Performance of LOCI® Assays for FSH*, LH*, and Prolactin* on the Dimension Vista® System

N. Zolotarjova, L. Amsel, E. Garcia, C. Moran, M. Wasson, and J. Pierson-Perry
Siemens Healthcare Diagnostics Inc. Newark, DE. U.S.A.

SIEMENS

Abstract

We describe the design and analytical performance of fully automated homogeneous sandwich immunoassays for follicular stimulating hormone (FSH*), luteinizing hormone (LH*), and prolactin (PRL*) on the Dimension Vista® system. The methods are based on LOCI technology. Each method uses three reagents: two synthetic bead reagents and a biotinylated analyte-specific tag antibody fragment. The first bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye. The second bead reagent (Chemibeads) is coated with the respective method's capture antibody and contains chemiluminescent dye. Test sample is incubated with Chemibeads and biotinylated antibody to form bead-analyte-biotinylated antibody sandwiches. Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of the complexes at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal (measured at 612 nm) is directly related to the analyte concentration. Time to first result is 10 minutes for each method.

Sample volumes are 2 µL (LH, PRL) or 4 µL (FSH) of serum or plasma. Analytical measurement ranges are 0.2-200 mIU/mL (FSH), 0.2-150 mIU/mL (LH), and 0.2-250 ng/mL (PRL), standardized respectively to WHO 92/510, 80/552, and 84/500. No high-dose hook effect was observed to at least 2,000 mIU/mL (FSH, LH) or 30,000 ng/mL (PRL). Precision was assessed per CLSI EP5-A2 using patient pools and commercial QC materials. Repeatability < 2.4 %CV and within-lab precision < 4.5 %CV were observed across the assay ranges for the respective methods. No significant interference (<10 % bias) was seen from lipemia (1000 mg/dL triglycerides), hemolysis (1000 mg/dL hemoglobin), or icterus (40 mg/dL conjugated bilirubin or 60 mg/dL unconjugated bilirubin). Comparison of results from patient samples processed by the three methods (Y) to their respective predicate methods on the ADVIA Centaur® system (X) showed good agreement by linear regression across the respective assay ranges: Y = 1.05X + 0.20, r = 0.99 for FSH;. Y = 0.84X + 1.9, r = 0.99 for LH; and Y = 1.14X + 0.37, r = 0.99 for PRL.

We conclude that the LOCI methods for FSH, LH, and prolactin on the Dimension Vista system provide acceptable accuracy, precision, turnaround time, and assay range for quantitation of the analytes in serum and plasma.

LOCI Technology

LOCI technology enables high sensitivity homogenous immunoassays in either a sandwich or a competitive format. LOCI reagents include two latex bead reagents and a biotinylated analyte-specific receptor. A generic bead reagent (Sensibead) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibead) is coated with a binding partner specific for the method and contains chemiluminescent dye. During an assay, the three reactants combine with analyte to form a bead-aggregated immunocomplex. Illumination of the complex releases singlet oxygen from the sensibead, which channels into the chemibead and triggers chemiluminescence.

Method Specifics

	FSH	LH	Prolactin
Sample Volume	4 µl	2 µl	2 µl
Specimens	Serum, Li, Na Heparin & EDTA Plasma	Serum, Li, Na Heparin & EDTA Plasma	Serum, Li, Na Heparin & EDTA Plasma
Analytical Measurement Range	0.2 - 200 mIU/ml	0.2 - 150 mIU/ml	0.2 - 250 ng/ml
Assay Time	10 minutes	10 minutes	10 minutes
Standardized To	WHO 92 / 510	WHO 80 / 552	WHO 84 / 500
Hook Effect	Not observed up to 40,000 mIU/ml	Not observed up to 20,000 mIU/ml	Not observed up to 50,000 ng/ml

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.

Analyte	Sample	Mean	Repeatability (% CV)	Within-Lab (% CV)
FSH (mIU/ml)	QC 1*	7.0	1.9	2.5
	QC 2*	18.9	1.8	2.1
	QC 3*	46.9	1.4	2.2
	Serum L1	129.9	1.6	2.2
	Serum L2	164.2	1.7	2.0
	Plasma	4.3	1.8	2.7
LH (mIU/ml)	QC 1*	4.3	1.5	2.1
	QC 2*	21.9	1.4	2.3
	QC 3*	85.3	1.7	2.2
	Serum L1	105.8	1.3	1.8
	Serum L2	132.7	1.2	1.8
	Plasma	4.5	2.4	3.0
Prolactin (ng/ml)	QC 1*	6.7	1.5	3.1
	QC 2*	17.5	1.4	2.9
	QC 3*	47.0	1.5	4.4
	Serum L1	27.8	1.7	2.9
	Serum L2	141.3	1.2	1.8
	Plasma	2.6	2.1	3.0

QC 1* - Bio-Rad Liquichek™ Immunoassay Plus Control Level 1

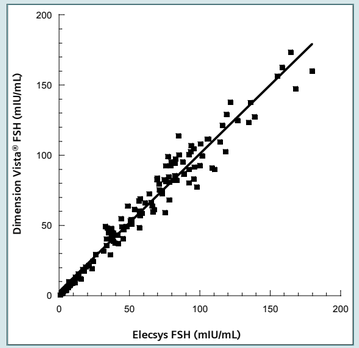
QC 2* - Bio-Rad Liquichek™ Immunoassay Plus Control Level 2

QC 3* - Bio-Rad Liquichek™ Immunoassay Plus Control Level 3

Method Comparison: FSH

FSH method on the Dimension Vista System was compared to the Roche Elecsys® FSH method through a method comparison study. Results show good agreement between the two systems.

FSH Assay	Least Squares Regression	Passing Bablok Regression
Slope	0.98	1.03
Y-Intercept	2.98	-0.05
r	0.98	0.98
N	137	137



FSH ASSAY - SERUM AND PLASMA EQUIVALENCE

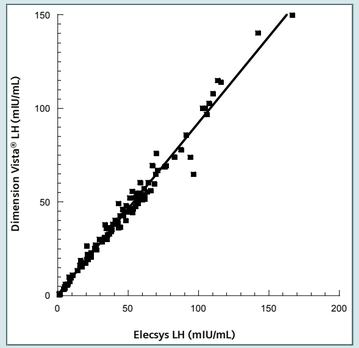
Comparison testing results on the Dimension Vista System for lithium heparin, sodium heparin, and EDTA plasma samples versus serum samples are provided below.
Testing was based on CLSI/NCCLS EP9-A2 protocol. Linear regression analysis showed excellent agreement among the specimen types.

Comparative Specimen	Slope	Intercept mIU/ml	Correlation Coefficient	N
Lithium Heparin Plasma	0.99	-0.05	0.999	68
Sodium Heparin Plasma	0.99	0.04	0.999	68
EDTA Plasma	0.96	-0.29	0.999	68

Method Comparison: LH

LH method on the Dimension Vista System was compared to the Roche Elecsys LH method through a method comparison study. Results show good agreement between the two systems.

LH Assay	Least Squares Regression	Passing Bablok Regression
Slope	0.92	0.92
Y-Intercept	0.58	0.19
r	0.99	0.99
N	111	111



LH ASSAY - SERUM AND PLASMA EQUIVALENCE

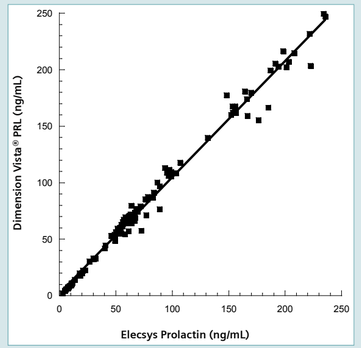
Comparison testing results on the Dimension Vista System for lithium heparin, sodium heparin, and EDTA plasma samples versus serum samples are provided below.
Testing was based on CLSI/NCCLS EP9-A2 protocol. Linear regression analysis showed excellent agreement among the specimen types.

Comparative Specimen	Slope	Intercept mIU/ml	Correlation Coefficient	N
Lithium Heparin Plasma	1.00	-0.07	0.999	72
Sodium Heparin Plasma	1.01	-0.26	0.998	72
EDTA Plasma	1.00	-0.24	0.999	72

Method Comparison: Prolactin

Prolactin method on the Dimension Vista System was compared to the Roche Elecsys Prolactin method through a method comparison study. Results show good agreement between the two systems.

Prolactin Assay	Least Squares Regression	Passing Bablok Regression
Slope	1.03	1.07
Y-Intercept	2.41	-0.13
r	0.99	0.99
N	121	121



PROLACTIN ASSAY - 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 ng/ml	Correlation Coefficient	N
Lithium Heparin Plasma	1.04	-0.25	0.999	69
Sodium Heparin Plasma	1.02	-0.10	0.999	69
EDTA Plasma	1.00	-0.22	0.997	69

Specificity

HEMOLYSIS, ICTERUS, LIPEMIA (HIL) INTERFERENCE

The FSH, LH and Prolactin methods were evaluated for interference according to CLSI/NCCLS EP7-A2. Tests were run at two concentrations of hormones. Bias is the difference in the results between the control samples (without the interferent) and the test sample (contains the interferent), expressed in percent.

Substance Tested	Substance Concentration	FSH mIU/ml	LH mIU/ml	Prolactin ng/ml	Bias
Hemoglobin (hemolysate)	1000 mg/dL	6.4 43.5	7.2 107.4	22.8 122.1	< 10%
Bilirubin (unconjugated)	60 mg/dL	6.6 44.6	7.8 116.3	23.1 121.9	< 10%
Bilirubin (conjugated)	40 mg/dL	———	7.8 116.9	———	< 10%
Bilirubin (conjugated)	60 mg/dL	6.6 45.2	———	23.3 121.1	< 10%
Lipemia (Intralipid®)	1000 mg/dL	———	7.4 112.5	———	< 10%
Lipemia (Intralipid®)	3000 mg/dL	6.4 47.7	———	21.7 112.4	< 10%

Cross-Reactivity

The following substances in the amounts indicated were evaluated and found to have insignificant cross-reactivity with the FSH, LH and Prolactin methods. Tests were run at FSH - 0 mIU/ml, LH - 0 mIU/ml and Prolactin - 0 ng/ml. Bias is the difference in the results between the control samples (without the cross-reactant) and the test sample (contains the cross-reactant).

Substance	Concentration	FSH Bias, mIU/ml	LH Bias, mIU/ml	Prolactin Bias, ng/ml
TSH	2500 µIU/ml	0.6	0.1	0.0
hCG	500,000 mIU/ml	0.0	0.4	0.0
FSH	2000 mIU/ml	X	0.0	0.0
LH	1100 mIU/ml	0.2	X	0.0
βLH	6.3 ng/ml	ND	1.1	ND
hGH	500 ng/ml	0.1	0.0	0.0
hPL	50 µg/ml	0.0	ND	0.0
Prolactin	400 ng/ml	0.1	0.0	X

Conclusions

The LOCI methods for FSH, LH, and Prolactin on the Dimension Vista system provide acceptable accuracy, precision, turnaround time, and assay range for quantitation of the analytes in serum and plasma.

*Product under development - Not available for sale.

www.siemens.com/diagnostics