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EXHIBIT D

SUMMARY OF SAFETY AND EFFECTIVENESS

I. GENERAL INFORMATION

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

Common or usual name - Bioluminescent immunoassay (BIA)

Classification name - FDA has classified LH test systems intended for the measurement of LH in the diagnosis of gonadal dysfunction.

II. INDICATIONS FOR USE

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

III. DEVICE DESCRIPTION

The AquaLite® LH Bioluminescent Immunoassay Kit uses a polyclonal anti-LH antibody that is pre-coated onto polystyrene tubes (solid phase). Samples (serum or plasma and appropriate calibrators or controls, are pipetted (50 μ L) into the pre-coated tubes. Anti-LH Conjugate (150 μ L) 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-LH monoclonal antibody. LH 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 LH in the sample. To calculate results, the light intensity (in relative light units, RLU) of the LH calibrators is plotted against LH 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 LH concentration in IU/L. Note that the numerical value for LH 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.

IV. ALTERNATIVE PRACTICES AND PROCEDURES

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

V. MARKETING HISTORY

The modified SeaLite Sciences, Inc. AquaLite® LH that is the subject of this submission is not currently marketed.

VI. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Caution: 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. SUMMARY OF STUDIES

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

Performance Characteristics

1. Sensitivity

The sensitivity or detection limit of the AquaLite® LH assay is 0.01 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 LH concentration (IU/L) corresponding to this calculated signal is defined as the analytical sensitivity of the assay.

2. Specificity

The AquaLite® LH assay measures intact LH. The following human sialoglycoprotein hormones were supplied by the World Health Organization's National Institute for Biological Standards and Control (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 (IU/L)</u>	<u>% Cross-reactivity</u>
TSH	2nd IRP 80-558	1,000	4.9
FSH	I.S. 83-575	1,000	0.68
hCG	IRP 75-551	2,500	1.2

3. **High Dose Hook Effect** - No high dose hook effect occurs prior to 5,000 IU/L LH.

4. Precision

(a) Intra-assay precision. Tri-level commercial controls containing LH at the following concentrations were assayed to determine intra-assay precision. (Total N = 20 per solution.)

<u>Mean LH Level (IU/L)</u>	<u>SD</u>	<u>% CV (calibration values)</u>
1.069	0.0807	7.55 %
9.821	0.578	5.9 %
40.129	2.728	6.8 %

- (b) Inter-assay precision. Tri-level commercial controls containing LH 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 LH Level (IU/L)</u>	<u>SD</u>	<u>% CV</u>
1.585	0.131	8.2%
11.303	0.924	8.17%
40.09	3.60	8.9%

5. Method Comparison

The AquaLite® LH assay was used to test patient samples (N=62) that were previously assayed by a commercially available kit. The samples ranged from 0.5 to 62.0 IU/L. A slope of 0.89 with a y-intercept of 1.53 was obtained. The correlation coefficient was 0.938.

6. Linearity and Nonparallelism

Three human serum samples containing the levels of endogenous LH shown below were diluted in parallel using Calibrator A with 0.9% sodium chloride added.

Sample ID	Dilution Factor	Found (IU/L)	Expected (IU/L)	Percent (%)
SLS#4	Undiluted	80.83	--	--
	1:2	39.55	40.42	97.9
	1:4	19.575	20.21	96.9
	1:8	13.4	10.1	130
SLS#18	Undiluted	63.82	--	--
	1:2	30.436	31.91	95.4
	1:4	15.84	15.95	99.4
	1:8	9.09	8.0	113
SLS#26	Undiluted	75.46	--	--
	1:2	44.4	37.73	115
	1:4	19.65	18.86	104
	1:8	10.4	9.43	111

7. Spike and Recovery

Normal male human serum samples were spiked with LH in the amounts noted below. The spiked samples were assayed using the AquaLite® LH assay. All values are in IU/L.

<u>Endogenous</u> <u>LH</u>	<u>Added</u> <u>LH</u>	<u>Measured</u> <u>LH</u>	<u>Expected</u> <u>LH</u>	<u>Percent</u> <u>Recovered</u>
6.83	50	60.35	56.83	106.1
11.82	50	56.59	61.82	91.6
3.14	50	57.69	53.14	108.6
0.922	50	48.88	51.0	96
2.17	50	53.29	52.17	101.5
0.16	50	52.7	50.2	104.6
2.06	50	56.89	52.06	109.3
2.53	50	56.02	52.53	106.6

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. LH was quantified using the AquaLite® LH assay. Recovered LH was compared with LH 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, and citrate plasmas when using the AquaLite® LH assay. Oxalate plasmas is not recommended.

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 LH were spiked into normal male serum as well as the normal male serum aliquots containing potential interferents. LH was quantified using the AquaLite® LH assay. Recovered LH was compared with the LH recovered in normal male serum. The data (in IU/L) demonstrate that the AquaLite® LH assay is not

significantly affected by hemoglobin, bilirubin, human serum albumin or triglycerides at the levels tested.

LH <u>Spike</u>	Neat <u>Serum</u>	Hemoglobin (at 500 mg/dL)		Bilirubin (at 20 mg/dL)		Albumin (at 12 mg/dL)		Triglycerides (at 3000 mg/dL)	
		<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	4.8	-	-	-	-	-	-	-	-
65	70.0	68.7	98.2	72.4	103	71.0	101	69.1	98.7

VIII. CONCLUSIONS DRAWN FROM STUDIES

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