

# Multiplexed Fertility Marker Assays: LH, FSH and Progesterone

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# Multiplexed Fertility Marker Assays: LH, FSH and Progesterone

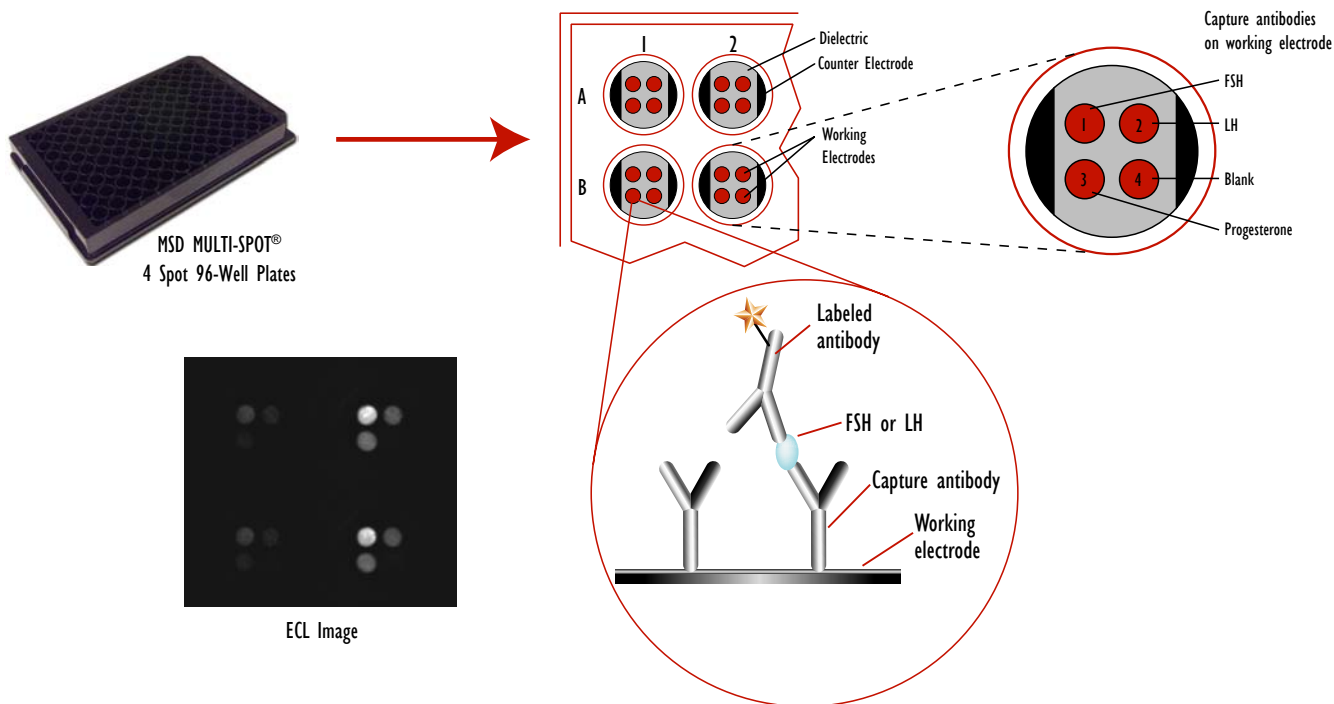
## 1 Abstract

We have developed simultaneous, multiplexed assays for Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and Progesterone on the MSD MULTI-ARRAY™ platform for measurement in serum. LH and FSH are measured in sandwich immunoassays, whereas Progesterone is measured in a competition format. An electrochemiluminescent tag is used to label the detection antibodies and the progesterone competitor. The assay protocol involves a single 1-hour incubation followed by a wash step. Assays are performed in MSD 4-Spot 96-well MULTI-ARRAY Plates, and are read on MSD's SECTOR™ Imager 6000, with a throughput of 80 samples per minute.

Assay ranges are 0.02 – 200 mIU/ml for LH, 0.1 – 200 mIU/ml for FSH and 0.1 – 70 ng/ml (0.3 – 225 nM) for Progesterone. All assays are highly specific, and cover the expected clinical ranges.

## 2 Background and Assay Format

LH and FSH are formatted as sandwich immunoassays using monoclonal antibodies, and progesterone is formatted as a competitive assay using a polyclonal capture antibody and a labeled progesterone competitor. A 4-spot 96-well MULTI-ARRAY plate is used which is precoated with the three capture antibodies on separate spots within each well. The progesterone competitor and the detection antibodies for FSH and LH are labeled with a  $\text{Ru}(\text{bpy})_3^{2+}$  compound, and electrochemiluminescence is used as the detection technology.



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## 3 Assay Protocol

### Assay Components:

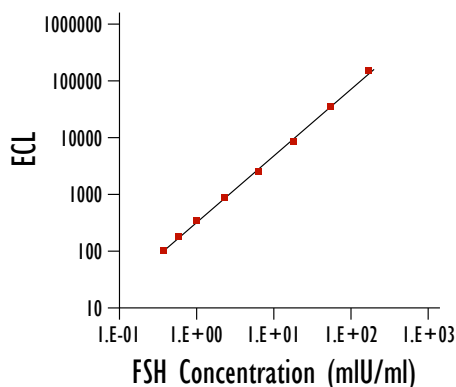
- Plate: 4-spot 96-well MULTI-ARRAY plate, pre-coated with all three capture antibodies.
- Calibration: 8-level multi-analyte calibrator set (serum based matrix).
- Detection Antibodies: labeled antibody solution containing labeled reagents (two labeled antibodies and labeled progesterone) and additives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

### Protocol:

- Add 25  $\mu$ l of labeled antibody solution to each well.
- Add 25  $\mu$ l of serum sample or calibrators to each well.
- Incubate with shaking for 60 minutes.
- Wash 3X with PBS.
- Add MSD-T Read Buffer.
- Read plate on SECTOR Imager 6000 Reader (1 minute read time).

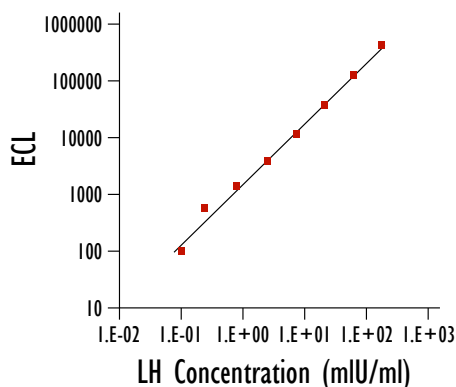
## 4 Assay Performance

FSH Calibration Curve



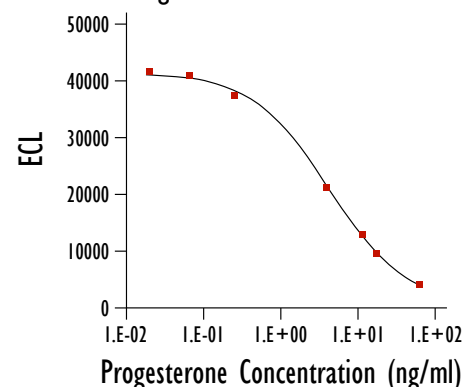
Sensitivity:  0.13 mIU/ml  
Dynamic Range:  0.13 to 200 mIU/ml  
Variability:  5% CV

LH Calibration Curve



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

Progesterone Calibration Curve



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



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## 5 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.

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

LH Dilution Linearity				
Sample #	Dilution Factor	Expected Concentration (mIU/ml)	Measured Concentration (mIU/ml)	Mean Recovery Percentage
1	1/1	23.2	23.2	100
	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	61.2	100
	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

## 6 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.

Average recovery is 102% for LH and 96% for FSH over all three samples and 3 spike levels.

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

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



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## 7 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.

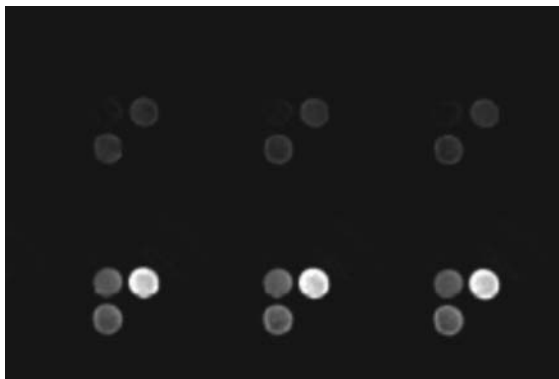
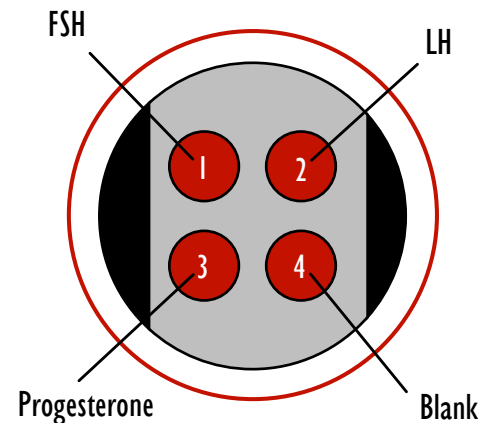
The progesterone assay was unaffected by the tested levels of FSH and LH, and elevated progesterone levels had no measurable effect on the other immunoassays.

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

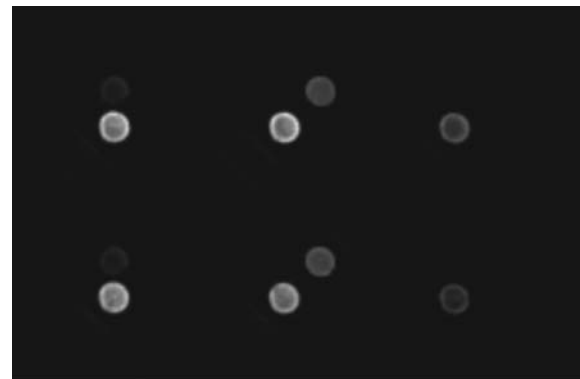
## 8 Multiplexing

Images of ECL from multiplexed fertility marker assays in MSD 4-Spot Plates.

Note: Progesterone is formatted as a competitive assay, therefore high ECL signal on the progesterone spot indicates low progesterone concentration. LH and FSH are sandwich immunoassays: increased concentration leads to increased ECL emission from their respective spots.



FSH, LH, and Progesterone in sample



FSH Only      LH Only      Progesterone Only



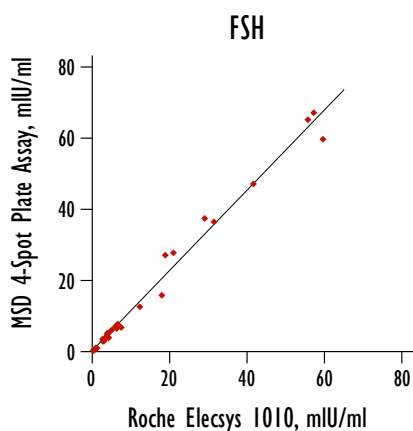
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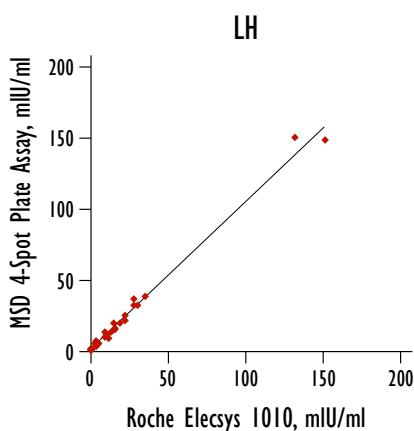
## 9 Method Comparison

Individual female serum samples were measured on both the MSD plate-based assay and the Roche Elecsys 1010 clinical laboratory analyzer.

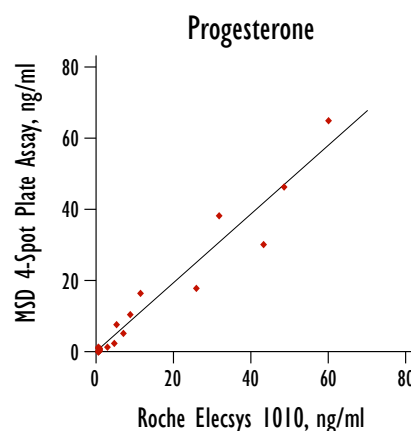
Good correlations were obtained for all three assays.



Slope: 1.123  
Intercept: 0.1267  
r: 0.99



Slope: 1.051  
Intercept: 0.350  
r: 0.99



Slope: 0.971  
Intercept: -0.054  
r: 0.95

## 10 Performance: Comparison to a Clinical Laboratory Analyzer

Assay	MSD Multiplexed Assay		Roche Elecsys	
	Analytical Sensitivity	Range	Analytical Sensitivity	Range
FSH	0.1 mIU/ml	200 mIU/ml	0.1 mIU/ml	200 mIU/ml
LH	0.2 mIU/ml	200 mIU/ml	0.1 mIU/ml	200 mIU/ml
Progesterone	0.1 ng/ml	70 ng/ml	0.15 ng/ml	100 ng/ml

Analytical sensitivity = 2.5 SD above background

Clinical Range: FSH: 1 - 110 mIU/ml  
LH: 1 - 100 mIU/ml  
Progesterone: 0.1 - 255 ng/ml



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## II Conclusions

1. Multiplexed FSH, LH, and Progesterone immunoassays have been demonstrated on the MSD platform.
2. Assays are rapid (1 hour incubation), simple (one addition/one wash), use small sample volumes (25  $\mu$ l), are very sensitive and have wide dynamic ranges ( $>3$  logs).
3. Performance is comparable to clinical laboratory analyzers.



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