Development and Performance of an Estradiol Assay* on the Dimension Vista® System.

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Abstract

We describe the design and analytical performance of a fully automated homogeneous competitive immunoassay for 17ß-estradiol (E2) on the Dimension Vista System. The E2* method utilizes LOCI® technology and three reagents: two synthetic bead reagents and a biotinylated monoclonal antibody specific for estradiol. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with estriol (E3), an estradiol analog, and contains chemiluminescent dye. In the first step, sample is incubated with biotinylated antibody which allows E2 from the sample to saturate a fraction of the biotinylated antibody that is directly related to the E2 concentration. In a second step, E3 chemibeads are added and form bead/antibody immunocomplexes with the non-saturated fraction of the biotinylated antibody. Sensibeads are then added and bind to the biotin to form bead pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from sensibeads, which diffuses into chemibeads, triggering a chemiluminescent reaction. The resulting signal (measured at 612 nm) is an inverse function of the E2 concentration in the sample.

The method uses a 12 µL sample volume of serum or plasma and has an analytical range of 10-1500 pg/mL, with results traceable to an ID/GC/MS reference method. The time to first result is 10 minutes. Precision was evaluated per CLSI EP5 using serum pools and commercial quality control materials. Repeatability and within-lab precision were ≤ 3.6 %CV and ≤ 7.1 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus three different systems by Ordinary Least Squares regression analysis : Dimension Vista E2 = 1.01 * ID/GC/MS Estradiol + 0.4 pg/mL (r = 0.999, n = 54), Dimension Vista E2 = 0.99 * IMMULITE® 2000 E2 + 5.4 pg/mL (r = 0.986, n = 59), and Dimension Vista E2 = 0.91 * Roche ELECSYS® 2010 E2 – 2.6 pg/mL (r = 0.999, n = 51). Minimal cross reactivity (<0.1%) was observed with key compounds including: 17ą-estradiol, estrone, estrone-3-sulfate, estriol, estriol-3-sulfate, DHEA, DHEA-sulfate, androstenedione, androsterone, testosterone, 5ą-dihydrotestosterone, progesterone, 17ą-hydoxyprogesterone, cortisol, 11-deoxycortisol, corticosterone, ethinyl estradiol, clomiphene and prednisolone.

Conclusions. We conclude that use of LOCI technology provides acceptable sensitivity, precision, accuracy, turnaround time, and dynamic range suitable for measurement of estradiol.

Reaction Scheme

The method is a homogeneous competitive immunoassay based on LOCI technology. Sample is incubated with biotinylated anti-E2 antibody and estriol (E3)-coated Chemibeads. The E2 in sample competes with E3-coated chemibeads for a limited amount of biotinylated anti-E2 antibody. After incubation, Sensibeads are added to form bead pair immunocomplexes. Following a final incubation step, the reaction is flashed with a light source to stimulate generation of singlet oxygen. The resulting chemiluminescent signal is inversely proportional to the E2 concentration in the sample.

E2 + Ab-Biotin ——> E2•Ab-Biotin + Ab-Biotin

E2•Ab-Biotin + Ab-Biotin + CB-E3 ——> E2•Ab-Biotin + CB-E3•Ab-Biotin

CB-E3•Ab-Biotin + SA-SB ——> CB-E3•Ab-Biotin•SA-SB

CB-E3•Ab-Biotin•SA-SB —light—> chemiluminescent signal

where:
Ab-Biotin = Monoclonal tag antibody covalently bound to biotin
CB-E3 = Chemibead particle coated with E3 (E2 analog)
SA-SB = Sensibead particle coated with streptavidin

Method Specifics

Assay Time	10 minutes
Assay Range	11 - 1500 pg/mL
Analytical Sensitivity	11 pg/mL
Sample Volume	12 µL
Specimen Types	serum, heparinized / EDTA plasma

Precision

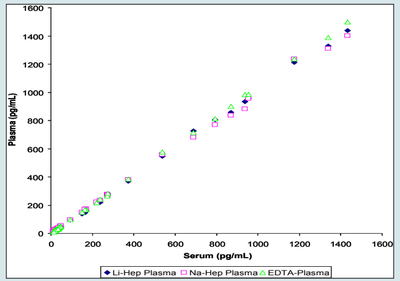
Repeatability and within-lab precision were assessed per CLSI EP5-A2 protocol over 20 days testing with two separate runs each day. Patient pools and commercial QC materials were used. The data were treated by Analysis of Variance (ANOVA) to yield reproducibility estimates for repeatability and within-lab precision.

Sample	Mean (pg/mL)	Precision (%CV)	
		Repeatability	Within-Lab
Bio-Rad Liquichek L1	25.4	6.8	12.3
Bio-Rad Liquichek L2	246.7	1.4	2.3
Bio-Rad Liquichek L3	695.2	1.1	1.8
Plasma	138.1	1.9	3.5
Serum L1	18.7	11.0	15.6
Serum L2	41.5	5.2	7.6
Serum L3	1295.9	1.3	2.1

Serum and Plasma Equivalence

Comparison testing results on the Dimension Vista System for lithium heparin, sodium heparin, and EDTA plasma samples versus serum samples are provided below. Testing was based on CLSI/NCCLS EP9-A2 protocol. Linear regression analysis showed excellent agreement among the specimen types.

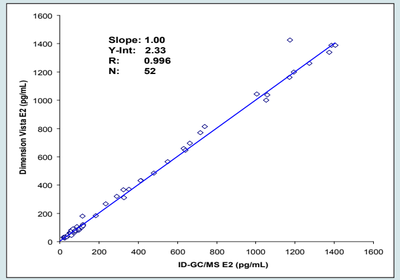
Comparative Specimen	Slope	Intercept pg/mL	Correlation Coefficient	N
Lithium Heparin Plasma	1.01	-1.2	0.9992	53
Sodium Heparin Plasma	0.99	1.6	0.9997	53
EDTA Plasma	1.04	-2.2	0.9999	53

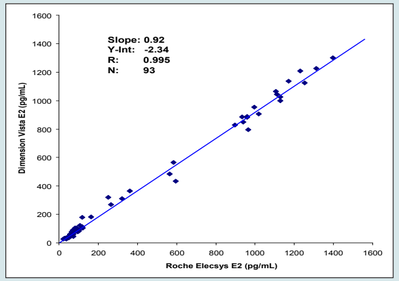


Method Comparison

The E2 method on the Dimension Vista system was compared to the ID-GC/MS reference method, Roche Elecsys 2010 and Siemens Immulite 2000 E2 method in a patient correlation study. Samples were analyzed in duplicate by the Dimension Vista and ID-GC/MS methods and singlicate by the Roche Elecsys and Siemens Immulite methods. Passing and Bablok linear regression analysis showed good agreement between Dimension Vista E2 and the other three E2 methods.

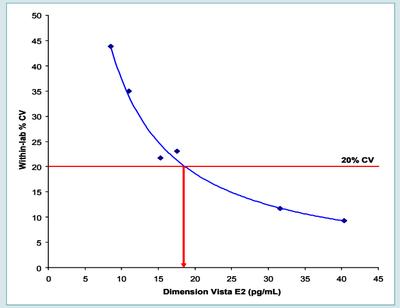
Comparative Method	Slope	Intercept pg/mL	Correlation Coefficient	N
ID-GC/MS	1.00	2.33	0.996	52
Roche Elecsys	0.92	-2.34	0.996	97
Siemens Immulite	1.00	3.43	0.990	46





Functional Sensitivity

Functional sensitivity is defined as the lowest concentration of E2 with a winthin-lab precision (%CV) of 20%, based on a two replicate per run, one run per day, 20 day reproducibility study. Six serum samples were tested, and the E2 concentration with a 20% total CV was read from the curve. The functional sensitivity was determined to be approximately 18.7 pg/mL.



HIL Interference

The Dimension Vista E2 method was evaluated for interference according to CLSI/NCCLS EP7-A2 at E2 levels of 200 pg/mL and 600 pg/mL. Bias is the difference in the results between the control samples (without the interferent) and the test sample (contains the interferent), expressed in percent. Results were deemed not significant if the bias (test mean vs. control mean) was < 10%.

Substance	Test Substance Concentration (mg/dL)	Bias (%) at [E2]	
		200 pg/mL	600 pg/mL
Hemoglobin (hemolysate)	1000	8.1	1.9
Bilirubin (unconjugated)	60	9.0	4.7
Bilirubin (conjugated)	20	5.2	3.6
Lipemia (Intralipid®)	1000	-6.7	-6.6

Cross-Reactivity

The following substances were evaluated for cross-reactivity in the E2 method when present in serum at the concentrations indicated. The substances were spiked into an estradiol-free sample and into a serum pool of nominal E2 concentration of 200 pg/mL. Biases due to these substances were expressed as % cross-reactivity. Maximum % cross-reactivities at the two E2 concentrations are presented for all substances.

$$\%\ \text{Cross-reactivity} = \frac{[\text{measured estradiol}] - [\text{control estradiol}]}{[\text{substance}]} \times 100$$

Substance	Test Substance Concentration (pg/mL)	Maximum %Cross-Reactivity
Aldosterone	100,000	ND
Androstenedione	10,000,000	ND
Cortisol	1,000,000	ND
11-deoxycortisol	1,000,000	ND
Cortisone	200,000	ND
Danazol	100,000	ND
DHEA	4,000,000	ND
DHEA-S	10,000,000	ND
17α-estradiol	100,000	0.015
17β-estradiol 3-β-D-glucuronide	10,000	ND
17β-estradiol 17-β-D-glucuronide	10,000	ND
17β-estradiol 3-β-sulfate	10,000	ND
17β-estradiol 3-β-sulfate-17-glucuronide	50,000	ND
17β-estradiol 17-propionate	10,000	0.39
17β-estradiol 17-valerate	3,600	ND
Estriol	20,000	0.23
Estriol 3-β-D-glucuronide	5,000	ND
Estriol 3-sulfate	100,000	ND
Estrone	3,600	ND
Estrone 3-β-D-glucuronide	100,000	ND
Ethinyl Estradiol	20,000	0.081
Norethindrone	100,000	ND
Norethindrone acetate	50,000	ND
Pregnenolone	50,000	ND
Progesterone	500,000	ND
17α-hydroxyprogesterone	100,000	ND
Testosterone	10,000,000	ND
5α-dihydrotestosterone	10,000,000	0.0002

Conclusions

We conclude that use of LOCI technology provides acceptable sensitivity, precision, accuracy, turnaround time, and dynamic range suitable for measurement of 17ß-estradiol.

*Product under development - Not available for sale.

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