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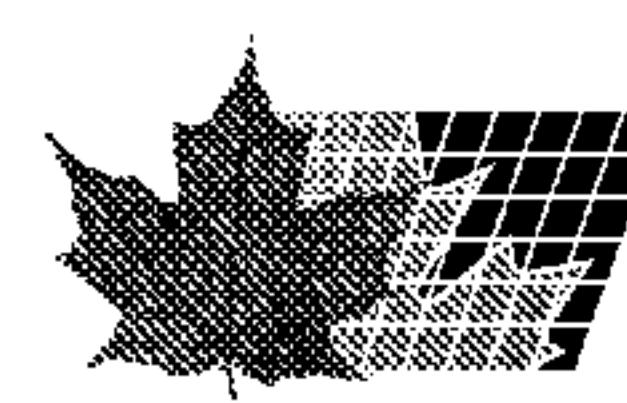
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(54) **Title: MEASUREMENT AND USES OF OXIDATIVE STATUS**

(57) Abrégé/Abstract:

An electrochemical test sensor for detecting the analyte concentration of a fluid test sample includes a base, a dielectric layer, a reagent layer and a lid. The base provides a flow path for the test sample having on its surface a counter electrode and a working electrode adapted to electrically communicate with a detector of electrical current. The dielectric layer forms a dielectric window therethrough. The reagent layer includes an enzyme that is adapted to react with the analyte. The lid is adapted to mate with the base and to assist in forming a capillary space with an opening for the introduction of the test sample thereto. At least a portion of the width of the counter electrode is greater than the width of the working electrode.



ABSTRACT

An electrochemical test sensor for detecting the analyte concentration of a fluid test sample includes a base, a dielectric layer, a reagent layer and a lid. The base provides a flow path for the test sample having on its surface a counter electrode and a working electrode adapted to electrically communicate with a detector of electrical current. The dielectric layer forms a dielectric window therethrough. The reagent layer includes an enzyme that is adapted to react with the analyte. The lid is adapted to mate with the base and to assist in forming a capillary space with an opening for the introduction of the test sample thereto. At least a portion of the width of the counter electrode is greater than the width of the working electrode.

MEASUREMENT AND USES OF OXIDATIVE STATUS

FIELD OF THE INVENTION

The invention relates to a method of determining the overall oxidative status of a body fluid or a tissue of a patient by measuring the oxidation-reduction potential (ORP) of the body fluid or tissue. In particular, the invention relates to methods for the diagnosis, evaluation and monitoring of patients who have suffered a trauma (such as a head injury), patients suspected of being critically-ill or who are critically ill, patients who have a viral infection, and patients suspected of having a myocardial infarction (MI) or who have an MI. The invention also relates to methods for the evaluation and monitoring of the exercise performance of patients. The invention further relates to methods for the evaluation and monitoring of stored blood products and of patients who will receive such products.

BACKGROUND

Oxidative stress is caused by a higher production of reactive oxygen and reactive nitrogen species or a decrease in endogenous protective antioxidative capacity. Oxidative stress has been related to various diseases and aging, and it has been found to occur in all types of critical illnesses. See, e.g., Veglia et al., *Biomarkers*, 11(6): 562-573 (2006); Roth et al., *Current Opinion in Clinical Nutrition and Metabolic Care*, 7:161-168 (2004); U.S. Patent No. 5,290,519 and U.S. Patent Publication No. 2005/0142613. Several investigations have shown a close association between the oxidative status of a critically ill patient and the patient's outcome. See Roth et al., *Current Opinion in Clinical Nutrition and Metabolic Care*, 7:161-168 (2004).

Oxidative stress in patients has been evaluated by measuring various individual markers. See, e.g., Veglia et al., *Biomarkers*, 11(6): 562-573 (2006); Roth et al., *Current Opinion in Clinical Nutrition and Metabolic Care*, 7:161-168 (2004); U.S. Patent No. 5,290,519 and U.S. Patent Publication No. 2005/0142613. However, such measurements are often unreliable and provide conflicting and variable measurements of the oxidative status of a patient. See Veglia et al., *Biomarkers*, 11(6): 562-573 (2006); Roth et al., *Current Opinion in Clinical Nutrition and Metabolic Care*, 7:161-168 (2004). The measurement of multiple markers which are then used to provide a score or other assessment of the overall oxidative status of a patient has been developed to overcome the

problems of using measurements of single markers. See Veglia et al., *Biomarkers*, 11(6): 562-573 (2006); Roth et al., *Current Opinion in Clinical Nutrition and Metabolic Care*, 7:161-168 (2004). Although such approaches are more reliable and sensitive than measurements of a single marker, they are complex and time consuming. Thus, there is a need for a simpler and faster method for reliably measuring the overall oxidative status of a patient.

SUMMARY OF THE INVENTION

One aspect of the invention relates to a method of diagnosing, evaluating or monitoring a patient who has suffered a trauma comprising the following steps:

measuring the oxidation-reduction potential (ORP) of a body fluid of the patient, a tissue of the patient or both; and

performing one or both of the following steps:

determining if the ORP is significantly different than the ORP of the same body fluid, tissue or both from normal patients; and/or

if the ORP of the body fluid, tissue or both of the patient has been measured at least one time prior to the current measurement, determining if the ORP has increased or decreased compared to the prior measurement(s).

In some embodiments, the ORP is monitored to determine the efficacy of a treatment of the patient.

In some embodiments, the ORP is monitored to determine whether the condition of the patient is improving or worsening.

In some embodiments, the ORP is measured once per day or a plurality of times per day.

In some embodiments, the ORP is measured in a body fluid, and the body fluid is plasma.

In some embodiments, the trauma is a head injury.

In some embodiments, the ORP is measured once per day or a plurality of times per day.

In some embodiments, the ORP is measured in a body fluid, and the body fluid is plasma.

In some embodiments, the ORP is measured in a body fluid, and the body fluid is cerebrospinal fluid.

Another aspect of the invention relates to a method of diagnosing, evaluating or monitoring a patient who has a viral infection comprising the following steps:

measuring the oxidation-reduction potential (ORP) of a body fluid of the patient, a tissue of the patient or both; and

performing one or both of the following steps:

determining if the ORP is significantly different than the ORP of the same body fluid, tissue or both from normal patients; and/or

if the ORP of the body fluid, tissue or both of the patient has been measured at least one time prior to the current measurement, determining if the ORP has increased or decreased compared to the prior measurement(s).

In some embodiments, the ORP is monitored to determine the efficacy of a treatment of the patient.

In some embodiments, the ORP is monitored to determine whether the condition of the patient is improving or worsening.

In some embodiments, the ORP is measured once per day or a plurality of times per day.

In some embodiments, the ORP is measured in a body fluid, and the body fluid is plasma.

Another aspect of the invention relates to a method of diagnosing, evaluating or monitoring a patient suspected of being critically-ill or of evaluating or monitoring a patient who has been diagnosed as being critically ill, the method comprising the following steps:

measuring the oxidation-reduction potential (ORP) of a body fluid of the patient, a tissue of the patient or both; and

performing one or both of the following steps:

determining if the ORP is significantly different than the ORP of the same body fluid, tissue or both from normal patients; and/or

if the ORP of the body fluid, tissue or both of the patient has been measured at least one time prior to the current measurement, determining if the ORP has increased or decreased compared to the prior measurement(s).

In some embodiments, the ORP is used as part of an evaluation to determine whether the patient is or is not critically ill.

In some embodiments, the ORP is monitored to determine the efficacy of a treatment of the patient.

In some embodiments, the ORP is monitored to determine whether the condition of the patient is improving or worsening.

In some embodiments, the ORP is measured once per day or a plurality of times per day.

In some embodiments, the ORP is measured in a body fluid, and the body fluid is plasma.

Another aspect of the invention relates to a method of diagnosing, evaluating or monitoring a patient suspected of having a myocardial infarction or of evaluating or monitoring a patient who has been diagnosed as having a myocardial infarction, the method comprising the following steps:

measuring the oxidation-reduction potential (ORP) of a body fluid of the patient, a tissue of the patient or both; and

performing one or both of the following steps:

determining if the ORP is significantly different than the ORP of the same body fluid, tissue or both from normal patients; and/or

if the ORP of the body fluid, tissue or both of the patient has been measured at least one time prior to the current measurement, determining if the ORP has increased or decreased compared to the prior measurement(s).

In some embodiments, the ORP is used as part of an evaluation to determine whether the patient is or is not having a myocardial infarction.

In some embodiments, the ORP is monitored to determine the efficacy of a treatment of the patient.

In some embodiments, the ORP is measured once per day or a plurality of times per day.

In some embodiments, the ORP is measured in a body fluid, and the body fluid is plasma.

Another aspect of the invention relates to a method of evaluating the exercise performance of a patient comprising:

measuring the oxidation-reduction potential (ORP) of a body fluid of the patient, a tissue of the patient or both before and after exercise and determining if the ORP is significantly different after exercise as compared to before exercise; or

measuring the ORP of a body fluid of the patient, a tissue of the patient or both before and during exercise and determining if the ORP is significantly different during exercise as compared to before exercise; or

measuring the ORP of a body fluid of the patient, a tissue of the patient or both before, during and after exercise and determining if the ORP is significantly different during exercise, after exercise or both, as compared to before exercise.

In some embodiments, the ORP is measured in a body fluid, and the body fluid is plasma.

Another aspect of the invention relates to a method of evaluating or monitoring a blood product which is stored comprising:

measuring the oxidation-reduction potential (ORP) of the blood product after it has been stored for one or more days; and

determining if the ORP of the stored blood product is significantly different than the ORP of the same blood product immediately after it was obtained from a donor.

In some embodiments, the blood product is blood.

In some embodiments, the blood product is packed red blood cells.

In some embodiments, the ORP of the blood product is measured once per day during storage.

In some embodiments, the method further comprises:

measuring the oxidation-reduction potential (ORP) of a body fluid of a patient who is to receive the blood product, a tissue of the patient or both; and

determining if the ORP is significantly different than the ORP level in the same body fluid, tissue or both from normal patients.

BRIEF DESCRIPTION OF THE INVENTION

The present invention provides such a method. In particular, the invention provides a method of determining the overall oxidative status of a body fluid or a tissue of a patient by measuring the oxidation-reduction potential (ORP) of the body fluid or tissue. The method has been found to be useful in the diagnosis, evaluation and monitoring of patients who have suffered a trauma (such as a head injury), patients suspected of being critically-ill or who have been diagnosed as being critically ill, patients who have a viral infection, and patients suspected of having a myocardial infarction (MI) or who have been diagnosed as having an MI. The method has also been found useful in monitoring and evaluating exercise performance in patients. In addition, the method has been found

useful in monitoring and evaluating stored blood products and patients who will receive such a product.

DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS OF THE INVENTION

As used herein, "patient" means a mammal, such as a dog, cat, horse, cow or human. Most preferably, the patient is a human.

Any body fluid of a patient can be used in the method of the invention. Suitable body fluids include a blood sample (*e.g.*, whole blood, serum or plasma), urine, saliva, cerebrospinal fluid, tears, semen, vaginal secretions, amniotic fluid and cord blood. Also, lavages, tissue homogenates and cell lysates can be utilized and, as used herein, "body fluid" includes such preparations. Preferably, the body fluid is blood, plasma, serum or cerebrospinal fluid. For head injuries, the body fluid is most preferably cerebrospinal fluid or plasma. In cases other than head injuries, the body fluid is most preferably plasma.

Any tissue of a patient can be used in the method of the invention. Suitable tissues include skin, eye and mouth tissues and tissue from biopsies.

As used herein, "normal," "normal patient" or "control" means a mammal of the same species as the patient (*e.g.*, the normal will be a human when the patient is a human), and who is not suffering from any disease. Since ORP increases with age, normals should be of the same age or age range as the patient(s) being tested.

An oxidation-reduction system, or redox system, involves the transfer of electrons from a reductant to an oxidant according to the following equation:



where ne^- equals the number of electrons transferred. At equilibrium, the redox potential (E), or oxidation-reduction potential (ORP), is calculated according to the Nernst-Peters equation:

$$E(\text{ORP}) = E_0 - \frac{RT}{nF} \ln \frac{[\text{reductant}]}{[\text{oxidant}]} \quad (2)$$

where R (gas constant), T (temperature in degrees Kelvin) and F (Faraday constant) are constants. E_0 is the standard potential of a redox system measured with respect to a hydrogen electrode, which is arbitrarily assigned an E_0 of 0 volts, and n is the number of electrons transferred. Therefore, ORP is dependent on the total concentrations of reductants and oxidants, and ORP is an integrated measure of the balance between total oxidants and reductants in a particular system. As such, ORP provides a measure of the overall oxidative status of a body fluid or tissue of a patient.

An ORP measurement which is significantly higher than that of normals will indicate the presence of oxidative stress. Oxidative stress has been related to many diseases, and it has been found to occur in all types of critical illnesses. Accordingly, an ORP level significantly higher than that of normals indicates the presence of a disease and perhaps a critical illness. An ORP measurement which is the same as or lower than that of normals indicates the absence of oxidative stress and the absence of a disease or critical illness. Thus, the ORP level of a patient can be used by a medical doctor or veterinarian as an aid in diagnosing or ruling out the presence of a disease, particularly a serious illness. Sequential measurements of ORP over time can be used to monitor the progression of a disease and the effectiveness or lack of effectiveness of treatment of the disease. If a patient's ORP does not decrease after treatment, or especially if it increases despite treatment, this may indicate a poor prognosis and the need for more aggressive and/or additional and/or different treatments. In the case of a measurement made by a patient, such as a patient experiencing symptoms of myocardial infarction, the ORP level may indicate the need for the patient to see a doctor or to immediately proceed to an emergency room for treatment.

The ORP of a body fluid or a tissue can be easily measure by contacting an ORP or redox electrode with the body fluid or tissue. Such electrodes are available commercially from, *e.g.*, Microelectrodes, Inc., Bedford, NH. Such electrodes can suffer from day-to-day variability, and the use of references standards will be necessary. Suitable references standards include saturated quinhydrone at several pH's. The electrode is connected to a meter which provides a readout of the ORP in millivolts and, optionally, other parameters, such as pH and temperature. Such meters are available commercially from, *e.g.*, Hanna Instruments, Woonsocket, RI.

The method of the invention has been found to be useful in the diagnosis, evaluation and monitoring of patients who are suffering from, or who are suspected of having, certain diseases. ORP can be used, in combination with a medical history, symptoms and other test results, as an aid in diagnosing, evaluating and monitoring these diseases and treatments of them. In particular, a normal ORP may be very helpful in ruling out the presence of a disease, particularly a serious illness, and in saving medical resources that might otherwise be devoted to unnecessarily treating patients. In addition, a significantly high ORP (indicating oxidative stress) may be used to identify those patients who are in need of immediate or more aggressive treatment of their disease and/or treatment to reduce oxidative stress.

An ORP that is significantly high compared to normals may indicate the need or desirability of performing tests for one or more individual markers of oxidative stress to better understand the reason or source of, and therefore the best treatment for, the oxidative stress or disease. Thus, the invention also includes the use of ORP in combination with tests for one or more individual markers of oxidative stress (collectively referred to herein as an "oxidative stress panel of tests"). Such markers of oxidative stress and methods of measuring them are known. See, e.g., Veglia, et al., *Biomarkers*, 11(6):562-573 (2006); Rana et al., *Mol. Cell Biochem.*, 291:161-166 (2006); Roth et al., *Curr. Opin. Clin. Nutr. Metab. Care*, 7:161-168 (2004); Horton, *Toxicology*, 189:75-88 (2003); Winterbourn et al., *Crit. Care Med.*, 28:143-149 (2000); Ghiselli et al., *Free Radic. Biol. Med.*, 29(11):1106-1114 (2000); Rice-Evans, *Free Radic. Res.*, 33 Suppl.:S59-S66 (2000); Prior and Cao, *Free Radic. Biol. Med.*, 27(11-12):1173-1181 (1999); Galley et al., *Crit. Care Med.*, 24:1649-53 (1996); Goode et al., *Crit. Care Med.*, 23:646-651 (1995).

Trauma injury is a leading cause of death and disability for all age groups under 60 years of age. In the United States, trauma injuries account for more than 160,000 deaths each year and millions more survivable injuries. For many people, the injury causes temporary pain and inconvenience. For others, the injury leads to suffering, disability, chronic pain, and a profound change in quality of life, including substantial financial consequences. The economic costs of trauma injuries include the costs associated with medical treatment as well as lost productivity costs. In 2000 alone, the 50 million injuries that required medical treatment will ultimately cost \$406 billion. This includes estimates of \$80.2 billion in medical care costs and \$326 billion in productivity losses.

The method of the present invention has been found to be useful in the diagnosis, evaluation and monitoring of patients who have suffered a trauma. As used herein, "trauma" refers to physical injury to any part of a patient's body or to multiple parts of a patient's body. Trauma injuries include head injuries, internal injuries, blunt trauma, multiple trauma, broken bones and burns.

The present invention provides a means for the determination and monitoring of the oxidative status of traumatized patients and provides medical doctors and veterinarians with real-time information to guide trauma treatment and care. In particular, the existence and degree of oxidative stress in traumatized patients can be determined and monitored. For example, an ORP level that is statistically the same as, or lower than, that of normals

upon first examination of a trauma patient (e.g., by a paramedic in the field or by a medical doctor in an emergency room) indicates that oxidative stress is not present and that the patient may not need aggressive treatment or may not even need to be admitted to a hospital. In this manner, medical resources can be saved and costs lowered. On the other hand, an ORP level significantly higher than that of normals upon first examination of a trauma patient indicates the presence of oxidative stress and the need for immediate treatment of the patient and for continued monitoring of the patient's ORP. The higher the ORP level, the higher the level of oxidative stress, and the greater the need for aggressive treatment of the patient. An ORP level that decreases with treatment indicates that the patient is improving and that the treatment is working. An ORP level that increases despite treatment indicates that the patient is getting worse and that more aggressive treatment, additional treatment and/or a different treatment is needed. An ORP level that has decreased to the point where it is no longer significantly higher than that of normals indicates that the patient may be discharged from the hospital. Of course, the ORP level is only one diagnostic parameter, and it should be used in combination with other symptoms, results of a physical examination, a medical history, and the results of any other laboratory tests, to determine the proper treatment for a trauma patient.

The method of the invention has also been found to be useful in the diagnosis, evaluation and monitoring of patients who have a viral infection. Viral infections include infections caused by human immunodeficiency virus, encephalitis virus, hepatitis viruses, influenza viruses, pneumonia virus and other viruses that cause serious viral illnesses.

In a patient suffering from a viral infection, an ORP level that is statistically the same as, or lower than, that of normals upon examination of such a patient indicates that oxidative stress is not present and that the patient may need only standard treatments. On the other hand, an ORP level significantly higher than that of normals upon examination of a patient indicates the presence of oxidative stress and the need for more aggressive treatment of the patient, including possibly hospital admission, and for continued monitoring of the patient's ORP. The higher the ORP level, the higher the level of oxidative stress, and the greater the need for aggressive treatment of the patient. An ORP level that decreases with treatment indicates that the patient is improving and that the treatment is working. An ORP level that increases despite treatment indicates that the patient is getting worse and that more aggressive treatment, additional treatment and/or a different treatment is needed. An ORP level that has decreased to the point where it is no longer significantly higher than that of normals indicates that aggressive treatment of the

patient may be discontinued, including discharge of the patient from the hospital. Of course, the ORP level is only one diagnostic parameter, and it should be used in combination with other symptoms, results of a physical examination, a medical history, and the results of any other laboratory tests, to determine the proper treatment for a patient having a viral infection.

Each year in the United States, approximately 6-8 million people present to a hospital emergency room (ER) with chest pain or other cardiac symptoms (e.g., shortness of breath and pain or tingling in the left arm). Unfortunately, about 2-5% of the 3-4 million that are sent home from the ER are mistakenly diagnosed. Chest pain diagnostic errors are the leading cause of emergency medicine malpractice awards. Of the other 3-4 million that are hospitalized, about 60-75% do not have cardiac disease. The minimum cost for each hospitalized patient is \$3,000-5,000, which means that over 6 billion healthcare dollars are wasted each year because of these unnecessary hospitalizations. With a non-diagnostic electrocardiogram (ECG), reliable early biomarkers do not exist. Troponin I or troponin T levels are unreliable during the first 6-24 hours after the onset of symptoms due to low sensitivity, and creatine kinase isoenzymes (CK-MB) and myoglobin are not cardiac specific. It would be highly desirable to have a laboratory test result that could aid in the diagnosis of myocardial infarction or rule it out.

The method of the invention provides such a test result and has been found to be useful in the diagnosis, evaluation and monitoring of patients suspected of having a myocardial infarction (MI). The method of the present invention is particularly useful for the early diagnosis of MI. By "early diagnosis" is meant ascertaining the presence or absence of MI during the first few hours (less than 24 hours, especially less than 12 hours) following the onset of symptoms indicative of MI, such as chest pain, shortness of breath and pain or tingling in the left arm. The method of the invention also has been found to be useful in the evaluation and monitoring of patients who have been diagnosed with an MI.

In particular, the existence and degree of oxidative stress in patients presenting with symptoms of MI can be determined and monitored according to the present invention. For example, an ORP level that is statistically the same as, or lower than, that of normals upon first examination of a suspected MI patient (e.g., by a paramedic in the field or by a medical doctor in an emergency room) indicates that oxidative stress is not present and that the patient is not experiencing an MI. In such a case, the patient may not need treatment and may not need to be kept in an ER or admitted to the hospital. In this manner, medical resources can be saved and costs lowered. On the other hand, an ORP

level significantly higher than that of normals upon first examination of a suspected MI patient indicates the presence of oxidative stress and that the patient may be experiencing an MI. Such an ORP level indicates the need for immediate treatment of the patient and continued monitoring of the patient's ORP. The higher the ORP level, the higher the level of oxidative stress, and the greater the need for aggressive treatment of the patient. An ORP level that decreases with treatment indicates that the patient is improving and that the treatment is working. An ORP level that increases despite treatment indicates that the patient is getting worse and that more aggressive treatment, additional treatment and/or a different treatment is needed. An ORP level that has decreased to the point where it is no longer significantly higher than that of normals indicates that the patient may be discharged from the hospital. Of course, the ORP level is only one diagnostic parameter, and it should be used in combination with other symptoms, results of a physical examination, a medical history, and the results of any other laboratory tests, to determine the proper treatment for a suspected MI patient or a patient diagnosed as actually experiencing an MI.

The method of the invention has also been found to be useful in the diagnosis, evaluation and monitoring of patients suspected of being critically ill and evaluation and monitoring of patients who are found to be critically ill. It is well known that the presence of oxidative stress in critically ill patients is positively correlated with poor outcomes. See Roth et al., *Curr. Opin. Clin. Nutr. Metab. Care*, 7:161-168 (2004). Accordingly, the ORP of patients who are, who are suspected of being, or who are likely to become, critically-ill should be monitored. An ORP level of a patient that is statistically the same as, or lower than, that of normals indicates that oxidative stress is not present and that the patient is not critically ill. Such an ORP level indicates that aggressive treatment of the patient is not needed. An ORP level that is significantly higher than that of normals indicates the presence of oxidative stress and that the patient is critically ill. Such an ORP level indicates the need for aggressive treatment of the patient and for continued monitoring of the patient's ORP. The higher the ORP level, the higher the level of oxidative stress, and the greater the need for aggressive treatment of the patient. An ORP level that decreases with treatment indicates that the patient is improving and that the treatment is working. An ORP level that increases despite treatment indicates that the patient is getting worse and that more aggressive treatment, additional treatment and/or a different treatment is needed. An ORP level that has decreased to the point where it is no longer significantly higher than that of normals indicates that the patient is no longer critically ill and may be

discharged from the hospital. Of course, the ORP level is only one diagnostic parameter, and it should be used in combination with other symptoms, results of a physical examination, a medical history, and the results of any other laboratory tests, to determine the proper treatment for a patient.

The use of banked blood products is a common practice employed by the medical community worldwide for obvious beneficial reasons. However, there is a risk of adverse side effects from the transfusion of blood products into patients, including the possible development of transfusion-related acute lung injury (TRALI), multiple organ failure (MOF), and acute respiratory distress syndrome (ARDS). Brittingham et al., *J. Am. Med. Assoc.*, 165:819-825 (1957); Sauaia et al., *Arch. Surg.*, 129:39-45 (1994); Miller et al., *Am. Surg.*, 68:845-850 (2002). It would be desirable to have a means of avoiding or reducing these side effects.

The method of the invention provides such a means, and the method of the present invention has been found useful in monitoring and evaluating stored (banked) blood products. Blood products that can be monitored and evaluated according to the present invention include whole blood, packed red blood cells, platelets and fresh frozen plasma. In particular, using the method of the present invention, it has been found the ORP of stored blood products increases with the time of storage. For example, packed red blood cells have a significantly increased ORP on day 42 as compared to day 1. An increased ORP indicates an increased level of oxidants in the blood product, and the increased level of oxidants may contribute to the development of side effects in patients receiving the blood product, since transfusion of such a blood product would be expected to increase the level of oxidants and oxidative stress in the patient.

The method of the invention is also useful in monitoring and evaluating patients who will receive stored blood products. In particular, an ORP level in such a patient which is significantly higher than that of normals indicates the presence of oxidative stress, and such a patient should not be transfused or should be transfused with blood products that contain lower levels of oxidant species (*i.e.*, a blood product that has a lower ORP level, preferably the same as that of fresh blood product). The ORP level of a blood product may be less critical for patients who have an ORP level that is statistically the same as, or lower than, that of normals, since such a level indicates that oxidative stress is not present. Determining the oxidative status of the patient and of the blood product should result in a decrease in transfusion-related side effects.

Exercise is associated with an enhanced aerobic and/or anaerobic metabolism which results in an increased formation of reactive oxygen species (ROS). Strenuous exercise, excessive exercise and overtraining generate ROS to a level that can overwhelm antioxidant defense systems. See Sen, *Sports Med.*, 31:891-908 (2001); Margonis et al., *Free Radical Biol. Med.*, 43(6):901-910 (September 15, 2007); Gomez-Cabrera et al., *Free Radical Biol. Med.*, 44(2):126-131 (2008); Radak et al., *Ageing Res. Rev.*, 7(1):34-42 (2008). The result is oxidative stress, and oxidative stress can cause extensive molecular, cellular and tissue damage. One possible outcome is oxidative damage to muscle tissues. Preventing or reducing muscle tissue damage during exercise training should help optimize the training effect and eventual performance. Regular exercise of moderate intensity and duration, while generating ROS, also induces an improvement in natural antioxidant enzymes and proteins and upregulation of antioxidant defense systems. See Ji, *Proc. Soc. Exp. Biol. Med.*, 222:283-292 (1999); Rahnama et al., *J. Sports Med. Phys. Fitness*, 47:119-123 (2007); Gomez-Cabrera et al., *Free Radical Biol. Med.*, 44(2):126-131 (2008); Ji, *Free Radical Biol. Med.*, 44:142-152 (2008); Radak et al., *Ageing Res. Rev.*, 7(1):34-42 (2008). These adaptations result in decreased oxidative challenge to the body and maintenance of the oxidant-antioxidant homeostasis. Further, it appears that exercise-induced modulation of the redox state is an important means by which exercise benefits the body, increasing the resistance against, and facilitating recovery from, oxidative stress. Radak et al., *Appl. Physiol. Nutr. Metab.*, 32:942-946 (2007); Radak et al., *Free Radical Biol. Med.*, 44:153-159 (2008). From the foregoing, it can be seen that the maximum benefits of exercise can be realized from exercise that does not cause oxidative stress, and that exercise that does cause oxidative stress is to be avoided, whenever possible.

The method of the invention can be used to monitor and evaluate exercise performance in patients. The ORP of patients before and after, before and during, or before, during and after exercise is measured. An ORP level of a patient during or after exercise that is statistically the same as, or lower than, that of the patient before exercise indicates that oxidative stress is not present. Such an ORP level indicates that the exercise need not be changed. An ORP level of a patient during or after exercise that is significantly higher than that of the patient before exercise indicates the presence of oxidative stress. Such an ORP level indicates that the exercise may be causing damage and should be changed in some way, such as changing the frequency of the exercise, length of the exercise or even the type of exercise. As can be seen, monitoring the ORP of

a patient can result in the design of an optimum exercise program for the patient so that the patient can achieve his/her desired physical fitness goals without experiencing the adverse effects and damage caused by oxidative stress.

EXAMPLES

EXAMPLE 1: Diagnosis Of Acute Myocardial Infarction

Whole blood was collected from normal subjects and patients with acute myocardial infarction (AMI) by venipuncture using a Vacutainer™ containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was aliquoted in 1 mL quantities and stored at -80°C for future use.

Oxidation-reduction potential (ORP) measurements were recorded using a micro Pt/AgCl combination MI-800/410 cm Redox Electrode (Microelectrodes, Inc., Bedford, NH, USA) connected to an HI4222 pH/mV/Temperature bench meter (Hanna Instruments, Woonsocket, RI, USA). The electrode was immersed in a plasma sample, and a steady-state ORP reading in millivolts (mV) was recorded.

The results are presented in Table 1 below. The data were analyzed using student's two-tailed t test assuming uneven variances. As can be seen from Table 1, the ORP of the plasmas from AMI patients was significantly different than that of the plasmas from normals. Thus, a plasma ORP measurement can be used as an aid in distinguishing patients who are actually experiencing an AMI from those whose AMI-like symptoms are due to another cause.

TABLE 1

AMI Plasmas	
Sample #	ORP
GR-358	-24.5
GR-379	-30.5
GR-397	-34.0
GR-1266-05	-15.0
GR-1328	-4.1
GR-1435-02	-11.7
Average:	-20.0
SD:	11.6
Normal Plasmas	
Sample #	ORP
GR-1347-02	-41.7
GR-1425	-65.3
GR-1426	-52.6
GR-1427	-52.7
GR-1428	-59.2
GR-1429	-55.3
GR-1430	-53.2
GR-1431	-58.4
GR-1432	-30.6
GR-1433	-50.9
Average:	-52.0
SD:	9.7
Comparison AMI samples with normal samples	
p-value	0.0003

EXAMPLE 2: Monitoring And Identifying Critically Ill Trauma Patients

The oxidation-reduction potential (ORP) in a biological system is an integrated measure of the balance between total pro- and antioxidant components of the system. In plasma, many constituents contribute to the ORP. Reactive oxygen species (ROS), such as the superoxide ion, hydroxyl radical, hydrogen peroxide, nitric oxide, peroxynitrite, transition metal ions, and hypochlorous acid, contribute to the oxidative potential. Plasma antioxidants include thiols, vitamin C, tocopherol, β -carotene, lycopene, uric acid, bilirubin, and flavinoids. Enzymes, such as SOD, catalase, and glutathione peroxidase, are

involved in the conversion of ROS into less reactive species. ORP monitoring of plasma provides a single measurement that integrates the overall quantitative balance among the pro- and antioxidant components of the system, and the ORP level is an indicator of a patient's overall oxidative status.

Critically ill patients suffer from oxidative stress, reflecting an imbalance in favor of the pro-oxidant components in the intra- and extracellular environments. The biological consequences of this imbalance favor certain chemical reactions which could be both beneficial and detrimental to the system depending upon the system involved and the disease process. Previous attempts at assessing the redox status of critically ill patients have been limited to measurements of single parameters, such as concentrations of individual antioxidants (Goode et al., *Crit. Care Med.*, 23:646-51 (1995)) or amount of lipid peroxidation (Rana et al., *Mol. Cell Biochem.*, 291:161-6 (2006)). Although these parameters could be helpful, they might not give the clinician a complete assessment of the amount of oxidative stress occurring in a critically ill patient. Additionally, the measurement of these various parameters would prove to be laborious, time consuming, and, hence, impractical in the clinical setting. Here, a method is described that measures the overall oxidative status of critically ill trauma patients using an electrode that measures ORP in the plasma of the patients on a possible real-time basis.

Materials And Methods

This study received approval by the HCA-HealthOne Institutional Review Board according to the guidelines published by the HHS Office for Protection from Research Risk. Blood was collected from normal subjects (N = 10) and critically ill patients who had suffered severe traumas (N = 39) by venipuncture using a Vacutainer™ containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). For critically ill patients, blood was collected on an almost daily basis until discharge. Plasma was aliquoted in 1 mL quantities and stored at -80°C for future use. Patient demographics are listed in Table 2.

TABLE 2

	Patients	Controls
Number (n)	39	10
Age	43.8 yrs \pm 2.7 SEM	46.4 yrs \pm 3.5 SEM
Sex	31 males 8 females	7 males 3 females
Injury Severity Score (ISS)	30.7 \pm 2.4 SEM	N/A

Oxidation-reduction potential (ORP) measurements were recorded at room temperature using a micro Pt/AgCl combination MI-800/410 cm Redox Electrode

(Microelectrodes, Inc., Bedford, NH, USA) connected to an HI4222 pH/mV/Temperature bench meter (Hanna Instruments, Woonsocket, RI, USA). Plasma samples were thawed, and the ORP electrode was immersed in the plasma. A reading was recorded in millivolts (mV) after the ORP value was stable for 5 seconds.

Total protein was quantified in all plasma samples using the bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL, USA). All plasma samples were diluted 1:100 with 1X phosphate-buffered saline, pH 7.4, prior to application to a 96-well plate. All samples were analyzed in duplicates.

Paraoxonase (PON) is a calcium-dependent aryl esterase (AE) present in plasma. When PON is associated with high density lipoproteins (HDL), an antioxidant effect against lipid peroxidation has been observed (Ferretti et al., *Biochim. Biophys. Acta*, **1635**:48-54 (2003)). Indeed, a lower PON activity has been associated with a higher susceptibility of HDL to peroxidation in patients affected by diseases characterized by increases in oxidative damage (Ferretti et al., *J. Clin. Endocrinol. Metab.*, **89**:2957-2962 (2004); Ferretti et al., *J. Clin. Endocrinol. Metab.*, **90**:1728-1733 (2005)).

Plasma PON-AE activity was measured as previously described. Ahmed et al., *J. Biol. Chem.*, **280**:5724-5732 (2005). Briefly, plasma was diluted 1:20 with 1X phosphate-buffered saline, pH 7.4. Diluted plasma was then combined 1:1 with 4 mM *para*-nitrophenolacetate (Sigma-Aldrich, St. Louis, MO) in a 96-well plate in duplicates. The plate was immediately read on a pre-warmed (37°C) plate reader (FL600 Microplate Fluorescence Reader, Bio-Tek Instruments Inc., Winooski, VT) set at 410 nm. Absorbance readings were taken every 2 minutes for 20 minutes. The slope of the linear portion of the kinetic plot ($R^2 \geq 0.99$) was used to generate PON-AE activity. PON-AE activity was normalized to plasma protein. PON-AE activity is reported in units (U), where a unit equals the change in milli-absorbance units at 410 nm per minute per mg of total plasma protein.

The ORP, PON-AE and total protein data were analyzed using Matlab R14 (Mathworks, Natick, MA). A one-way ANOVA was used to compare all patient data versus normal data to test for significant differences with a Tukey-Kramer correction for multiple comparisons with a significance level of 0.05. All data are reported as \pm standard error of the mean (SEM).

Results And Conclusions

Plasma was collected from critically ill patients who had suffered severe traumas from the time of admission until discharge. ORP was measured in the complete series of

plasma samples collected from a particular patient after the patient was discharged in order to limit any day-to-day variability in the ORP electrode.

A rapid increase in ORP was observed for all of the patients after an initial ORP reading of -19.9 mV (± 3.0 SEM) on admission. The admission ORP value is significantly higher than that of normal plasmas (-52.0 mV ± 3.1 SEM, $p < 0.05$). The ORP maximum, reached at day 6 (± 0.5 SEM), was significantly higher than the admission value, with a value of +13.7 mV (± 2.5 SEM). The ORP maximum was also significantly higher than normal plasmas (-52.0 mV ± 3.1 SEM, $p < 0.05$). During the remaining course of the hospitalization, the ORP of the plasma of these severely traumatized patients steadily approached the ORP of normal plasma. At the time of discharge, the ORP of plasma obtained from the severely traumatized patients was not significantly different from that of normal plasma (-52.0 mV ± 3.1 SEM).

For method validation purposes, PON-AE activities and total protein levels were measured. PON-AE activities were significantly higher in the admission samples (740.0 U/mg protein ± 20.2 SEM) compared to the ORP maxima samples (649.1 U/mg protein ± 18.8 SEM). Thus, the results show a correlation between trauma and a decrease in PON-AE activity. Similarly, protein levels were higher in the admission samples (47.3 mg/ml ± 1.6 SEM) compared to the ORP maxima samples (41.6 ± 1.3 SEM).

The presence of markers of oxidative stress in critically ill patients is associated with a poor prognosis (Roth et al., *Curr. Opin. Clin. Nutr. Metab. Care*, 7:161-8 (2004)). However, no single parameter can accurately predict the overall redox status for a critically ill patient. The laborious performance of multiple assays for the quantification of pro-oxidants and antioxidants is not practical in a clinical setting. Therefore, a quick and simple diagnostic test is warranted.

Here, the oxidation-reduction potential (ORP) of plasma collected on consecutive days from critically ill, traumatized patients was measured from hospital admission until the time of discharge. Daily ORP levels correlated with clinical events recorded in the medical records of each particular patient, with increases in ORP indicating a worsening of a patient's condition and decreases in ORP indicating an improvement in a patient's condition. Accordingly, monitoring ORP should be a useful tool for assessing and monitoring the presence and degree of oxidative stress, the severity of injury, a patient's prognosis, and the efficacy of treatment(s). ORP monitoring could be used to determine the appropriate clinical conditions and timing that warrant treatment (e.g., administration of antioxidants) of trauma patients. In particular, ORP monitoring could be used to aid in

the identification of patients who are critically ill and those who need more aggressive treatment than may be indicated by their symptoms and other diagnostic test results.

EXAMPLE 3: ORP Of Plasmas Before And After Exercise

The purpose of this experiment was to determine if exercise had an effect on oxidation-reduction potential (ORP) in plasma. Whole blood was collected from members of a women's college soccer team before and after exercise. The exercise consisted of an intense one-hour cardiovascular workout. The blood was collected by venipuncture using a Vacutainer™ containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). Blood tubes were centrifuged at 2000 rpm for 10 minutes, and plasma was collected and aliquoted in 1 mL quantities and stored at -80°C for future use.

The plasma samples were thawed at room temperature. ORP measurements were recorded using an MI-800/410 cm Redox Electrode (Microelectrodes, Inc., Bedford, NH, USA) connected to an HI4222 pH/mV/Temperature bench meter (Hanna Instruments, Woonsocket, RI, USA). The electrode was immersed in a plasma sample, and a steady-state ORP reading in millivolts (mV) was recorded.

The results are presented in Table 3 below. As can be seen from Table 3, the ORP of the plasmas of these high performance athletes decreased after exercise.

TABLE 3

Patient Sample	Exercise	ORP (mV)	Change (+/-)
GR 1814-01	No	-39.4	
GR 1814-02	Yes	-46.0	-6.6
GR 1815-01	No	-35.5	
GR 1815-02	Yes	-38.3	-2.8
GR 1816-01	No	-40.7	
GR 1816-02	Yes	-44.4	-3.7
GR 1817-01	No	-49.5	
GR 1817-02	Yes	-49.2	+0.3
Average change			-3.2

EXAMPLE 4: ORP Of Plasma From Critically-Ill Patient Suffering Viral Infection

Patient GR-1029 was admitted to the intensive care unit of Swedish Hospital, Englewood, Colorado with flu-like symptoms, pneumonia and respiratory failure triggered by exposure to rat droppings and urine while cleaning the cage of a pet rat. The patient was diagnosed as likely having a viral infection transmitted by rodents.

This study received approval by the HCA-HealthOne Institutional Review Board according to the guidelines published by the HHS Office for Protection from Research Risk.

Blood was collected from this patient on several days during his hospitalization (see Table 4 below) by venipuncture using a Vacutainer™ containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was aliquoted in 1 mL quantities and stored at -80°C for future use.

The plasma samples were thawed at room temperature. ORP measurements were recorded using an MI-800/410 cm Redox Electrode (Microelectrodes, Inc., Bedford, NH, USA) connected to an HI4222 pH/mV/Temperature bench meter (Hanna Instruments, Woonsocket, RI, USA). The electrode was immersed in a plasma sample, and a steady-state ORP reading in millivolts (mV) was recorded.

The results are presented in Table 4 below. As can be seen from Table 4, ORP was highest during the first 8 days after admission and then declined until the patient was discharged, in a similar manner to critically ill trauma patients (see Example 2). However, the ORP level for patient GR-1029 did not return to normal levels (-52.0 mV \pm 3.1 SEM) before discharge. The ORP levels correlated with clinical events recorded in the medical records of the patient, with increased ORP levels indicating a worsening of the patient's condition and decreased ORP levels indicating an improvement in the patient's condition.

Table 4

TIME (days)	ORP (mV)
1.0	73.0
1.5	78.8
8.0	59.5
9.0	36.6
10.0	60.6
11.0	17.7
12.0	33.6
16.0	3.8
22.0	-13.2
23.0	-12.4
24.0	-26.3
25.0	-14.0
26.0	1.7
30.0	-34.2
31.0	-20.9
33.0	-7.8
36.0	-9.5
37.0	-15.8
38.0	-20.0

EXAMPLE 5: Monitoring Of Stored Blood Product (PBRC)

Transfusion-related acute lung injury (TRALI) is an adverse effect of transfusion and is the leading cause of transfusion-related death. Silliman et al., *Blood*, 105:2266-2273 (2005). Longer storage times of packed red blood cells (PBRCs) and other blood products have been associated with an increased risk in developing TRALI in transfused patients. See Biffl et al., *J. Trauma*, 50:426-432 (2001).

A total of 10 transfusion bags containing PBRCs stored in adenine, citrate and dextrose (ACD) buffer at 4°C according to American Association of Blood Banks criteria were obtained from Bonfils Blood Center (Denver, CO). At Bonfils, a sample of each bag of PBRCs was collected on storage days 1 and 42. Samples were immediately centrifuged at 1000 g at 4°C for 10 minutes, and the supernatants were collected and stored at -80°C until further analysis.

Oxidation-reduction potential (ORP) was measured at room temperature in both the day 1 and day 42 sample supernatants. ORP measurements were recorded using a micro Pt/AgCl MI-800/410 cm Redox Electrode (Microelectrodes, Inc., Bedford, NH, USA) connected to an HI4222 pH/mV/Temperature bench meter (Hanna Instruments, Woonsocket, RI, USA). The electrode was immersed in a sample supernatant, and a steady-state ORP reading in millivolts (mV) was recorded.

The results are presented in Table 5 below. A student t-test was used to compare day 1 versus day 42 data to test for significant differences ($p < 0.05$, Microsoft Excel). As can be seen, ORP was significantly increased ($p < 0.05$) in the day 42 samples ($98.1 \text{ mV} \pm 21.9 \text{ SD}$) as compared to the day 1 samples ($62.6 \text{ mV} \pm 21.5 \text{ SD}$).

For method validation purposes, protein oxidation in the sample supernatants was determined by measuring plasma proteins in the supernatants by mass spectrometry (MS). Sample supernatants were analyzed by HPLC (Waters 2795 Separations Module, Milford, MA, USA) coupled to positive electrospray ionization time of flight mass spectrometry (+ESI-TOF MS, LCT, Micromass, UK) using a method described previously. Bar-Or et al., *Crit. Care Med.*, 33:1638-1641 (2005). $10\mu\text{L}$ of each sample was injected onto a YMC-Pack Protein-RP HPLC column (Waters, Milford, MA, USA) heated to 50°C. A 20-minute linear gradient from 10 to 40% B using water/0.1% trifluoroacetic acid (A) and AcN/0.1% TFA (B) was utilized with a flow rate of 1 mL/min. For each plasma protein detected, the MS spectrum was deconvolved to the uncharged, parent mass using MaxEnt 1 software (Micromass, UK). The parent mass spectrum was then integrated and relative

proportions of each species were calculated using an advanced, proprietary MS integration software package developed in-house.

Oxidation modifications of human serum albumin (HSA) include cysteinylation of cysteine 34 and dehydroalanine (DHA) modification of lysine 487. The percentage of oxidized HSA species increased significantly in the supernatants from day 1 (44.1% \pm 6.9 SD) to day 42 (72.1% \pm 8.4 SD).

Other plasma proteins identified in the supernatants by MS were α -chain of hemoglobin (α Hb), β -chain of hemoglobin (β Hb), apolipoprotein A1 (ApoA1) and transthyretin (TTR). Significantly higher levels of oxidation modifications of α Hb, β Hb and TTR were observed in the day 42 supernatants as compared to the day 1 supernatants ($p < 0.05$). Also, for α Hb and ApoA1, species which had a cleaved C-terminal amino acid were observed, indicating the presence of carboxypeptidase activity, a marker of inflammation.

The data demonstrate the presence of an oxidative environment in PBRCs, which increases with storage time. This could partially explain the increased risk of developing TRALI related to the transfusion of older blood products.

Accordingly, the ORP of PBRCs and other stored blood products should be monitored, and the ORP of patients that are to receive the blood products should also be monitored. A patient that has significant oxidative stress (*i.e.*, has a high ORP level) should be transfused with fresher blood products that contain less pro-oxidant species (*i.e.*, has a lower ORP level). Taking the oxidative status of the patient and of the blood product into account should result in a decrease in transfusion-related risk factors such as TRALI.

Table 5

PBRC Sample No.	Day 1 ORP (mV)	Day 42 ORP (mV)
054A	41.4	88.5
056A	48.9	113.8
057A	105.2	124.6
058A	77.4	131.2
059A	70.3	115.5
060A	88.0	100.7
061A	48.8	80.6
062A	52.2	73.5
063A	46.8	75.2
064A	46.7	77.1
Average:	62.6	98.1
Standard deviation (SD)	21.5	21.9
Standard error of the mean (SEM)	6.8	6.9
p-value	0.0018	
% Change	+56.7%	

EXAMPLE 6: Monitoring And Identifying Traumatic Brain Injury Patients

In this experiment, the overall oxidative status of patients with isolated, traumatic brain injuries (ITBI) was determined by measuring the oxidation-reduction potential (ORP) of the plasma of the patients.

Serial whole blood samples were obtained from severe ITBI patients (Abbreviated Injury Score (AIS) \geq 3, N = 32) and demographically similar non-head injury traumatized patients (N = 26) on an almost daily basis until discharge from the hospital. Whole blood was also collected from patients with minor to moderate ITBI (AIS \leq 2, N = 18) and healthy volunteers (N = 22). Plasma was aliquoted in 1 mL quantities and stored at -80°C for future use.

ORP measurements were recorded at room temperature using a micro Pt/AgCl combination MI-800/410 cm Redox Electrode (Microelectrodes, Inc., Bedford, NH, USA) connected to an HI4222 pH/mV/Temperature bench meter (Hanna Instruments, Woonsocket, RI, USA). Plasma samples were thawed, and the ORP electrode was immersed in the plasma. A reading was recorded in millivolts (mV) after the ORP value was stable for 5 seconds.

Plasma ORP increased during the first few days of hospitalization and approached normal levels upon discharge in all trauma groups. ORP upon presentation was significantly elevated in all traumatized patients compared to healthy controls ($p < 0.05$).

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 ($+8.5\text{mV} \pm 3.4 \text{ SEM}$) compared to the non-head injury group ($-5.2\text{mV} \pm 2.9 \text{ SEM}$).

These results demonstrate the presence of an oxidative environment in the plasma of traumatized patients, including especially severe ITBI patients. Therefore, monitoring ORP is a useful tool for assessing the degree of oxidative stress, severity of injury and efficacy of treatment in ITBI patients.

The embodiments of the present invention for which an exclusive property or privilege is claimed are defined as follows:

1. A method of evaluating or monitoring a blood product which is stored comprising:
 - a. measuring the oxidation-reduction potential (ORP) of the blood product after it has been stored for one or more days; and
 - b. determining if the ORP of the stored blood product is significantly different than the ORP of the same blood product immediately after it was obtained from a donor.
2. The method of claim 1, wherein the blood product is blood.
3. The method of claim 1, wherein the blood product is packed red blood cells.
4. The method of claim 1, wherein the ORP of the blood product is measured once per day during storage.