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To: Dr. Gary Samuelson
ASEA

Re: ASEA Composition

**In the course of many years of investigations into the production of salt-water based redox signaling molecules, consisting of Reactive Oxygen Species [ROS] and Reduced Species [RS], We have performed numerous biological and biochemical analyses of the compounds generated from the same patented production method employed to make the ASEA product, including fluorometric determination of ROS, standard analytics to determine the RS content and analysis to detect such redox signaling molecules in blood and plasma.**

**ASEA is a clear, colorless liquid generated from preservative-free and endotoxin-free, nonpyrogenic, sterile, saline by using a patented electro-catalytic process. It contains numerous highly reactive species. Reactive oxygen species [ROS] are of immense interest because there is compelling evidence linking them to aging and fundamental life processes. The experimental procedure to measure ROS is outlined briefly.**

**We have modified a simple but extremely sensitive method of Cao et. al. [1] to determine total reactive oxygen content of ASEA. In this assay, R-Phycoerythrin [an algal protein] is exposed to varying levels of a standard ROS generating compound [AAPH] wherein the level of fluorescence quenching is logarithmically related to ROS content. This provides a standard curve from which to estimate the ROS content of unknown samples.**

**Determination of ROS content in ASEA has been successful and is used routinely to monitor production quality for ROS levels. The assay has the following characteristics: ease of use, sensitivity, and quantitation. The assay is linear over a 100 fold range of ROS concentrations. For ASEA, the starting saline was used as a negative control, AAPH served as a positive control and allowed the generation of a standard curve, and ASEA or other samples comprised the unknowns.**

**The following analysis was employed to answer another important question: How long does the ROS contained in ASEA exist in the blood and tissues of a mammal? A measure of this length is called the "biological half-life" that defines,**

[1] Cao, G et al. Clinical Chemistry 1998; 44:6 1309-1315

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**in part, the pharmacokinetic behavior of ROS contained in ASEA. Protected hydroxylamine probes have been developed to measure ROS in vivo, both in the blood and tissues. [2, 3] The parent compound, ACP, is subject to hydrolysis by esterases to an intermediate compound which is then subject to oxidation forming the N-oxide by ROS. ROS was measured by its action on a surrogate indicator compound, ACP. We have developed a highly sensitive high-performance liquid chromatography [HPLC] methodology combined with positive ion electrospray mass spectrometry for detection/quantitation of ACP in the blood of animals. An innovative quantitative technology with excellent sensitivity and linearity was developed to measure ACP as a surrogate marker for the ROS contained in ASEA in blood plasma and can be extended to the determination of ACP in tissues as well. This technology extended the level of detection to the extent that $T_{1/2}$ could be comfortably be detected.**

**In conclusion, we have been able to document the existence of Reactive Oxygen Species, belonging to a family of molecules called redox signaling molecules, in ASEA. Using highly sensitive fluorescent assay, we measured the levels of ROS in ASEA on a regular and consistent basis. Secondly, we established a highly sensitive assay to detect and quantify indirectly the levels and the half-life of ROS present in the blood of a mammal.**

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[2] Belkin, S. et. al. Biochem. Biophys. 1987; 256:232-243

[3] Saito, K. et. a. Free Radical Biol. & Med. 2003;36:517-525

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**James Clagett Bio**

Shortly after James A Clagett, Ph.D. received his Ph.D. from Nebraska University, he was quickly appointed Affiliate and a full Tenured Professorship at the University of Washington where he taught graduate and undergraduate Immunology and Immunopathology to Microbiology. After over a decade of teaching and research achievements, Dr. Clagett branched out into the medical diagnostics industry where he gained recognition and experience in developing clinical studies, patents and research necessary for the commercialization of new technologies. In this capacity Dr. Clagett has achieved a long and distinguished career marked by many successes, helping commercialize developing technologies like the Tissue Bone matix, Gen Sci Regeneration Sciences, Inc. as an example. He has been instrumental in establishing several technologies and businesses instrumental to medical research all over the globe. Dr. Clagett is now a sought after consultant for businesses developing new technologies and research protocols for a variety of companies. He first developed the experimental techniques and analytical methods used to test for redox signaling molecules in liquids.


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