason, any value below the LoD is reported by the ADVIA Centaur eE2 assay as “less than 11.8 pg/mL.”

Functional Sensitivity

Curves used to derive FS are displayed in Figure 4 (pooled samples) and Figure 5 (individual samples). Although there are small differences between lots and sample type, under all conditions, FS was less than 19.0 pg/mL (69.8 pmol/L) as originally reported in the instructions for use documentation, and thus FS should still be considered as 19.0 pg/mL.

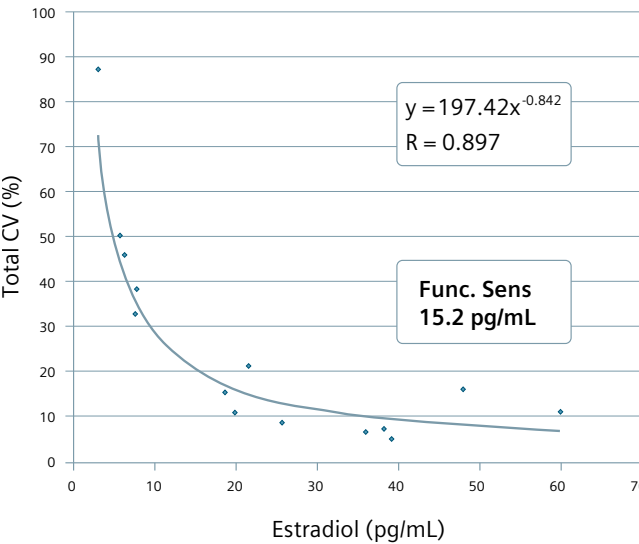


Figure 4. Functional sensitivity derived from pooled samples

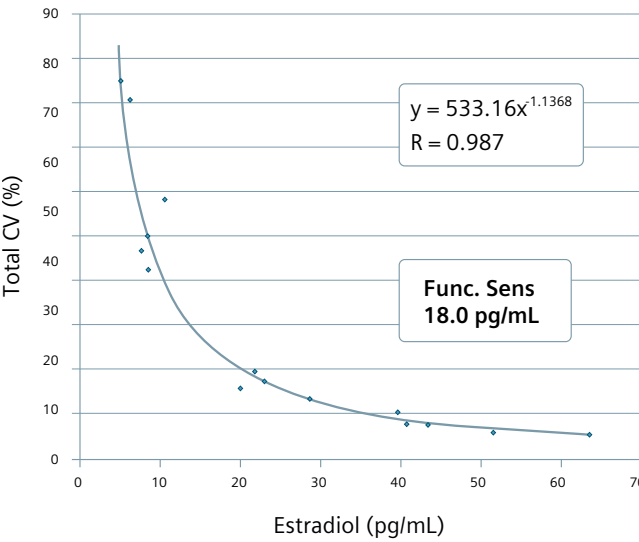


Figure 5. Functional sensitivity derived from individual samples

The UM results were reasonably comparable, with the FS of lot 98 = 20.04 pg/mL and the FS of lot 99 = 22.82 pg/mL (Figure 6).

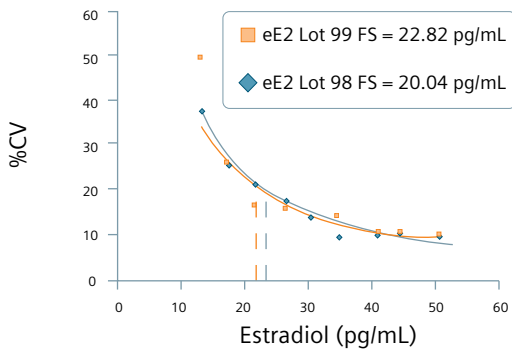


Figure 6. Functional sensitivity of the Siemens ADVIA Centaur eE2 assay for two reagent lots

Method Comparisons

Figure 7 displays the results from the UM eE2 lot-to-lot comparison study. Correlation between the two lots was 99% at the lower range of the assay (11.8–350 pg/mL) and 99.9% over the entire range of the assay (11.8–3000 pg/mL). The correlation between the two lots thus met the generally accepted industry standard of no more than 5% variability.^{2,3,5}

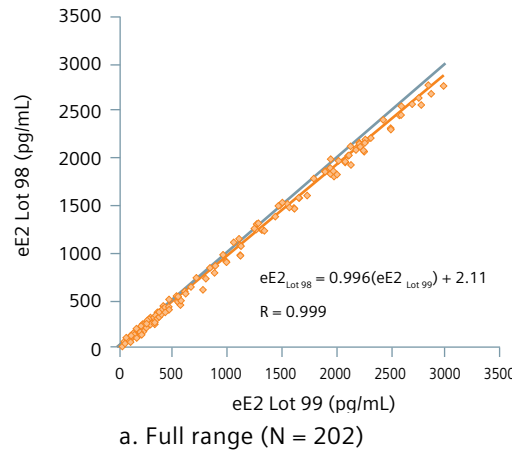
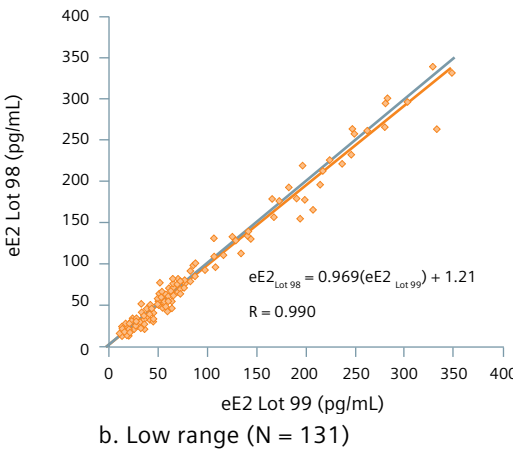
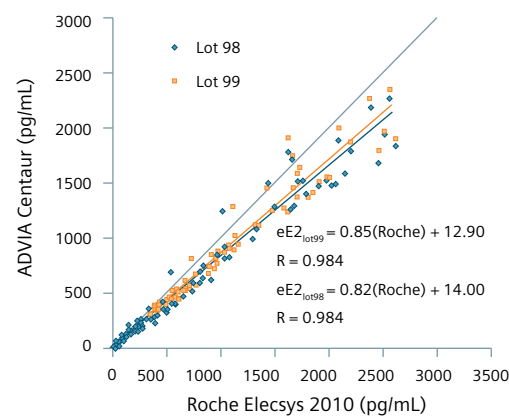


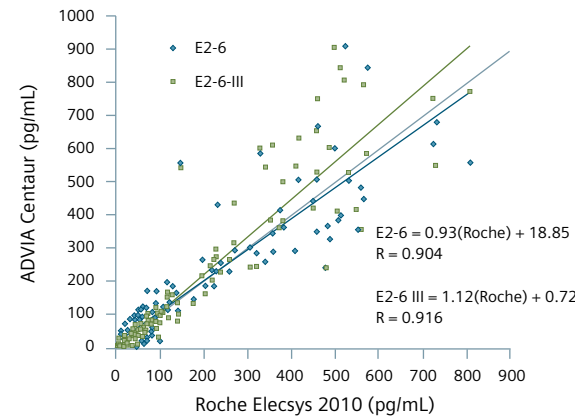
Figure 7. ADVIA Centaur eE2 lot-to-lot comparison. Regression lines are in orange; identity lines are in gray.



Method comparison results comparing each of the ADVIA Centaur Estradiol assays to the Roche Elecsys 2010 assay over their full range are shown in Figure 8, and over only the low end of the range in Figure 9.



a. Comparison to the ADVIA Centaur eE2 assay, lots 98 (N = 186) and 99 (N = 187)

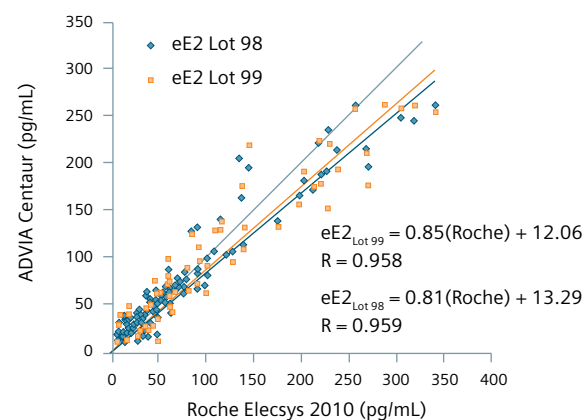


b. Comparison to the ADVIA Centaur E2-6 (N = 137) and E2-6 III (N = 133) assays

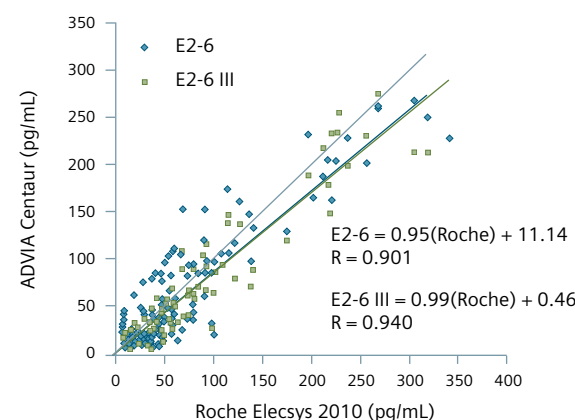
Regression lines are in orange (Lot 99) and blue (Lot 98); the identity line is in gray.

Regression lines are in blue (E2-6) and green (E2-6 III); the identity line is in gray.

Figure 8. Siemens Estradiol assays compared to the Roche Elecsys assay over the full assay range (11.8–3000 pg/mL for eE2, 10–1000 pg/mL for E2-6, and 7–1000 pg/mL for E2-6 III)



a. Comparison to the ADVIA Centaur eE2 assay, lots 98 (N = 124) and 99 (N = 125)



b. Comparison to the ADVIA Centaur E2-6 (N = 109) and E2-6 III (N = 104) assays

Regression lines are in orange (Lot 99) and blue (Lot 98); the identity line is in gray.

Regression lines are in blue (E2-6) and green (E2-6 III); the identity line is in gray.

Figure 9. Siemens Estradiol assays compared to the Roche Elecsys assay over each assay's range (11.8–350 pg/mL, eE2; 10–350 pg/mL, E2-6; 7–350 pg/mL, E2-6 III).

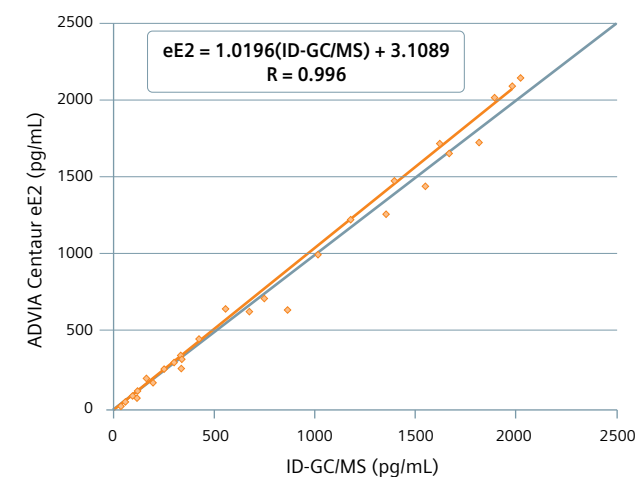
Because the eE2 method has an extended upper range, regression analysis was also performed for the ADVIA Centaur eE2 assay in the 11.8–1000 pg/mL range (Table 4) to compare more directly to the E2-6 and E2-6 III assays.

Table 4. Regression data for the ADVIA Centaur eE2 assay in the 11.8–1000 pg/mL range (two lots)

Lot	N	Slope (m)	Intercept (b)	Correlation Coefficient (R)
99	159	0.833	11.54	0.983
98	158	0.799	13.34	0.982

As can be seen, there was greater than 90% correlation for each of the Siemens estradiol assays with the Roche assay throughout the low and full ranges of the assays, although correlation between the ADVIA Centaur eE2 assay and the Roche assay was greater than for the earlier estradiol assays. Comparison of Figures 8a and 9a suggests that the eE2 assay correlates better with the ID-GC/MS standardized assay over the full range of the assay. This is because the eE2 assay is calibrated directly to ID-GC/MS–traceable samples, rather than to calibrators traceable to ID-GC/MS.

Despite the good correlation between the Siemens eE2 and Roche assays, at first glance Figures 8a and 9a suggest that the Siemens assay under-reports in comparison to the Roche assay. When the Siemens eE2 assay is compared directly to ID-GC/MS, however, no such bias is observed (Figure 10).



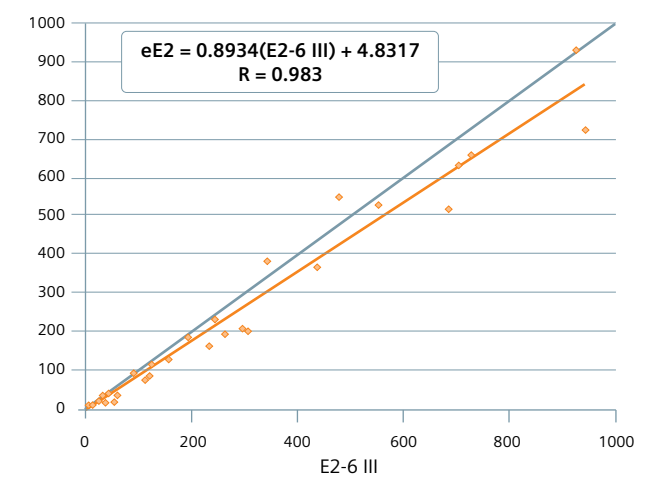
Regression line is in orange; the identity line is in gray.

Figure 10. ADVIA Centaur eE2 correlation ID-GC/MS

Some literature reports that the Roche Elecsys estradiol method demonstrates a positive bias (over-reports by 11–20%) on serum samples with ID-GC/MS–assigned values.^{8,19} Accordingly, the linear regression between the ID-GC/MS–standardized ADVIA Centaur eE2 and the Roche Elecsys estradiol methods demonstrates a slope between approximately 0.80 and 0.90, as would be expected. Rather than under-reporting, slope behavior combined with the direct comparison of the Siemens eE2 method to ID-GC/MS suggests that the Siemens eE2 assay accurately reports estradiol levels.

ADVIA Centaur eE2 vs. E2-6 III Method Comparison

Because the eE2 assay was optimized for enhanced agreement with ID-GC/MS and improved linearity over the dynamic range of the assay, eE2 does not demonstrate optimum agreement with the E2-6 III and E2-6 assays. Thus, while the two assays correlate well with each other, a negative dose bias of ~10% to ~20% has been noted between the Siemens eE2 and E2-6 III methods. Deming regression derived from a method comparison study conducted at Wright Patterson Air Force Base is representative of both correlation and bias (Figure 11).

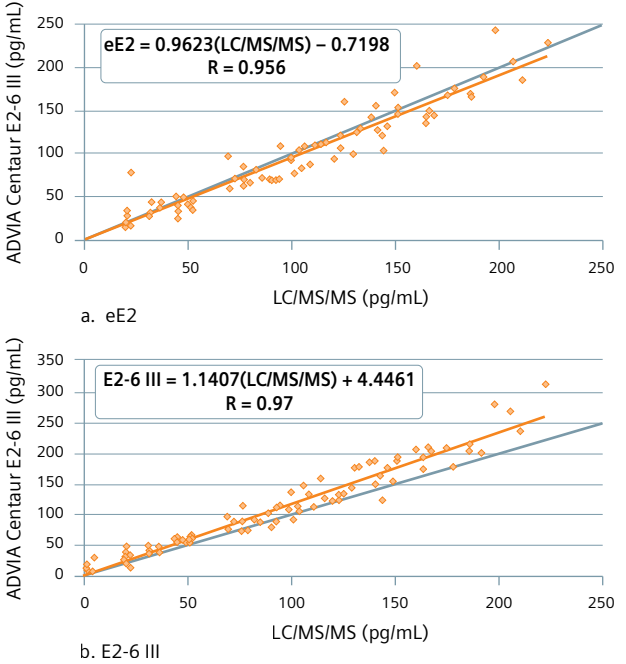


Regression line is in orange; the identity line is in gray.

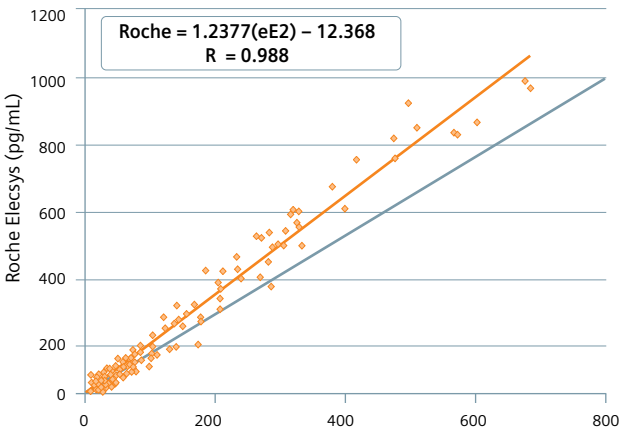
Figure 11. Correlation study conducted at Wright Patterson Air Force Base between the ADVIA Centaur eE2 and E-26 III methods (N = 35)

This bias also can be clearly observed in a low assay range study comparing the eE2 and E2-6 III assays to LC/MS/MS (Figure 12). While both demonstrate similar correlation to LC/MS/MS, the slope difference between the regression and identity lines for the eE2 assay is much closer to 1 than it is for the E2-6 III assay, as is the y intercept.

Tables 5 and 6 show method comparison statistics for patient sample distribution throughout the assay range, and indicate that the dose bias of the eE2 method with the E26 method is fairly consistent across the dynamic range of the assays. A consistent 25% bias was observed between the ADVIA Centaur eE2 assay and the Roche Elecsys Estradiol assay (Figure 13).



Regression lines are in orange; identity lines are in gray.
Figure 12. Low end standardization of the eE2 and E2-6 III methods



Regression line is in orange; identity line is in gray.
Figure 13. Consistent linear regression slope observed between the Roche Elecsys and the Centaur eE2 between the eE2 LoD and over 700 pg/mL

Table 5. Impact of sample dose segmentation of the mean bias observed between ADVIA Centaur eE2 and E2-6

	pg/mL			pmol/L		
	1000	500	300	3670	1835	1101
Slope	0.75	0.82	0.76	0.75	0.82	0.76
Intercept	16.25	9.13	13.0	59.64	33.51	47.71
R	0.94	0.95	0.91	0.94	0.95	0.91

Table 6. Impact of sample dose segmentation of the mean bias observed between ADVIA Centaur eE2 and E2-6 III

	pg/mL			pmol/L		
	1000	500	300	3670	1835	1101
Slope	0.62	0.79	0.84	0.62	0.79	0.84
Intercept	31.46	17.27	13.60	115.46	63.38	49.91
R	0.92	0.94	0.93	0.92	0.94	0.93

Conclusion

Siemens has recently released an enhanced estradiol assay for the ADVIA Centaur CP system. The studies discussed in this document highlight the improved precision, sensitivity (LoB, LoD, FS), and dynamic range of this new assay, and demonstrate its improved standardization to ID-GC/MS over earlier assays and another commercial ID-GC/MS-standardized assay. ID-GC/MS measurement is considered a preferred method for measuring steroid hormones because of its excellent sensitivity and specificity.^{8,10,11,14-16} Many commercial assays have demonstrated considerable variability in accuracy of E2 measurements and are prone to overestimation of the analyte in clinical samples.^{7,8} Much of this overestimation results from many assays’ lower specificity for E2, which bears chemical and structural similarities to a range of other steroid hormones, and consequently cross-reactivity.

In contrast, ID-GC/MS methods of quantification have been shown to be highly specific for different steroid hormones, including E2. Thus, standardization to ID-GC/MS of automated immunoassays is a much sought-after goal, as assays with good correlation should reflect greater clinical accuracy.

The ADVIA Centaur eE2 assay has been shown by Siemens, independent customers, and an independent clinical trial site to be well standardized to ID-GC/MS across the low range of the assay (11.8–350 pg/mL) as well as the full range of the assay (11.8–3000 pg/mL). Correlation studies suggest that the new eE2 assay approaches the accuracy observed with ID-GC/MS. This is an improvement over the E2-6 III assay, which may over-report by 11–15%, especially at E2 concentrations at the higher range of the assay. Additionally, unlike the earlier E2-6 and E2-6 III assays, results are linear across the entire range of the assay. Furthermore, the ADVIA Centaur eE2 assay correlates well with the ID-GC/MS-standardized Roche Elecsys assay. Unlike the Roche assay, however, the Siemens assay does not over-report.

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Finally, the ADVIA Centaur eE2 method demonstrated superior performance characteristics when compared to the existing ADVIA Centaur E2-6 and E2-6 III methods in all metrics. This includes improved imprecision results, notably for estradiol levels ≤50 pg/mL, a low LoB, and a low LoD. Functional sensitivity was under 25 pg/mL, whereas the functional sensitivity for E2-6 has been reported to be ≥45 pg/mL.

Overall, these improved features convey reliability of ADVIA Centaur eE2 assay as a method for E2 quantification, and are indicative of its suitability for clinical practice.

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