The Redox SYS™ Diagnostic System

A Novel Diagnostic Instrument for
Clinical Applications & Scientific Research
About Luoxis Diagnostics

Luoxis Diagnostics is an in-vitro diagnostics company focused on the development and global commercialization of the Redox SYS™ Diagnostic System. This novel diagnostic platform is comprised of a first-in-class diagnostic device and disposable testing sensors that together measure the presence of oxidative stress and antioxidant reserves in critically ill and injured patients. More information is available at www.luoxis.com.

The Redox SYS™ Diagnostic System is the only diagnostic system that measures human Oxidation Reduction Potential (ORP), an important, novel measure implicated in both critical and chronic illnesses. As demonstrated over decades in multiple peer-reviewed publications, ORP is an important homeostatic parameter that serves as a marker in the assessment of oxidative stress and patient morbidity across a wide range of diseases and conditions. There are numerous clinical, laboratory, and research applications for this homeostatic parameter for which there is no currently available testing platform.

About Oxidative Stress
Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling. In humans, oxidative stress is known to be involved in the development of numerous chronic diseases including cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome, Sickle Cell Disease, lichen planus, vitiligo, autism, and chronic fatigue syndrome. Oxidative stress has also been shown to manifest in acute illnesses, traumatic bodily injuries, and traumatic brain injury.
Numerous scientific studies confirm the clinical value of measuring oxidative stress. Recently, a large assortment of blood and cell tests have been used in research studies to measure separate biomarkers of oxidative stress, such as lipid peroxidation, protein oxidation and total antioxidants, but currently multiple, separate biomarker test results are needed to assess total oxidative stress — yielding incomplete analyses as many oxidants and antioxidants are not yet identified.

Despite the importance of assessing oxidative stress, we are not aware of any practical or efficient method for measuring these oxidative stress biomarkers in a clinical setting. Oxidative stress is often a marker for inflammation, which in turn indicates the presence of disease-related processes or developing conditions across a wide array of diseases.

Oxidative stress is studied in multiple research settings, but traditional test methods assess individual markers that are incomplete and time consuming.

Traditional laboratory tests assessing oxidative stress include:

- Catalase Activity Assays
- Cellular Antioxidant Assays
- Glutathione Assays
- Hydroxyl Radical Antioxidant Capacity Assays
- Oxygen Radical Antioxidant Capacity Assays
- Superoxide Dismutase (SOD) Assays

**About Oxidation-Reduction Potential**

Oxidation-reduction potential (ORP) is an overall measure of the oxidative stress to which a biological component is subjected and correlates with organ dysfunction. Specifically, ORP in biological systems has been described as an integrated measure of the balance between total oxidants (e.g., oxidized thiols, superoxide radical, hydroxyl radical, hydrogen peroxide, nitric oxide, peroxynitrite, and transition metal ions), and total reductants (e.g., free thiols, ascorbate, a-tocopherol, b-carotene, and uric acid). Oxidation-Reduction Potential (ORP) is an established, integrated measure of all elements of oxidative stress, inclusive of all known and unknown oxidants and reductants.
The current measurement of ORP is difficult, costly, and time consuming. Until the development of the Redox SYS™ Diagnostic System, ORP applications were limited to studies unrelated to medical and clinical research.

About the Redox SYS™ Diagnostic System

Redox SYS™ Diagnostic System Overview
The Redox SYS™ Diagnostic System is comprised of two distinct, patented components that enable a system capable of measuring the ORP (Oxidation-Reduction Potential) and antioxidant capacity of a biological fluid: a small, portable reader and disposable sensor strips. In mechanical terms ORP is defined as the potential between a working electrode and a reference electrode at equilibrium. The Redox SYS™ Diagnostic System has been specifically studied in human whole blood, serum, and plasma. The Redox SYS™ Diagnostic System measures distinct elements to determine a patient’s oxidation reduction potential:

- **Static ORP** – the standard potential between a working electrode and a reference electrode with no driving current (or extremely small current) this is proportional to the balance of redox agents and is what is classically defined as ORP; Low ORP values mean that the biological sample is in the normal range of oxidative stress. Higher than normal ORP values means that the biological sample is in a higher state of oxidative stress.
- **Capacity** – the measure of antioxidant reserve available in the body’s system; High capacity values mean that the biological sample has antioxidant reserves in the normal range. Lower than normal capacity values means that the biological sample has below normal antioxidant reserves.

Both of these values are uniquely measured and reported by the Redox SYS™ Diagnostic System and show statistically significant correlations with important clinical features in critical injuries. More information is presented on the Clinical Applications section.

The Redox SYS™ Reader
The Redox SYS™ reader consists of both a small, portable desktop platform that may be used at a patient’s point-of-care. The reader is a small device that accepts an inserted sensor that then collects a small sample of human plasma as obtained by heparin-based blood collection procedures. The reader is battery powered and equipped with an AC adaptor for recharging or stationary laboratory use. The reader consists of a Galvanostat analog circuit with > 1012 MΩ input impedance.

The reader contains a 10 MHz external crystal (internal 4X PLL for 40 MHz operation), and a programming/serial header is externally accessible. The device has internal power/heart-beat indicator LED, primary storage of 128Mbit (16Mbyte) SPI Flash (3.3V) (Bulk data storage), and secondary storage of 2Mbit (256Kbyte) SPI FRAM (3.3V) (Hi-Speed Storage).

The Redox SYS™ reader contains a simple user interface that is flexibly designed to accommodate multiple endpoints depending upon the specific clinical condition being considered. The interface is LCD, 20x4, with a white backlight with on/off control via user selection or optional, variable delay auto-off time-out. Two status LED indicators are visible through front panel mounted lenses. Further, the reader contains multiple push-button switches, power on button for battery mode operation, switch usage switch, audible alerts, strip detection, and test completion signals.
The ORP reader’s power management consists of an external 5VDC power jack with input capacitance and filtering, a boost converter supplied by external 5VDC power or internal Li-Ion battery, and provides main 5VDC digital board supply. The reader functions with or without the battery connected.

Additional technical specifications of the ORP reader include:
- µC managed on/off power control
- Li-Ion battery charger with automatic temperature and current limiting
- Power supply
- SPI bus
- Temperature monitor (analog input)
- RH monitor (analog input)
- Real-Time Clock, SPI bus, backup with main battery
- 4.096V reference voltage
- Internal AFE board temperature monitor
- Internal AFE board RH sensor monitor
- Battery voltage monitor
- Battery temperature monitor
- ESD protection on externally accessible pins
Sensor Strips
The Redox SYS™ sensor strips receive a plasma sample (40 µL) from which the ORP clinical analysis is performed. The Redox SYS™ sensor strips are small, disposable, and biocompatible and consist of a ceramic substrate and a five-lead configuration.

- Lead 1 is the working electrode.
- Lead 2 is the reference electrode.
- Lead 3 is the counter electrode.
- Lead 4 and 5 are used as loopback strip detectors.

The electrodes are multi-layered and consist of the following:

- Platinum vacuum sputtering with a titanium/tungsten adhesion layer
- Ag/AgCl Reference electrode (printable conductive ink)
- Two layer reference isolation/electrolyte gels
- Vinyl electrical and physical isolation
- Small pore size fiberglass blood separation filter and salt bridge

Image of the Redox SYS™ Sensor Strip
The ORP Laboratory System performs comprehensive oxidative stress assessment with a simple test.

Clinical Studies
Significant research has been performed on the oxidation-reduction potential diagnostic platform, and numerous peer-reviewed publications demonstrate the various considerations made in the development of this application in a clinical setting. Further, the research conducted to date demonstrates the clinical relevance of ORP as a diagnostic marker in trauma, the development of TRALI via blood transfusions, and other conditions. Over the past twenty years Ampio Pharmaceuticals, Inc. employees Dr. Bar-Or, Raphael Bar-Or, Leonard Rael, and their colleagues have employed the resources of two Level 1 trauma centers in the state of Colorado. Specific, select studies reporting on the clinical role of ORP as it relates to trauma, acute lung injury related to blood transfusions, and traumatic brain injury include:


Abstract
The amount of oxidative stress in severely traumatized patients is usually based on various individual parameters such as total antioxidants and lipid peroxidation. Serial measurements of plasma oxidation–reduction potential (ORP) in severely traumatized patients as a simple mean of assessing overall oxidative stress is described. Serial whole blood samples were obtained from multi-trauma patients (N = 39) and healthy individuals (N = 10). Plasma ORP in multi-trauma patients increased during the first few days of hospitalization and approached normal ORP levels upon discharge. On the ORP maxima day (5.8 days ± 0.5 SEM), a statistically significant decrease (p < 0.05) was observed for negative acute phase reactants such as plasma paraoxonase–arylesterase (PON–AE) activity and total plasma protein in comparison with admission plasma levels. Monitoring ORP could be a useful tool for assessing the degree of oxidative stress, inflammation, severity of injury, and potential efficacy of treatment.

Abstract:
Background: Transfusion-related acute lung injury (TRALI) is a life-threatening condition characterized by oxidative stress. Longer storage times of packed red blood cells (PRBC) and other blood products have been implicated with an increased risk in developing TRALI in transfused patients. Methods: A total of 10 units of blood containing PRBC stored in citratephosphate-dextrose buffer at 4°C were included in the study. At Bonfils Blood Center (Denver, CO), samples were collected on storage day 1 and day 42. Samples were immediately centrifuged, and the supernatants were collected and stored at 80°C until further analysis. Oxidation reduction potential and protein oxidation were measured in both the day 1 and day 42 samples. Results: Oxidation-reduction potential significantly increased (p < 0.05) in the day 42 sample (98.1 mV ± 21.9 SD) versus the day 1 sample (62.6 mV ± 21.5 SD). The oxidation of human serum albumin increased by 63.6% during the storage time. Other serum proteins such as apolipoprotein A1 and transthyretin demonstrated similar increases in oxidation. Also, proteins with a cleaved C-terminal amino acid were observed indicating the presence of carboxypeptidase activity, a marker of inflammation. Conclusions: The presence of an oxidative environment in transfused PRBC increases with storage time. This could partially explain the increased risk of developing TRALI related to the transfusion of older blood products.


Abstract
The amount of oxidative stress in patients with an isolated traumatic brain injury (ITBI) can be estimated by measuring several biochemical parameters, such as total antioxidants, lipid peroxidation, protein oxidation, and others. Unfortunately, measuring these parameters is time-consuming, impractical in a clinical setting, and may miss important factors contributing to the overall redox balance. Here we suggest that the overall oxidative status in ITBI patients can be assessed by measuring plasma oxidation-reduction potential (ORP). Daily whole blood samples were obtained from severe ITBI patients (abbreviated injury score [AIS] 3, n=32), and demographically similar non-head injury traumatized patients (n=26) until discharge. Whole blood was also collected from patients with minor to moderate ITBI (AIS 2, n=18) and healthy volunteers (n=22). Admission plasma ORP was significantly elevated in all traumatized patients compared to controls. Maximum ORP was detected on day 6 for severe ITBI and non-head injury traumatized patients. However, maximum ORP values were significantly higher (p<0.05) in the severe ITBI group (þ8.5mV± 3.4 SD) compared to the non-head injury group (5.2mV± 2.9 SEM). Additionally, a significantly higher oxidation of human serum albumin (HSA) was measured in all trauma patients compared to controls. These results demonstrate the presence of an oxidative environment in the plasma of traumatized patients, specifically in severe ITBI patients. Therefore monitoring ORP is a potentially useful tool for assessing the degree of oxidative stress, inflammation, severity of injury, and potential efficacy of treatment in ITBI patients.
Rael, LT et al., Injury Severity And Serum Amyloid A Correlate With Plasma Oxidation-Reduction Plasma In Multi-Trauma Patients: A Retrospective Analysis. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine, 17:57 (November 2009)

Abstract
Background: In critical injury, the occurrence of increased oxidative stress or a reduced antioxidant status has been observed. The purpose of this study was to correlate the degree of oxidative stress, by measuring the oxidation-reduction potential (ORP) of plasma in the critically injured, with injury severity and serum amyloid A (SAA) levels. Methods: A total of 140 subjects were included in this retrospective study comprising 3 groups: healthy volunteers (N = 21), mild to moderate trauma (ISS < 16, N = 41), and severe trauma (ISS ≥ 16, N = 78). For the trauma groups, plasma was collected on an almost daily basis during the course of hospitalization. ORP analysis was performed using a microelectrode, and ORP maxima were recorded for the trauma groups. SAA, a sensitive marker of inflammation in critical injury, was measured by liquid chromatography/mass spectrometry. Results: ORP maxima were reached on day 3 (± 0.4 SEM) and day 5 (± 0.5 SEM) for the ISS < 16 and ISS ≥ 16 groups, respectively. ORP maxima were significantly higher in the ISS < 16 ( -14.5 mV ± 2.5 SEM) and ISS ≥ 16 groups ( -1.1 mV ± 2.3 SEM) compared to controls ( -34.2 mV ± 2.6 SEM). Also, ORP maxima were significantly different between the trauma groups. SAA was significantly elevated in the ISS ≥ 16 group on the ORP maxima day compared to controls and the ISS < 16 group. Conclusion: The results suggest the presence of an oxidative environment in the plasma of the critically injured as measured by ORP. More importantly, ORP can differentiate the degree of oxidative stress based on the severity of the trauma and degree of inflammation.

Applications of the ORP Laboratory System
Luoxis’ Laboratory System has broad application in clinical settings across human health, animal health, and banked blood. The system has further application in multiple research settings where oxidative stress analytes are studied.
The Redox SYS™ Laboratory System; Intellectual Property

Multiple broad US patent applications have been awarded that describe methods and systems for measuring oxidation-reduction potential of a fluid sample. Additional patent applications include methods for determining ORP values of banked blood, ORP as a diagnostic marker for trauma, viral infection, critical illness, myocardial infection, and exercise. Further applications have been filed for the ORP as a risk assessment tool in making hospitalization decisions. Other applications are being actively considered. Two patents surrounding the ORP Diagnostic System were issued in November of 2012.

A summary of the active and pending intellectual property surrounding the ORP Diagnostic System is depicted below.
Device-related Intellectual Property
This patent family includes two issued United States patents, a pending United States application and a pending PCT application for foreign protection. Patents issuing in this family will not expire until 2032. The issued United States patents broadly cover ORP test devices, including the ORP Diagnostic System and methods for determining the ORP of a fluid sample without limitation on the use for which the ORP is determined. The second issued patent broadly covers an ORP test strip. The pending application has been filed to pursue additional related subject matter in this family that has not already been protected in the two issued patents. The PCT application will be filed in individual foreign countries by August and September of 2013. Related to this family is a pending United States provisional application protecting a specific design of the test strip. Regular United States and foreign applications will be filed for this invention and any related improvements in 2013 and the term of patents issuing from this family will continue until 2033.

Application-related Intellectual Property. This patent family includes three pending United States applications, and pending applications in Europe, Japan and Canada. Patents issuing in this family will not expire until 2028. The pending applications in the United States claim methods of evaluating hospital patient discharge decisions based on the results of ORP measurements in patients and methods of evaluating head injury trauma patients based on the results of ORP measurements. This patent family also provides support for the use of ORP measurements in diagnosing, evaluating or monitoring patients who have a variety of conditions, including trauma, viral infections, critical illness, and myocardial infarction. Other methods in this family include evaluating exercise performance and monitoring blood products.

Capacity-related Intellectual Property. The company has one pending United States provisional application covering methods and systems for evaluating oxidative stress in patients by determining the oxidation reduction potential capacity of a fluid sample. This technology is an improvement over determining the oxidation reduction potential at a single point in time. Regular United States and foreign applications will be filed for this invention in 2013 and the term of patents issuing from this family will continue until 2033.
The details of the patent families described above are in the following table. The ORP patents held by IMM have been assigned to us in exchange for 50,000 shares of Common Stock and $330,000 to be paid from the proceeds of this offering.

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**LUOXIS Diagnostics**
**ORP Laboratory System Specifications**

Luoxis is finalizing packaging configuration for the ORP Laboratory System and will collaborate with distributors on specific elements of the product’s configuration including pricing, packaged quantities, and distribution logistics. These items will be customized to distributors’ requirements based upon each market’s needs.