

SeaLite Sciences, Inc.

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**510(K) SUMMARY**

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**I. GENERAL INFORMATION**

**Trade or proprietary name** - SeaLite Sciences, Inc. AquaLite® FSH

**Common or usual name** - Bioluminescent immunoassay (BIA)

**Classification name** - FDA has classified FSH test systems intended for the measurement of FSH in the diagnosis of pituitary gland and gonadal function.

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**Legally Marketed Device to which Claim Substantial Equivalence:** SeaLite Sciences, Inc. AquaLite® FSH Assay

**II. DEVICE DESCRIPTION**

The AquaLite® FSH Bioluminescent Immunoassay Kit uses a polyclonal anti-FSH antibody that is pre-coated onto polystyrene tubes (solid phase). Samples (serum or plasma) and appropriate calibrators or controls, are pipetted (25 µL) into the pre-coated tubes. Anti-FSH Conjugate (150 µL) is then added to the tubes. The conjugate uses the photoprotein, AquaLite® (recombinant aequorin; Patent Nos. 5, 422, 266 and 5, 486, 455) which is covalently linked to an anti-FSH monoclonal antibody. FSH in the sample simultaneously combines with polyclonal antibody on the solid phase and conjugate antibody to form an immune complex or "sandwich" bound to the solid phase. Complex formation is complete after a 60-minute incubation period at room temperature (18°C to 25°C) on a standard orbital shaker. The tubes are then washed to remove unbound conjugate.

The washed tubes are placed in a luminometer that is capable of reading a triggered, flash-type reaction in 12 x 75 mm tubes. An injected calcium trigger solution causes AquaLite® to oxidize its self-contained luciferin molecule. This reaction produces a flash of light at 469 nm, which is measured by the luminometer. The intensity of the light is directly proportional to the concentration of the FSH in the sample. To calculate results, the light intensity (in relative light units, RLU) of the FSH calibrators is plotted against FSH concentration (in International Units per liter, IU/L) to yield a calibration curve. This curve is used to relate the light intensity generated from the samples and controls to FSH concentration in IU/L. Note that the numerical value for FSH in mIU/mL is the same as for IU/L (International System). For example, 15.6 mIU/mL equals 15.6 IU/L.

Note: Samples that generate signals greater than the signal from the highest calibrator are off-scale. These samples must be diluted with Calibrator A and re-assayed. Remember to multiply the results from diluted samples by the dilution factor used.

### **III. SUMMARY OF STUDIES AND TECHNOLOGICAL CHARACTERISTICS**

Studies on SeaLite Sciences, Inc. AquaLite® FSH were conducted at SeaLite Sciences. The results are summarized below:

#### **Performance Characteristics**

##### **1. Sensitivity**

The sensitivity or detection limit of the AquaLite® FSH assay is 0.03 IU/L. Sensitivity is determined by adding the mean signal of twenty (20) replicates of the zero level calibrator plus two (2) standard deviations above this mean. The FSH concentration (IU/L) corresponding to this calculated signal is defined as the analytical sensitivity of the assay.

##### **2. Specificity**

The AquaLite® FSH assay measures intact FSH. The following human sialoglycoprotein hormones were supplied by the World Health Organization's National Institute for Biological Standards and Controls (London, England). Aliquots of these preparations were diluted to the following levels in zero calibrator and assayed. Percent cross-reactivity (%) is reported below:

<u>Substance</u>	<u>WHO/NIBSC Lot Number</u>	<u>Tested at</u>	<u>% Cross-reactivity</u>
hCG	3rd IS 75/537	2,500 IU/L	0.02
LH	2nd IS 80/552	1,000 IU/L	0.10
TSH	2nd IRP 80/558	1,000 mIU/L	1.90

3. **High Dose Hook Effect** - No high dose hook effect occurs prior to 4,000 IU/L FSH.

4. **Precision**

(a) Intra-assay precision. Tri-level commercial controls containing FSH at the following concentrations were assayed to determine intra-assay precision. (Total N = 10 per solution.)

<u>Mean FSH Level (IU/L)</u>	<u>SD</u>	<u>% CV (calibration values)</u>
6.69	0.36	5.4
26.89	1.80	6.65
45.28	3.23	7.15

(b) Inter-assay precision. Tri-level commercial controls containing FSH at the following concentrations were assayed in duplicate repetitively. Ten assays were performed using ten sets of calibration values. Inter-assay precision observed for the solutions (Total N = 2 x 10 = 20) is shown below.

<u>Mean FSH Level (IU/L)</u>	<u>SD</u>	<u>% CV</u>
6.45	0.485	7.52
26.26	1.679	6.39
43.33	4.049	9.35

**5. Method Comparison**

The AquaLite® FSH assay was used to test patient samples (N=92) that were previously assayed by a commercially available kit. The samples ranged from 1.4 to 230 IU/L. A slope of 0.66 with a y-intercept of 0.78 was obtained. The correlation coefficient was 0.90.

**6. Linearity and Nonparallelism**

Five human serum samples containing the levels of endogenous FSH shown below were diluted in parallel using Calibrator A.

Sample ID	Dilution Factor	Found (IU/L)	Expected (IU/L)	Recovery (%)
2	Undiluted	179.6	--	--
	1:2	92.1	89.9	102
	1:4	42.1	44.9	94
	1:8	24.2	22.45	108
3	Undiluted	104.8	--	--
	1:2	48.8	52.4	93
	1:4	29.4	26.2	109
	1:8	12.7	13.1	97.6
6	Undiluted	54.1	--	--
	1:2	24.5	27	90.7
	1:4	14.9	13.5	110
	1:8	8.1	6.8	115
30	Undiluted	134.6	--	--
	1:2	75.1	67.3	111
	1:4	36.1	33.6	106
	1:8	19.7	16.8	113
42	Undiluted	163.5	--	--
	1:2	84.3	81.75	102
	1:4	41.4	41	101
	1:8	26.1	20.5	118

**7. Spike and Recovery**

Eight normal human serum samples were spiked with 25 IU/L FSH using WHO FSH (2nd IRP 78/549). The spiked samples were assayed using the AquaLite® FSH assay. All values are in IU/L.

<u>Sample ID</u>	<u>Unspiked</u>	<u>FSH Measured</u>	<u>FSH Expected</u>	<u>% Recovery</u>
1	4.2	29.2	29.2	100
12	4.4	34.3	29.4	115
14	7.3	37.4	32.3	115
17	4.9	32.6	29.9	109
20	7.1	35.5	32.1	110
54	7.9	42.4	32.9	128
57	7.4	35.1	32.4	108
81	7.3	37.2	32.3	115

**8. Recovery in Serum and Plasma**

Blood Samples from 2 normal subjects were prepared as sera (standard technique and SST tubes) as well as heparin, EDTA, oxalate, and citrate plasmas. FSH was quantified using the AquaLite® FSH assay. Recovered FSH was compared with FSH recovered in serum (standard technique). The data demonstrate that there are no significant differences among serum and SST serum nor among serum and heparin, EDTA, oxalate and citrate plasmas when using the AquaLite® FSH assay. All values are in IU/L.

<u>Sample</u>	<u>P1</u>	<u>%</u>	<u>P2</u>	<u>%</u>
Serum	9.35	100	9.59	100
SST	9.54	102	10.5	109
EDTA	9.07	97	8.62	90
Heparin	9.89	106	10.4	109
Oxalate	8.50	91	8.8	92
Citrate	7.75	83	9.1	95

**9. Effect of Common Interferents**

Pooled normal male human serum was spiked with preparations of hemoglobin, bilirubin, human serum albumin and triglycerides to the levels shown below. Equal amounts of FSH were spiked into normal male serum as well as the normal male serum aliquots containing potential interferents. FSH was quantified using the AquaLite® FSH assay. Recovered FSH was compared with the FSH recovered in normal male serum. The data (in IU/L) demonstrate that the AquaLite® FSH assay is not significantly affected by hemoglobin, bilirubin, human serum albumin or triglycerides at the levels tested.

FSH		Hemoglobin (at 500 mg/dL)		Bilirubin (at 20 mg/dL)		Albumin (at 12 mg/dL)		Triglycerides (at 3000 mg/dL)	
<u>Spike</u>	<u>Serum</u>	<u>IU/L</u>	<u>%</u>	<u>IU/L</u>	<u>%</u>	<u>IU/L</u>	<u>%</u>	<u>IU/L</u>	<u>%</u>
0	9.77	9.83	100	9.13	93	10.2	105	8.56	88
25.7	35.5	36.3	102	35.6	94	37.0	104	36.6	103

**IV. CONCLUSIONS DRAWN FROM STUDIES**

The data from the studies conducted demonstrated that the performance of SeaLite Sciences, Inc. AquaLite® FSH is similar and substantially equivalent to that of other commercially available assays for FSH.

**V. ALTERNATIVE PRACTICES AND PROCEDURES**

There are several assay technologies commonly employed to measure the presence of human FSH in serum or plasma. They include: radioimmunoassay (RIA) and enzyme-linked immunosorbent immunoassay (ELISA).

**VI. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH**

Use Universal Precautions. No known test method can offer complete assurance that products derived from human serum are pathogen-free; therefore, handle all materials of human origin as though they were potentially infectious.

Sodium azide is used as a preservative. This preservative may react with metallic plumbing to form explosive metal azides. Flush with large volumes of water when disposing of materials containing sodium azide.

As an *in vitro* diagnostic test, there are not direct adverse effects on the health of a patient from the use of this product. However, failure of the device to perform as indicated, the contamination of reagents, the use of reagents past the labeled expiration dates, the use of improper specimens, or human error during the performance of the test may lead to erroneous results and possible improper patient management.

## **VII. INDICATIONS FOR USE**

The AquaLite® FSH Bioluminescent Immunoassay (BIA) Kit (or the AquaLite® FSH assay) is intended to be used in clinical laboratories for the quantitative determination of human FSH in sera and plasma. The AquaLite® FSH assay is for *in vitro* diagnostic use.