

Multiplexed Fertility Marker Assays: LH, FSH and Progesterone



Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.
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Assay Protocol

Assay Components:

- Plate: MULTI-SPOT[®] 4-spot 96-well plate, pre-coated with all three capture antibodies
- Calibration: 8-level multi-analyte calibrator set (serum-based matrix)
- Detection Antibodies: solution containing 3 labeled reagents (two labeled antibodies and labeled progesterone) and additives such as HAMA blockers

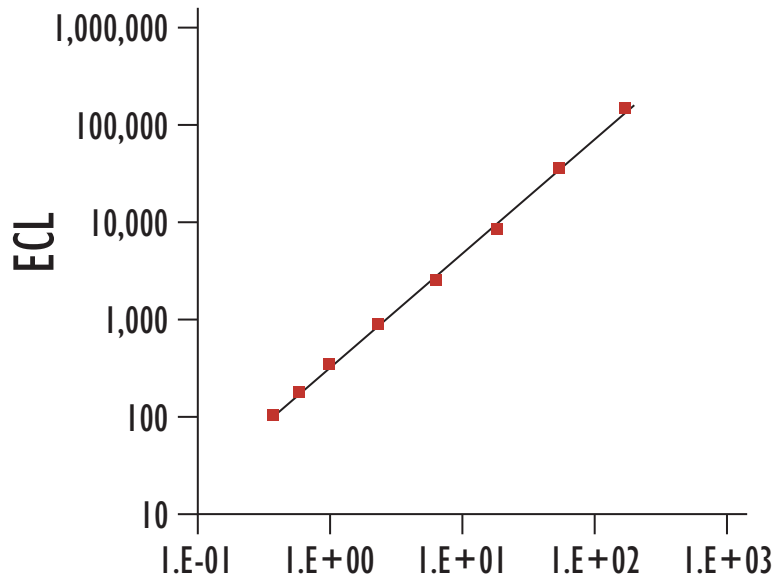
Protocol:

- 1 Add 25 μ L of labeled antibody solution to each well.
- 2 Add 25 μ L of serum sample or calibrators to each well.
- 3 Incubate with shaking for 60 minutes.
- 4 Wash 3X with PBS.
- 5 Add MSD Read Buffer T.
- 6 Read plate on SECTOR[™] Imager 6000.



FSH and LH Assay Performance

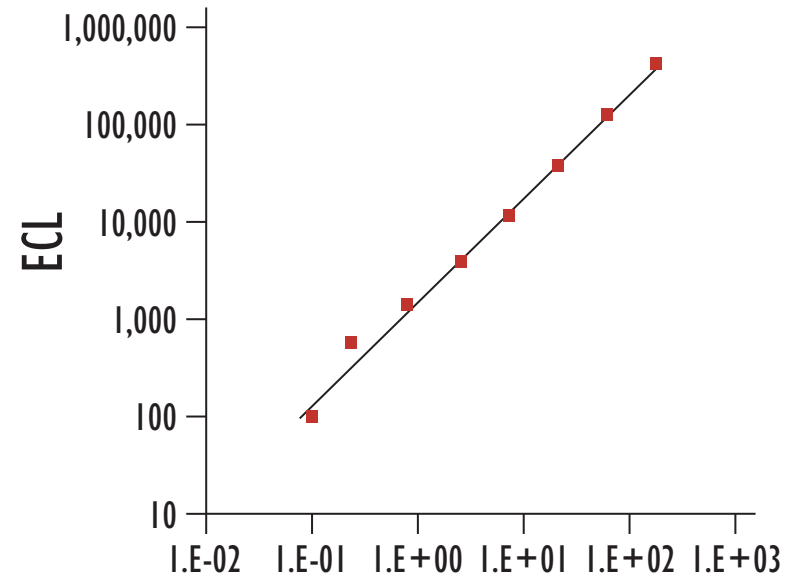
FSH Calibration Curve



FSH Concentration (mIU/mL)

Sensitivity: 0.13 mIU/mL
Dynamic Range: 0.13 to 200 mIU/mL
Variability: 5% CV

LH Calibration Curve

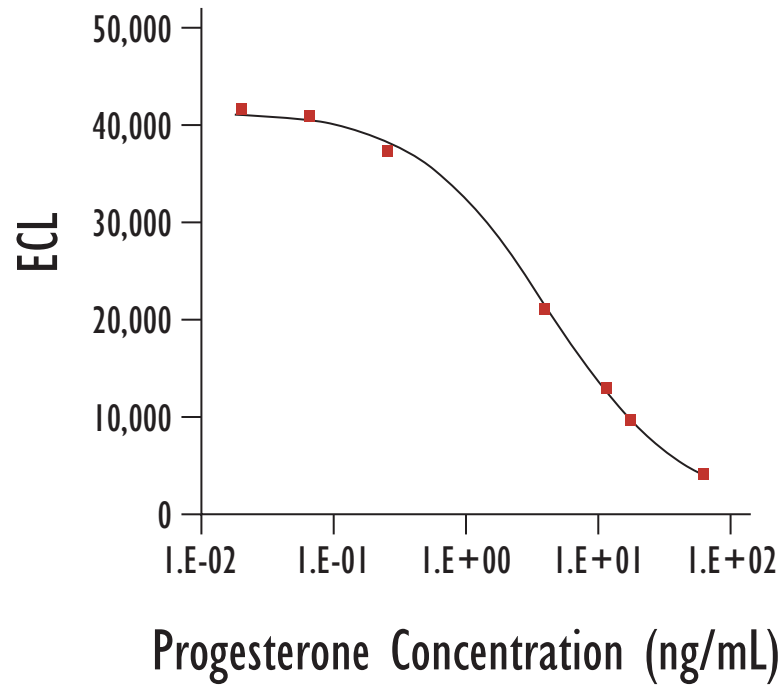


LH Concentration (mIU/mL)

Sensitivity: 0.02 mIU/mL
Dynamic Range: 0.02 to 200 mIU/mL
Variability: 5% CV

Progesterone Assay Performance

Progesterone Calibration Curve



Sensitivity: 0.1 ng/mL
Dynamic Range: 0.1 to 70 ng/mL
Variability: 5% CV



Dilution Linearity

Three female serum samples were diluted with calibrator matrix and measured three times. The average measured concentration is compared to the expected concentration.

LH Dilution Linearity				
Sample #	Dilution Factor	Expected Concentration (mIU/mL)	Measured Concentration (mIU/mL)	Mean Recovery Percentage
1	1/1	23.2	--	--
	1/2	11.6	11.9	103
	1/4	5.8	5.9	99
	1/8	2.9	3.2	110
2	1/1	21.2	21.2	100
	1/2	10.6	10.0	95
	1/4	5.3	5.1	101
	1/8	2.6	2.3	91
3	1/1	2.3	2.3	100
	1/2	1.2	1.1	90
	1/4	0.6	0.6	117
	1/8	0.3	0.3	95

FSH Dilution Linearity				
Sample #	Dilution Factor	Expected Concentration (mIU/mL)	Measured Concentration (mIU/mL)	Mean Recovery Percentage
1	1/1	61.2	--	--
	1/2	30.6	31.1	101
	1/4	15.3	17.8	115
	1/8	7.7	10.1	114
2	1/1	63.7	63.7	100
	1/2	31.9	29.6	93
	1/4	15.9	17.0	115
	1/8	8.0	8.1	95
3	1/1	5.8	5.8	100
	1/2	2.9	3.0	104
	1/4	1.4	1.9	128
	1/8	0.7	1.1	114

Dilutions are linear, and average recoveries are 109% for FSH and 100% for LH over all three samples and three dilutions.



Spike Recovery

Three female serum samples were spiked with known quantities of LH and FSH and measured three times. The measured average concentration is compared to the expected concentration.

FSH Recovery				
Sample #	Amount Spiked (mIU/ml)	Expected Concentration (mIU/mL)	Measured Concentration (mIU/mL)	Mean Recovery Percentage
1	1/1	1.2	--	--
	1/2	13.7	11.7	94
	1/4	26.2	23.0	92
	1/8	51.2	46.2	92
2	1/1	0.2	--	--
	1/2	12.7	12.5	110
	1/4	25.2	21.2	102
	1/8	50.2	42.9	106
3	1/1	0.5	--	--
	1/2	13.0	12.7	100
	1/4	25.5	23.4	85
	1/8	50.5	52.9	86

LH Recovery				
Sample #	Amount Spiked (mIU/ml)	Expected Concentration (mIU/mL)	Measured Concentration (mIU/mL)	Mean Recovery Percentage
1	0.0	0.1	--	--
	12.5	12.6	13.3	107
	25.0	25.1	25.6	102
	50.0	50.1	47.7	95
2	0.0	0.1	--	--
	12.5	12.6	13.4	107
	25.0	25.1	24.6	98
	50.0	50.1	43.8	88
3	0.0	5.4	--	--
	12.5	17.9	14.4	115
	25.0	30.4	26.0	104
	50.0	55.4	51.8	104

Average recovery is 102% for LH and 96% for FSH over all three samples and three spike levels.



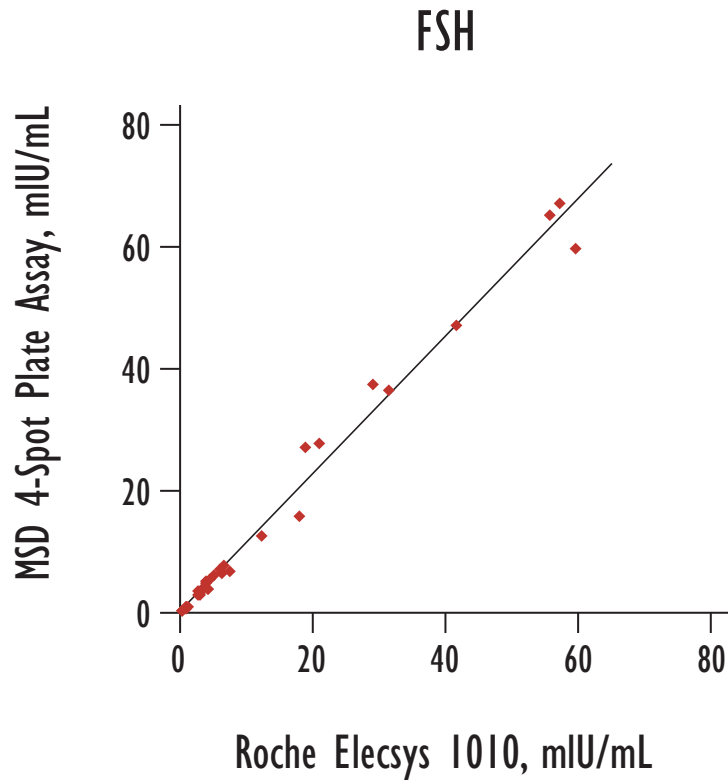
Cross Reactivity

- Inter-assay cross reactivity was evaluated by testing calibrators spiked with FSH (42 mIU/mL), LH (25 mIU/mL) and/or progesterone (12 ng/mL).
- Only a slight cross-reactivity between LH and FSH was noted at the tested concentrations.
- The progesterone assay was unaffected by the tested levels of FSH and LH, and elevated progesterone levels had no measurable effect on the other immunoassays.

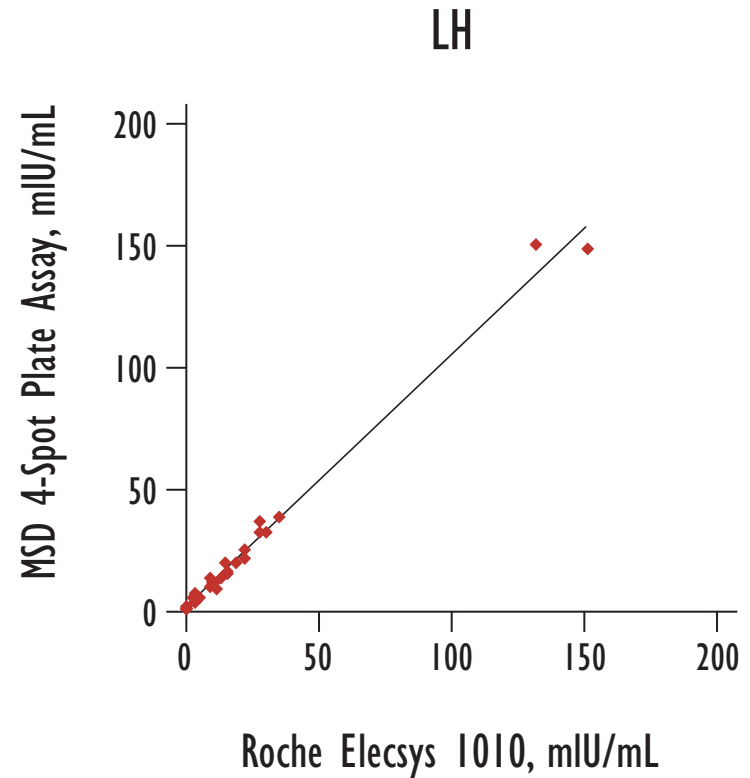
Assay	Analyte		
	FSH	LH	Progesterone
FSH	--	< 1%	0
LH	0.1%	--	0
Progesterone	0	0	--



Method Comparison



Slope: 1.123
Intercept: 0.1267
r: 0.99

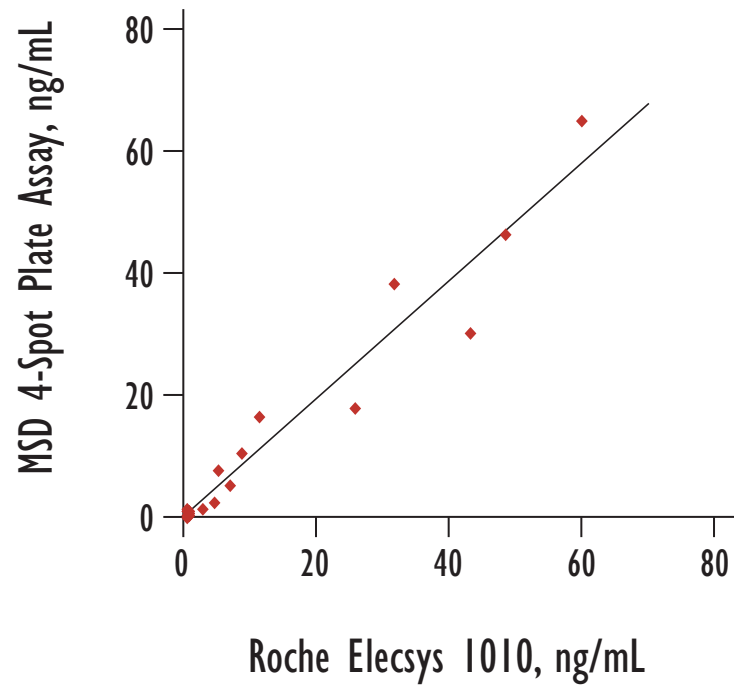


Slope: 1.051
Intercept: 0.350
r: 0.99

Individual female serum samples were measured using both the MSD plate-based assay and the Roche Elecsys 1010 clinical laboratory analyzer. Good correlations were obtained for all three assays.

Method Comparison (continued)

Progesterone



Slope: 0.971

Intercept: -0.054

r: 0.95



Summary

- Multiplexed FSH, LH, and Progesterone immunoassays have been demonstrated on the MSD platform.
- Assays are rapid (1 hour incubation), simple (one addition/one wash), use small sample volumes (25 μ L), are very sensitive and have wide dynamic range.
- Performance is comparable to clinical laboratory analyzers.

