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(54) **STEAP3 AS A BIOCHEMICAL MARKER OF RED BLOOD CELL STORAGE AND TOXICITY**

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(52) **U.S. Cl.**

CPC **G01N 33/5005** (2013.01); **C12Q 1/6883** (2013.01); **G01N 33/53** (2013.01); **G01N 33/80** (2013.01); **C12Q 2600/106** (2013.01); **C12Q 2600/156** (2013.01); **C12Q 2600/158** (2013.01); **G01N 33/48** (2013.01); **G01N 2333/82** (2013.01); **G01N 2333/90209** (2013.01); **G01N 2800/7066** (2013.01)

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See application file for complete search history.

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ABSTRACT

Compositions and methods for determining post-transfusion survival or toxicity of red blood cells and the suitability of red blood cell units for transfusion by measuring the levels of one or more markers in a red blood cell sample are provided.

10 Claims, 2 Drawing Sheets

Specification includes a Sequence Listing.

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FIG. 1A

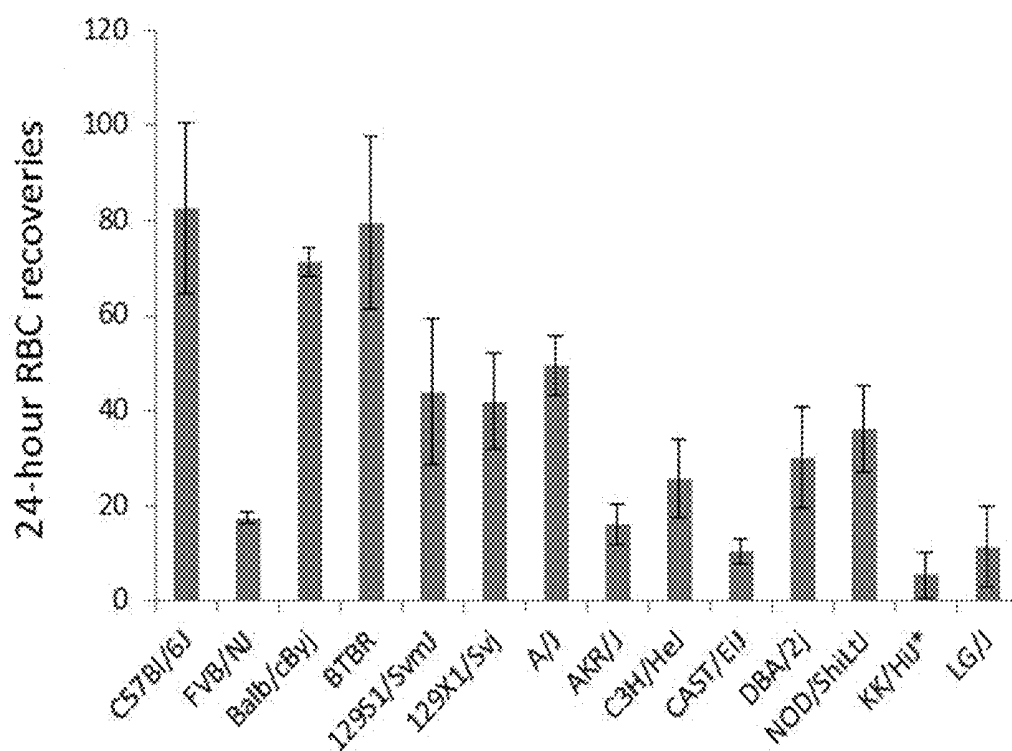


FIG. 1B

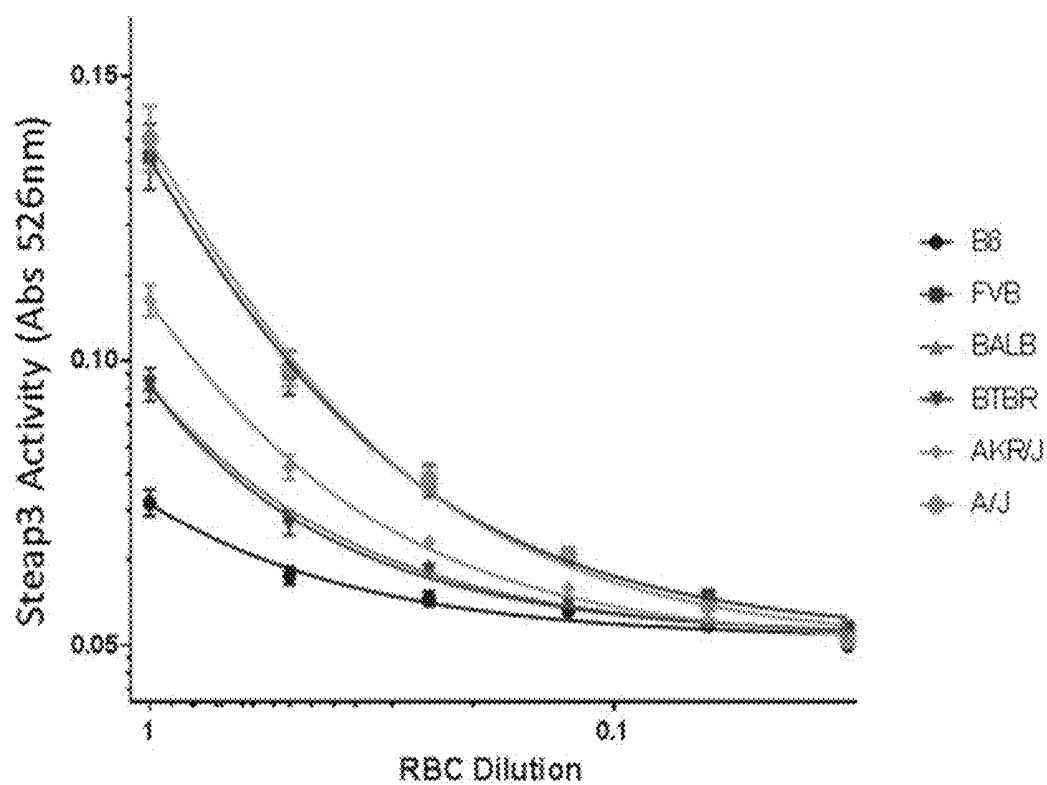
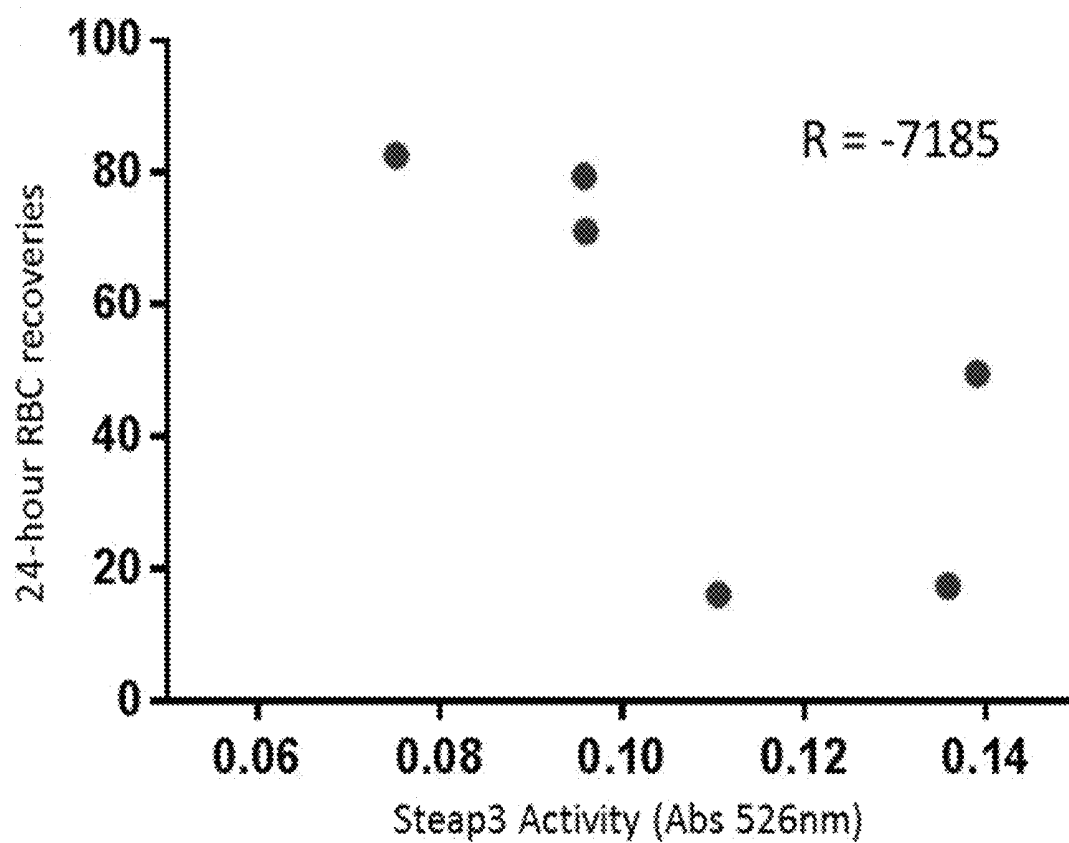


FIG. 1C



STEAP3 AS A BIOCHEMICAL MARKER OF RED BLOOD CELL STORAGE AND TOXICITY

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 15/308,836, filed on Nov. 3, 2016, which is a U.S. National Phase Patent Application based on International Patent Application No. PCT/US2015/029552, filed on May 6, 2015, which claims the benefit of U.S. Provisional Application No. 61/989,440, filed May 6, 2014, all of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

The application contains a Sequence Listing which has been filed electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 27, 2015, is named B212-0027PCT Sequence Listing and is 18,069 bytes in size.

FIELD OF THE INVENTION

The disclosure relates to compositions and methods for determining post-transfusion survival and toxicity of red blood cell (RBC) units by measuring the levels of one or more markers in a RBC sample.

BACKGROUND OF THE INVENTION

In excess of 15,000,000 units of RBCs are transfused in the USA each year into an excess of 5,000,000 patients (approximately 1 out of every 65 Americans). Currently, there are only 3 quality control measures utilized prior to release of a unit of RBCs: 1) testing negative for the screened pathogens, 2) compatibility with the patient regarding recipient antibodies to donor antigens, and 3) storage history of 4° C. FDA guidelines for RBC storage require that stored RBCs (up to 42 days) have less than 1% hemolysis and have 75% 24 hour post-transfusion survival, on average for a given storage system. However, it has been appreciated for over forty years that there is tremendous variability in how individual units of RBCs store from different human donors^{1,2}. Even for current blood storage solutions, 24 hour post-transfusion recoveries range from 35% to 100%². It has been further observed that RBC storage is reproducible from donation to donation for a given donor^{3,4}, suggesting a potential genetic component^{1,5}. Despite extensive study, there is no measurable entity known to predict how an RBC unit will do when transfused. For this reason, currently, there are no quality control measures (or unit release criteria) regarding quality of RBC units.

This is a medical problem for a number of reasons. First, RBCs that survive poorly post-transfusion result in a less efficacious product from the standpoint of RBC replacement. However, even more important is the notion that RBCs that are cleared from circulation represent a toxic insult to the recipient, which may result in morbidity and/or mortality. A second issue is what biochemical markers may predict RBCs that are going to be toxic from pathways other than simple RBC clearance.

There are currently no existing techniques to predict post-transfusion survival of RBC units or toxicity of said units. Thus, the present disclosure satisfies these and other needs. Disclosed herein is a method for assessing a RBC unit

(prior to transfusion) allowing the prediction of its post-transfusion survival and toxicity. Specifically, biochemical markers that predict if RBCs will survive well post-transfusion or will be toxic are presented herein.

SUMMARY

Described herein are compositions and methods for determining post-transfusion survival and toxicity of a RBC unit by measuring the levels of one or more markers, including Steap3, in a RBC sample.

In a first aspect, disclosed herein is a method of determining post-transfusion survival of red blood cells (RBC) prior to transfusion, the method comprising the steps of: a) providing a sample of RBC; b) measuring the level of activity of Steap3 protein in the RBC sample; c) comparing the level of activity of Steap3 protein in the RBC sample with the level of Steap3 protein activity in a control RBC sample, wherein a higher level of activity of Steap3 protein in the RBC sample as compared to the control sample is indicative of a lower RBC storage quality.

In a second aspect, disclosed herein is a method of determining the suitability of a red blood cell (RBC) unit for transfusion, the method comprising the steps of: a) providing a sample of RBC; b) measuring the level of activity of Steap3 protein in the RBC sample; c) comparing the level of activity of Steap3 protein in the RBC sample with the level of Steap3 protein activity in a control sample, wherein a higher level of activity of Steap3 protein in the RBC sample is indicative of a lower suitability for transfusion.

In various embodiments of the first and second aspects, the measurement is performed at the time of collection of the RBC sample.

In various embodiments of the first and second aspects, the measurement is performed during the time of storage of the RBC sample.

In various embodiments of the first and second aspects, the measurement of activity is performed by determining ferric reductase activity.

In various embodiments of the first and second aspects, the level is 2-200 fold higher than in the control sample.

In a third aspect, disclosed herein is a method of evaluating the suitability of a human subject to be blood donor comprising: (a) providing a biological sample from the human subject, wherein the sample comprises all, or a portion of, an Steap3 gene; and (b) detecting the presence of a polymorphism in the Steap3 gene or the portion thereof in the sample, wherein the Steap3 gene comprises at least 90% sequence identity to SEQ ID NO: 2; and (c) evaluating the human subject for suitability as a blood donor based on the presence of a polymorphism in the Steap3 gene or the portion thereof.

In various embodiments of the third aspect, the polymorphism in the Steap3 gene results in a A>V substitution at position 350 of the protein sequence.

In various embodiments of the third aspect, the polymorphism in the Steap3 gene results in reduced activity of the Steap3 protein.

In a fourth aspect, disclosed herein is a method of evaluating the suitability of a human subject to be blood donor comprising: (a) providing a biological sample from the human subject; and (b) determining the level of Steap3 protein activity in the sample; and (c) evaluating the human subject as being an unsuitable blood donor based on the higher level of Steap3 protein activity in the sample as compared to the activity in a control sample.

In various embodiments of the fourth aspect, the protein activity is ferric reductase activity.

In a fifth aspect, disclosed herein is a method for determining RBC storage quality, the method comprising the steps of: obtaining a dataset associated with a sample of stored blood, wherein the dataset comprises a Steap3 marker; analyzing the dataset to determine data for the Steap3 marker, wherein the data is positively correlated or negatively correlated with RBC storage quality of the sample of stored blood.

In various embodiments of the fifth aspect, the data is protein level, mRNA level, protein activity level, or sequence of the Steap3 protein or nucleic acid.

In a sixth aspect, disclosed herein is a method for determining RBC storage quality, the method comprising the steps of: providing a sample of stored blood, wherein the sample comprises a Steap3 marker; contacting the sample with a reagent; generating a complex between the reagent and the Steap3 marker; detecting the complex to obtain a dataset associated with the sample, wherein the dataset comprises expression or activity level or sequence data for the Steap3 marker; and analyzing the expression or activity level data for the a Steap3 marker, wherein the expression or activity level or sequence of the a Steap3 marker is positively correlated or negatively correlated with RBC storage quality.

In a seventh aspect, disclosed herein is a computer-implemented method for determining RBC storage quality, the method comprising the steps of: storing, in a storage memory, a dataset associated with a stored blood sample, wherein the dataset comprises data for a Steap3 marker; and analyzing, by a computer processor, the dataset to determine the expression or activity levels or sequence of the Steap3 marker, wherein the expression or activity levels or sequence are positively correlated or negatively correlated with RBC storage quality.

In an eighth aspect, disclosed herein is a system for determining RBC storage quality, the system comprising: a storage memory for storing a dataset associated with a stored blood sample, wherein the dataset comprises data for a Steap3 marker; and a processor communicatively coupled to the storage memory for analyzing the dataset to determine the activity or expression levels or sequence of the Steap3 marker, wherein the activity or expression levels or sequence are positively correlated or negatively correlated with RBC storage quality.

In a ninth aspect, disclosed herein is a computer-readable storage medium storing computer-executable program code, the program code comprising: program code for storing a dataset associated with a stored blood sample, wherein the dataset comprises data for a Steap3 marker; and program code for analyzing the dataset to determine the activity or expression levels or sequence of the Steap3 marker, wherein the activity or expression levels or sequence of the markers are positively correlated or negatively correlated with RBC storage quality.

In a tenth aspect, disclosed herein is a method for predicting a negative transfusion outcome, the method comprising the steps of: obtaining a dataset associated with a sample of stored blood, wherein the dataset comprises data for a Steap3 marker; analyzing the dataset to determine data for the at least one marker, wherein the data is positively correlated or negatively correlated with a negative transfusion outcome if the blood sample is transfused into a patient.

In an embodiment of the tenth aspect, the data is protein level, mRNA level, protein activity level, or sequence of the Steap3 protein or nucleic acid.

In an eleventh aspect, disclosed herein is a method for predicting a negative transfusion outcome, the method comprising the steps of: providing a sample of stored blood, wherein the sample comprises a Steap3 marker; contacting the sample with a reagent; generating a complex between the reagent and the Steap3 marker; detecting the complex to obtain a dataset associated with the sample, wherein the dataset comprises expression or activity level or sequence data for the Steap3 marker; and analyzing the expression or activity level data for the markers, wherein the expression or activity level or sequence of the at least one marker is positively correlated or negatively correlated with a negative transfusion outcome if the blood sample is transfused into a patient.

In a twelfth aspect, disclosed herein is a computer-implemented method for predicting a negative transfusion outcome, the method comprising the steps of: storing, in a storage memory, a dataset associated with a stored blood sample, wherein the dataset comprises data for a Steap3 marker; and analyzing, by a computer processor, the dataset to determine the expression or activity levels or sequence of the Steap3 marker, wherein the expression or activity levels or sequence are positively correlated or negatively correlated with a negative transfusion outcome if the blood sample is transfused into a patient.

In a thirteenth aspect, disclosed herein is a system for predicting a negative transfusion outcome, the system comprising: a storage memory for storing a dataset associated with a stored blood sample, wherein the dataset comprises data for a Steap3 marker; and a processor communicatively coupled to the storage memory for analyzing the dataset to determine the activity or expression levels or sequence of the Steap3 marker, wherein the activity or expression levels or sequence are positively correlated or negatively correlated with a negative transfusion outcome if the blood sample is transfused into a patient.

In a fourteenth aspect, disclosed herein is a computer-readable storage medium storing computer-executable program code, the program code comprising: program code for storing a dataset associated with a stored blood sample, wherein the dataset comprises data for a Steap3 marker; and program code for analyzing the dataset to determine the activity or expression levels of the Steap3 marker, wherein the activity or expression levels or sequence of the markers are positively correlated or negatively correlated with a negative transfusion outcome if the blood sample is transfused into a patient.

In embodiments of the method or storage medium or system of the aspects above, the dataset is obtained at the time of collection of the RBC sample.

The method or storage medium or system of any one of the aspects above, wherein the dataset is obtained during the time of storage of the RBC sample.

In embodiments of the method or storage medium or system of the aspects above, the dataset is obtained by determining ferric reductase activity.

In a fifteenth aspect, disclosed herein is a method for determining post-transfusion survival of red blood cells (RBC) prior to transfusion comprising: a) generating data on the level of Steap3 in a sample from the subject; b) generating data on the level of at least one additional biomarker or indicator of blood storage c) generating a score by mathematically combining the data in (a) and (b), wherein the score is indicative of post-transfusion survival of red blood cells (RBC) in the sample.

In an embodiment of the fifteenth aspect, the score is used to determine whether the RBC sample will be administered to the subject.

In an embodiment of the fifteenth aspect, the score is generated by a computer processor.

In various embodiments of the above, the method further comprises the step of administering or not administering the RBC sample that has been tested.

In a sixteenth aspect, disclosed herein is a kit for use in predicting a negative transfusion outcome or red blood cell (RBC) storage quality, the kit comprising: a set of reagents comprising a plurality of reagents for determining from a stored blood sample data for Steap3 marker; and instructions for using the plurality of reagents to determine data from the stored blood sample.

In various embodiments of the sixteenth aspect, data is protein level, mRNA level, protein activity level, or sequence of the Steap3 protein or nucleic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1C shows the correlation between Steap3 activity and RBC storage. (FIG. 1A) Each strain had slightly different RBC storage characteristics, as measured by post-transfusion 24-hour recoveries of stored RBCs. (FIG. 1B) 6 particular stains, ranging in storage, were chosen for further analysis by ferrozine assay (measuring Steap3 activity). (FIG. 10) A Pearson's correlation coefficient of -0.7185 was observed between the ferrozine activity and the 24-hr RBC recoveries.

DETAILED DESCRIPTION

The present invention generally relates to compositions and methods for determining post-transfusion survival and toxicity of RBCs by measuring the levels of one or more markers in a RBC sample.

The invention described in this disclosure represents a method for assessing an RBC unit (prior to transfusion) allowing the prediction of its post-transfusion survival and toxicity. Among the specific aims are: (1) Biochemical markers that predict if RBCs will survive well post-transfusion; and (2) Biochemical markers that predict if RBCs are toxic post-transfusion.

Red blood cell (RBC) transfusion is a life-saving therapy, and refrigerated storage is crucial for maintaining an adequate supply of donor units. However, recent studies have focused on potential adverse clinical sequelae resulting from transfusing humans with RBC units stored for longer periods of time. Indeed, multiple observational studies in human patients provide data demonstrating inferior clinical outcomes when older, stored RBC units are transfused¹. Nonetheless, this issue remains controversial because other, similarly designed human studies, show no difference in clinical outcome when comparing patients receiving transfusions of older or fresher RBC units^{1,2}. To begin to address this controversy, several prospective human trials are currently ongoing, and one was recently completed³⁻⁵. However, it is not controversial that stored RBCs accumulate multiple factors that may be toxic when infused (e.g. microparticles, free iron, free hemoglobin, prostaglandins, and leukotrienes)⁶⁻¹⁴.

<See Ref List Below>

One complication in studying RBC transfusion is that there is considerable donor-to-donor variation in the effect of refrigerated storage on RBC function and quality. In addition, there is a general absence of robust analytic tests that

consistently and accurately predict the quality of a given RBC unit prior to transfusion¹⁵. Due to the genetic and environmental complexity of outbred human donor populations, and the difficulty in limiting the number of independent variables in studying human RBC transfusion, we developed a robust animal model to begin to address these issues. Using inbred mouse strains in defined environmental and dietary settings limits the experimental variability of the system, and allows for deliberate manipulation of independent variables.

It is to be understood that this invention is not limited to particular methods, reagents, compounds, compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural references unless the content clearly dictates otherwise.

The term "about" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein.

As used herein, "RBC storage quality" is defined as the extent of post-transfusion recovery of the stored RBCs; higher recovery is defined as higher quality. Examples of post-transfusion recovery include greater than zero and almost 100% recovery, i.e., recovery of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, and all percentages in between. In one embodiment, an acceptable RBC storage quality is an average of 75% post-transfusion recovery at 24 hours, as under FDA guidelines.

As used herein, "toxicity" of a RBC unit is defined as any adverse reaction associated with transfusion of a RBC unit, including, but not limited to, hemolytic transfusion reactions, exposure to free hemoglobin, iron overload, induction of recipient cytokines, introduction of procoagulant activity, and inhibition of recipient vascular relaxation, among others.

As used herein, a RBC unit is less suitable for transfusion if it has lower RBC quality (i.e., lower post-transfusion survival) or elevated toxicity as compared to other RBC units, e.g., as compared to a control.

An "analyte" or "target" refers to a compound to be detected. Such compounds can include small molecules, peptides, proteins, nucleic acids, as well as other chemical entities. In the context of the present invention, an analyte or target will generally correspond to the biochemical compounds disclosed herein, or a reaction product thereof.

To "analyze" includes determining a value or set of values associated with a sample by measurement of analyte levels in the sample. "Analyze" may further comprise comparing the levels against constituent levels in a sample or set of samples from the same subject or other subject(s). For example, the blood storage markers, such as Steap3, of the present teachings can be analyzed by any of various conventional methods known in the art.

The term “biomarker” refers to a molecule (typically small molecule, protein, nucleic acid, carbohydrate, or lipid) that is expressed and/or released from a cell, which is useful for identification or prediction. Such biomarkers are molecules that can be differentially expressed, e.g., overexpressed or underexpressed, or differentially released in response to varying conditions (e.g., storage). In the context of the present invention, this frequently refers to Steap3 as disclosed herein, which shows altered levels of activity in stored versus non-stored RBCs, for instance, 1-fold, 2-fold, 3-fold, 4-fold, 5-fold or more in stored RBCs versus non-stored RBCs.

A “sample” refers to any source which is suspected of containing an analyte or target molecule. Examples of samples which may be tested using the present invention include, but are not limited to, blood, serum, plasma, urine, saliva, cerebrospinal fluid, lymph fluids, tissue and tissue and cell extracts, cell culture supernatants, among others. A sample can be suspended or dissolved in liquid materials such as buffers, extractants, solvents, and the like. In the context of the present application, a sample is generally a stored RBC sample of varying length of storage.

The term “subject” encompasses a cell, tissue, or organism, human or non-human, whether in vivo, ex vivo, or in vitro, male or female.

The term “generating data” encompasses obtaining a set of data determined from at least one sample. Generating data encompasses obtaining a sample, and processing the sample to experimentally determine the data. The phrase also encompasses receiving a set of data, e.g., from a third party that has processed the sample to experimentally determine the data. Additionally, the phrase encompasses mining data from at least one database or at least one publication or a combination of databases and publications. Data can be obtained by one of skill in the art via a variety of known ways including stored on a storage memory. Obtaining data encompasses data which has been generated from a sample, or data which has been obtained from sources such as patient medical history and records, physical examinations, treatment history, and the like.

The term “clinical factor” refers to a measure of a condition of a subject, e.g., disease activity or severity. “Clinical factor” encompasses all indicators of a subject’s health status, including non-sample markers, and/or other characteristics of a subject, such as, without limitation, age and gender. A clinical factor can be a score, a value, or a set of values that can be obtained from evaluation of a sample (or population of samples) from a subject or a subject under a determined condition.

The term “algorithm” encompasses any formula, model, mathematical equation, algorithmic, analytical or programmed process, or statistical technique or classification analysis that takes one or more inputs or parameters, whether continuous or categorical, and calculates an output value, index, index value or score. Examples of algorithms include but are not limited to ratios, sums, regression operators such as exponents or coefficients, biomarker value transformations and normalizations (including, without limitation, normalization schemes that are based on clinical parameters such as age, gender, ethnicity, etc.), rules and guidelines, statistical classification models, and neural networks trained on populations. Also of use in the context of blood storage factors are linear and non-linear equations and statistical classification analyses to determine the relationship between (a) levels of blood storage markers, such as Steap3, detected in a subject sample and (b) the level of blood storage or blood quality from the respective subject.

“Antibody” refers to any immunoglobulin or intact molecule as well as to fragments thereof that bind to a specific epitope that may be used in the practice of the present invention. Such antibodies include, but are not limited to polyclonal, monoclonal, chimeric, humanized, single chain, Fab, Fab’, F(ab)’ fragments and/or F(v) portions of the whole antibody and variants thereof. All isotypes are encompassed by this term and may be used in the practice of this invention, including IgA, IgD, IgE, IgG, and IgM.

An “antibody fragment” refers specifically to an incomplete or isolated portion of the full sequence of the antibody which retains the antigen binding function of the parent antibody and may also be used in the present invention. Examples of antibody fragments include Fab, Fab’, F(ab’)₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

An intact “antibody” for use in the invention comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH₁, CH₂ and CH₃. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The term antibody includes antigen-binding portions of an intact antibody that retain capacity to bind. Examples of binding include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH1 domains; (ii) a F(ab’)₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., Nature, 341:544-546 (1989)), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR).

“Single chain antibodies” or “single chain Fv (scFv)” may also be used in the present invention. This term refers to an antibody fusion molecule of the two domains of the Fv fragment, V_L and V_H. Although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird et al., Science, 242:423-426 (1988); and Huston et al., Proc Natl Acad Sci USA, 85:5879-5883 (1988)). Such single chain antibodies are included by reference to the term “antibody” fragments can be prepared by recombinant techniques or enzymatic or chemical cleavage of intact antibodies.

A "monoclonal antibody" may be used in the present invention. Monoclonal antibodies are a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

In one embodiment, the antibody or fragment is conjugated to an "effector" moiety. The effector moiety can be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels.

A "label" or a "detectable moiety" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P , fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be made detectable, e.g., by incorporating a radiolabel into the peptide or used to detect antibodies specifically reactive with the peptide.

Samples of RBCs stored for various amounts of time are compared to "control" samples which can be freshly drawn RBCs or RBCs which have been minimally stored. Control samples are assigned a relative analyte amount or activity to which sample values are compared. Relevant levels of analyte elevation occur when the sample amount or activity value relative to the control is 110%, more preferably 150%, more preferably 200-500% (i.e., two to five fold higher relative to the control), more preferably 1000-3000% higher. Alternatively, relevant levels of analyte decrease occur when the sample amount or activity value relative to the control is 90%, more preferably 50%, more preferably 20-50% (i.e., two to five fold lower relative to the control), more preferably 20-100% lower.

Assays for many of the biochemical compounds disclosed herein are known or commercially available.

For example, antibody reagents can be used in assays to detect the levels of analytes in RBC samples using any of a number of immunoassays known to those skilled in the art.

Immunoassay techniques and protocols are generally described in Price and Newman, "Principles and Practice of Immunoassay," 2nd Edition, Grove's Dictionaries, 1997; and Gosling, "Immunoassays: A Practical Approach," Oxford University Press, 2000. A variety of immunoassay techniques, including competitive and non-competitive immunoassays, can be used. See, e.g., Self et al., *Curr. Opin. Biotechnol.*, 7:60-65 (1996). The term immunoassay encompasses techniques including, without limitation, enzyme immunoassays (EIA) such as enzyme multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), IgM antibody capture ELISA (MAC ELISA), and microparticle enzyme immunoassay (META); immunohistochemical (IHC) assays; capillary electrophoresis immunoassays (CEIA); radioimmunoassays (RIA); immunoradiometric assays (IRMA); fluorescence polarization immunoassays (FPIA); and chemiluminescence assays (CL). If desired, such immunoassays can be automated. Immunoassays can also be used in conjunction with laser induced fluorescence. See, e.g., Schmalzing et al., *Electrophoresis*, 18:2184-93 (1997); Bao, J. *Chromatogr. B. Biomed. Sci.*, 699:463-80 (1997). Liposome immunoassays, such as flow-injection liposome immunoassays and liposome immunosensors, are also suitable for use in the present invention. See, e.g., Rongen et al., *J. Immunol. Methods*, 204:105-133 (1997). In addition, nephelometry assays, in which the formation of protein/antibody complexes results in increased light scatter that is converted to a peak rate signal as a function of the marker concentration, are suitable for use in the methods of the present invention. Nephelometry

assays are commercially available from Beckman Coulter (Brea, Calif.; Kit #449430) and can be performed using a Behring Nephelometer Analyzer (Fink et al., *J. Clin. Chem. Clin. Biochem.*, 27:261-276 (1989)).

Specific immunological binding of the antibody to proteins can be detected directly or indirectly. Direct labels include fluorescent or luminescent tags, metals, dyes, radionuclides, and the like, attached to the antibody. A chemiluminescence assay using a chemiluminescent antibody specific for the protein is suitable for sensitive, non-radioactive detection of protein levels. An antibody labeled with fluorochrome is also suitable. Examples of fluorochromes include, without limitation, DAPI, fluorescein, Hoechst 33258, R-phycoerythrin, B-phycoerythrin, R-phycoerythrin, rhodamine, Texas red, and lissamine. Indirect labels include various enzymes well known in the art, such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β -galactosidase, urease, and the like. A horseradish-peroxidase detection system can be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB), which yields a soluble product in the presence of hydrogen peroxide that is detectable at 450 nm. An alkaline phosphatase detection system can be used with the chromogenic substrate p-nitrophenyl phosphate, for example, which yields a soluble product readily detectable at 405 nm. Similarly, a β -galactosidase detection system can be used with the chromogenic substrate o-nitrophenyl- β -D-galactopyranoside (ONPG), which yields a soluble product detectable at 410 nm. An urease detection system can be used with a substrate such as urea-bromocresol purple (Sigma Immunochemicals; St. Louis, Mo.).

A signal from the direct or indirect label can be analyzed, for example, using a spectrophotometer to detect color from a chromogenic substrate; a radiation counter to detect radiation such as a gamma counter for detection of ^{125}I ; or a fluorometer to detect fluorescence in the presence of light of a certain wavelength. For detection of enzyme-linked antibodies, a quantitative analysis can be made using a spectrophotometer such as an EMAX Microplate Reader (Molecular Devices; Menlo Park, Calif.) in accordance with the manufacturer's instructions. If desired, the assays of the present invention can be automated or performed robotically, and the signal from multiple samples can be detected simultaneously.

The antibodies can be immobilized onto a variety of solid supports, such as magnetic or chromatographic matrix particles, the surface of an assay plate (e.g., microtiter wells), pieces of a solid substrate material or membrane (e.g., plastic, nylon, paper), and the like. An assay strip can be prepared by coating the antibody or a plurality of antibodies in an array on a solid support. This strip can then be dipped into the test sample and processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

In some embodiments, the measurement of the markers of the present invention is performed using various mass spectrometry methods. As used herein, the term "mass spectrometry" or "MS" refers to an analytical technique to identify compounds by their mass. MS refers to methods of filtering, detecting, and measuring ions based on their mass-to-charge ratio, or "m/z". MS technology generally includes (1) ionizing the compounds to form charged compounds; and (2) detecting the molecular weight of the charged compounds and calculating a mass-to-charge ratio. The compounds may be ionized and detected by any suitable means. A "mass spectrometer" generally includes an ionizer and an ion detector. In general, one or more molecules of

interest are ionized, and the ions are subsequently introduced into a mass spectrographic instrument where, due to a combination of magnetic and electric fields, the ions follow a path in space that is dependent upon mass ("m") and charge ("z"). See, e.g., U.S. Pat. No. 6,204,500, entitled "Mass Spectrometry From Surfaces;" U.S. Pat. No. 6,107,623, entitled "Methods and Apparatus for Tandem Mass Spectrometry;" U.S. Pat. No. 6,268,144, entitled "DNA Diagnostics Based On Mass Spectrometry;" U.S. Pat. No. 6,124,137, entitled "Surface-Enhanced Photolabile Attachment And Release For Desorption And Detection Of Analytes;" Wright et al., Prostate Cancer and Prostatic Diseases 1999, 2: 264-76; and Merchant and Weinberger, Electrophoresis 2000, 21: 1164-67.

As used herein, the term "gas chromatography" or "GC" refers to chromatography in which the sample mixture is vaporized and injected into a stream of carrier gas (as nitrogen or helium) moving through a column containing a stationary phase composed of a liquid or a particulate solid and is separated into its component compounds according to the affinity of the compounds for the stationary phase.

As used herein, the term "liquid chromatography" or "LC" means a process of selective retardation of one or more

components of a fluid solution as the fluid uniformly percolates through a column of a finely divided substance, or through capillary passageways. The retardation results from the distribution of the components of the mixture between one or more stationary phases and the bulk fluid, (i.e., mobile phase), as this fluid moves relative to the stationary phase(s). Examples of "liquid chromatography" include reverse phase liquid chromatography (RPLC), high performance liquid chromatography (HPLC), and turbulent flow liquid chromatography (TFLC) (sometimes known as high turbulence liquid chromatography (HTLC) or high throughput liquid chromatography).

Steap3

The Steap3 gene encodes a metalloredutase with the activity of converting iron from an insoluble ferric (Fe^{3+}) to a soluble ferrous (Fe^{2+}) form.

Reference sequences for the mouse gene and protein have the NCBI accession numbers NM_001085409 and NP_001078878. The human gene and protein have the NCBI accession number NM_001008410. Representative sequences of Steap3 genes and proteins are shown below.

TABLE 1

Accession SEQ Number And ID NO Description Amino acid/nucleotide sequence	
1 NM_001008 410 Human protein	MPEEMDKPLISLHLVSDSSSLAKVPDEAPKVGILGSGDFARSLATRLVSGFKVVGSRNPKRT ARLFPSAAQVTFQEEAVSSPEVIFVAVFREHYSSLCSLSDQLAGKILVDVSNPTQEHLQHRES NAEYLASLFPTCTVVKAFNVI SAWTLQAGPRDGNRQVPI CGDQPEAKRAVSEMALAMGFMPVDM GSLASAVEVEAMPLRLLPWKVPTLLALGLFVCFYAYNFVRDVLQPYVQESQNKFFKLPSVSVN TTLPCVAYVLLSLVYLPGLAALQLRRGTYQRFPDWDHLQHRKQIGLLSFFCAALHALYS FCLPLRRAHRYDLVNLAVKQVLANKSHLWVEEVWRMETYLSLGLALGTSLLAVTSLPSIAN SLNWREFSFVQSSLGFLVALVSLTLTLTYGWTRAFESRYKFYLPPTFTLLVPCVVILAKAL FLLPCI SRRLARIRRGWERESTIKFTLPDHALAEKTSHV
2 NM_001008 410 Human nucleotide	gaggaggagc ctggggcgga gccaccgcct tcggccgga ccttcagctg ccgcggtcgctccgagcggc gggccgcaga gccacaaaa tgccagaaga gatggacaag ccactgatcagctccacct ggtggacagc gatagtagcc ttgccaaagt ccccgatgag gccccaaagtgggcatcct gggtagcggg gactttgccc gctccctggc cacacgcctg gtgggctctggctcaaaagt ggtggtggg agccgcaacc ccaaacgcac agccagctg ttccctcagcggcccaagt gactttccaa gaggaggcag tagctcccc ggaggtcatc ttgtggtgtgttccggga gcactactct tcactgtgca gtctcagtgca ccagctggcg ggcaagatcctggtgatgt gagcaaccct acagagcaag agcaccttca gcatcgtgag tccaatgctgagtacctgct ctcctcttc ccaacttgca cagtggtaaa ggccttcaat gtcatctctgctggacct gcaggtggc ccaaggatg gtaacaggca ggtgcccac tgcggtgaccacccagaagc caagcgtgct gtctcggaga tggcgctgcg catgggcttc atgcccgtggacatgggatc cctggcgtca gcctgggagg tggaggccat gccctgcgc ctcctccggcctggaaggt gccaccctg ctggccctgg ggctcttctg ctgcttctat gctcaactctctgctcggga cgttctgtag cctatgtgc aggaagccca gaacaagttc ttcaagctgcccgtgtcctt ggtcaacacc aactgcccgt gcgtggccta cgtgctgctg tcactgtgtacttgcccgg cgtgctggcg gctgcccctgc agctgcggcg cgcaccaag taccagcgcttccccgactg gctggaccac ttgctacagc accgcaagca cgtcggctg ctcagcttctctgctgcgcgc cctgcacgcc ctctacagct tctgcttgcc gctgcgcgc gccaccgcctacgacctggt caacctggca gtcaagcagg tcttgcccaa caagagccac ctctgggtggaggaggaggt ctggcggatg gagatctacc tctccctggg agtgcctggc ctcggcacgttgtccctgct ggccgtgacc tcactgccc ccatgcaaaa ctgcctcaac tggaggaggattcagcttctg tcagtcctca ctgggctttg tggccctcgt gctgagcaca ctgcacacgctcacctacgg ctggaccgc gccttcgagg agagccgcta caagttctac ctgctcccaccttcacgct cacgctgctg gtgcccgtgc tgcctatcct ggccaaagcc ctgtttctctgcccctgcat cagccgcaga ctgcgcagga tccggagagg ctgggagagg gagagcaccatcaagttcac gctgcccaca gaccacgccc tggccgagaa gacgagccac gtagaggtgctgcccctgg gctctggacc ccgggcacac gagggaagg gcccgtgacc cgtaggttttctttctctg gtggtgcaaa gtggtataac tgtgtgcaaa taggaggttt gagggtccaaattcctgggac tcaaatgtat gcagtactat tcagaatgat atacacacat atgtgtatatgtattacat atattccaca tatataacag gatttgcaat tatacatagc tagctaaaaagttgggtctc tgagatttca actttagatg ttaaaaacaa gtgcccgtacg ttaagagaaggcagatcat gctattgtga catttgaga gatatacaca cactttttgt acagaagaggcttgtgctgt ggtgggttcg atttatccct gccaccacca cccccacaac ttcccttttctacttcccc aaggctcttg cagagctagg gctctgaagg ggagggaagg caacggctctgcccagagcc atccctggag catgtgagca gcggctggtc tcttccctcc acctggggcagcagcaggag gctgggggag gaggaaaac aggcagtcgg cctggagctc gtgctggtcctttgcccgg tgggtggagg atggagggat tgggtgaag ctgctccacc tcactcctgctgagtggggg agacatttcc cctgaaagtc agaagtcacc atagagcctg

TABLE 1-continued

Accession						
SEQ ID NO	Number And Description	Amino acid/nucleotide sequence				
		caaatggatcctcctgtgag	agtgaagctca	cctcctttcc	agagccatta	gtgagcctgg
		cttggggaacaagtgtaattt	ccttccctcc	tttaacctgg	cgatgagcgt	cctttaaaccc
		actgtgccttctcaccctt	ccatcttcag	tttgaatgac	tcccaggaag	gcctagagca
		gacccttttagaaatcagccc	aagggggaga	gcaagagaaa	acactctagg	gagtaaagct
		ccccgggctcagagttgag	ccctgcctgg	gctgaaggac	tgtcttcacg	aagtcaagtc
		tgaggaaaaatattggggac	tccaaatgtc	ctctggcaga	ggaccagaa	aaccacactg
		gctccaaactctctcatg	gggcattaca	cttcaaaaca	gtggggagca	acttttccac
		caaagctacaaacctaaaat	gctgctgccc	caaagcacaa	gagggagag	caccgcccgg
		ggccacaggagctgtgtcctc	cagtcacagg	ccatccttgc	tgtccctac	tgactctagc
		ttacttccccctgtgaagaaa	caggtgttct	cggtgagcc	cccaacctc	tgacagaacca
		ggttgatctgccacagaaaa	agcatctttg	aagacaaaga	gggtgaggtc	ttcatgagtc
		tctgtgggccccaaagccatct	tctgatgga	ggaagagagt	agggccagtg	aaggctgccc
		agagagaatgtcacagatga	ggctgcccct	gcccccccc	cgccaggag	gtttcatgag
		ctcatgtctatgcagcacat	aagggttctt	cagtgaagag	caggagaaga	gccactgca
		aggatagctcattagggaca	tgaccgatgc	aggggaagcc	atgccgggga	agctcttctc
		gcaggtatttttccatctgct	gtgccaaagg	tgagcggcag	aaacttgtct	cataaattgg
		cactgatggagcatcagctg	tggcccacag	agagccttgc	tgagaagggg	gcaggtaaag
		cagagatttttagcattgctc	tggcataaaca	agggcccatc	gattccctac	taatgagagg
		caggagagcatgggcaatg	gagaccaccc	aatgatcccc	aaccccggtg	ggtagtggct
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		tgggtacaaaagtgcctcaa	cgacatgctc	tggaaatccc	aaatgccaca	gtctgagggt
		gatatctaaaatctatgcct	tcaaaagagt	ctctgttttt	tttttttaac	ctggtagaca
		gtataaaagcagtgcaata	aacacctaac	cttctgcaaa		
3	NP_001078 878 Mouse protein	MSGEMDKPLI SRRLVDSGDS	LAIEVPKEAPK	VGILGSGDFA	RSLATRLVGS	
		GFSVVVGSRNPKRTAGLFP	LAQVTFQEEA	VSSPEVIFVA	VFREHYSSLC	SLADQLAGKI
		LVDVSNPTKEHLQHRQNSA	EYLASLFPAC	TVVKAFNVIS	AWALQAGPRD	GNRQVLICSD
		QPEAKRTISEMARAMGFTPL	DMGSLASARE	VEAIPLRLLP	SWKVPTLLAL	GLFVCFYTYN
		FIRDVLQPIRKDENKFKYKM	PLSVVNTLP	CVAYVLLSLV	YLPGLVLAAL	QLRRGTYKQR
		FDPDLHDLQHRKQIGLLSF	FFAMHLALYS	FCLPLRRSHR	YDLVNLAVKQ	VLANKSRLWA
		EEEVWRMEIYLSLGLVALGM	LSLLAVTSLP	SIANSNLNKE	FSFVQSTLGF	VALILSTMHT
		LTYGWTRAFENHYKPYLPP	TFTLTLLLP	VIIILAKGLFL	LPCLNRLTK	IRRGWEKDGA
		VKFMLPGDHTQGEKTSHV				
4	NM_001085 409 Mouse nucleotide	tcacactcta tctcagacct	tgagctaaag	gactctccca	aggcgagggg	
		gccactgcttactggggccc	tccacctggg	aaggagaggt	taaaggtgac	ccagggttaa
		ggagaacctggtatgccaca	tctcaactta	tgatgtgagc	tccagccacc	cagcttcaga
		agaaatggctgcagaggccc	acaggcagca	gggtccttgc	cccaccatcc	cctcagaggg
		ctgtggaaagtccacagaga	agaaaggcag	tgccggccag	cttagacctg	gtgagctgga
		gtctgtggggcaggaggagaa	gcagggcata	gaagacacag	gaagcaactc	tgccttgatt
		ccagactccatctgcatgga	ctgattccag	gtgctagggt	tcctttctca	gaaacccccc
		aagtccacgaaggcactgct	atgtcggggg	agatggacaa	gccgctgac	agccgcgcgc
		tagtggcagtgatggcagt	ctggctgagg	tccccaagga	ggcccccaaa	gtgggcatcc
		tgggcagtggggattttgccc	cgttccctgg	ccacacgcct	gggtgggtct	ggcttcagtg
		tgggtggggggagccgtaac	cccaaacgca	cggtgggctc	cttccctctc	ttagctcaag
		tgactttccaggaggaagcc	gtgagctctc	cagaggtcat	ctttgtggcc	gtgttcgggg
		agcactatctctcactgtgc	agtctcgtcg	accagttggc	tggcaagatc	ctcgtggatg
		taagcaacccccacggagaag	gagcatcttc	agcaccgcga	gtctaacgct	gagtagcctg
		cctcactcttttctcgtgctc	actgtgggtga	aggccttcaa	cgctcatctc	gcatggggcc
		tacaggctggccccaaaggat	gggaacaggg	agggtgctcat	ctgcagtgat	cagccagaag
		ccaagcgcaccatctcagag	atggcacgag	ccatgggttt	cacacccctg	gacatgggat
		ccctggcctcagcgaggagg	gtagaagcca	taccctcgcc	cctccttcca	tcctggaagg
		tgccccacctcctggcactg	gggctctttg	tgtgcttcta	cacctacaac	ttcatccgag
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		gtgtgctggcagctgcgctt	cagctgcgga	gggggaccaa	gtaccagcgc	ttcccagact
		ggctggaccactggctgcag	catcgcaagc	agatcgggct	gtcagcttc	ttcttcgcga
		tgtgcacgctctctacagc	ttctgctgcg	cgctgcgcgc	ctcccaccgc	tacgacctgg
		tcaatctggctgtgaagcag	gtcctggcca	acaagagccg	cctctgggct	gaggaagaag
		tctggaggatggagatatac	ctgtccctgg	gtgtgctggc	cctgggcatg	ttgtcgctgc
		tggctgtcacctcgctcccg	tccattgcta	attccctcaa	ctggaaggag	ttcagcttcg
		tgcagtcacactgggcttc	gtggccctga	tactcagcac	aatgcacaca	ctcacctacg
		gctggaccgctgcctttgag	gaaaaccact	acaagtctta	cctgccgccc	acattcacac
		tcacgctgctcctgcctgtg	gtgatcatcc	tggccaaggg	cctcttctc	ctgccctgccc
		tcaaccgcagactcaccag	atcgcaggg	gctgggagaa	agatggggct	gtcaagttca
		tgtccccggcgaccacaca	cagggggaga	aaacaagcca	cggtgtgaggc	cctggaagtg
		gagatggctgtgtggggggccc	tgagctgggt	tgggtctct	ttcttgatg	ctgcacagcg
		aggtgatgatataatgcgtgg	gtggctgaga	tctcaattcc	tgggatgcag	gtgtaaaactg
		acatactcagaatgacaccc	catacatgtg	atatgtactt	acataatatt	cacataataa
		aagatttctctattattctta	cttagctaaa	aaaaaaaagt	gggtccctat	atttcagcgt
		aagcattttcaagcaaatgc	cacacattga	acagcagatc	ccacccttgt	ggtagctaca
		gaggcagacagacactctgg	tataggagaa	actgtcttct	gttggtattct	ctcctttaat

TABLE 1-continued

Accession SEQ Number And ID NO Description Amino acid/nucleotide sequence				
ctctatgctccttattagct	gaatcctaaa	gttggtgcaa	agctggggca	agaaatgcct
ctggtgcccctacccccat	cccagggcta	agaaagaagc	ctcgagtga	caggaacca
ggctctggactctgctgcttc	cctgggcgtg	cgtggggagg	ctcagcaaga	ccccctggga
tctatgcaggagctttttca	ggtccgtcct	ttcttcaggg	aagggctga	agctgccccca
tctgatccctagctgagctga	gaagattctt	ccccaccccc	tgaagtgcca	gagtcaccac
cggagcctgcaaatgatcc	ttctgcgaag	gtgtgaagtc	accgctcttc	cagagccatt
aatgaacctggtcttcggga	ggaggataat	tgtttctctc	ccattaagtt	gctggtgacc
ccccctttaaatcactgtgc	cttctcgctc	tttccatcat	taatttggac	atctccgtgg
agtggacacttgctcgggca	gtccgggggtg	ggggggagca	ttagagattg	cagagaataa
ccatcgaaatcctctctctgg	ggcaaccctc	cccttggatg	tgecccagge	ctgccttcac
taaaattggtccctgaggaga	ataataggga	cccttttcat	ttaccctgtc	gcctgtaggc
agaaaacctaccttctgagc	accagaaaaa	cacagtggcc	ccatgctctt	cttcaggggg
ttccacagcccccttccccc	tggttttggc	tcctctccctc	cttctccccc	tcctctccctc
cctcactgttacgttcaacc	acaaaagtct	tcaaatattg	tttttttgaa	ttcttaaaga
gacctcattttattacaaaa	aaaaaaaaaa	aa		

The sequence of Steap3 indicates that it is a six-trans-²⁰ membrane protein.

The quantity of Steap3 of the present teachings can be indicated as a value. The value can be one or more numerical values resulting from the evaluation of a sample, and can be derived, e.g., by measuring level(s) of Steap3 in a sample by an assay performed in a laboratory, or from data obtained from a provider such as a laboratory, or from data stored on a server. Steap3 levels can be measured using any of several techniques known in the art, such as those described herein.

The measurement of levels of Steap3 can be determined at the protein or nucleic acid level using any method known in the art. "Protein" detection comprises detection of full-length proteins, mature proteins, pre-proteins, polypeptides, isoforms, mutations, variants, post-translationally modified proteins and variants thereof, and can be detected in any suitable manner. Levels of Steap3 can be determined at the protein level directly or by measuring the enzymatic activities Steap3. Such methods are well-known in the art and include, e.g., immunoassays based on antibodies to proteins encoded by the genes, aptamers or molecular imprints.

Steap3 can also be determined at the nucleic acid level. For example, nucleic acid sequences that correspond Steap3 can be used to construct primers and probes for detecting and/or measuring Steap3 nucleic acids. These probes can be used in, e.g., Northern or Southern blot hybridization analyses, ribonuclease protection assays, and/or methods that quantitatively amplify specific nucleic acid sequences. As another example, Steap3 sequences can be used to construct primers for specifically amplifying Steap3 sequences in, e.g., amplification-based detection and quantitation methods such as reverse-transcription based polymerase chain reaction (RT-PCR) and PCR. When alterations in gene expression are associated with gene amplification, nucleotide deletions, polymorphisms, post-translational modifications and/or mutations, sequence comparisons in test and reference populations can be made by comparing relative amounts of the examined DNA sequences in the test and reference populations.

As an example, Northern hybridization analysis using probes which specifically recognize one or more of these sequences can be used to determine gene expression. Alternatively, expression can be measured using RT-PCR; e.g., polynucleotide primers specific for the differentially expressed Steap3 mRNA sequences reverse-transcribe the mRNA into DNA, which is then amplified in PCR and can be visualized and quantified. Steap3 RNA can also be

quantified using, for example, other target amplification methods, such as TMA, SDA, and NASBA, or signal amplification methods (e.g., bDNA), and the like. Ribonuclease protection assays can also be used, using probes that specifically recognize Steap3 mRNA sequences, to determine gene expression.

In some embodiments, Steap3 is detected by contacting a subject sample with reagents, generating complexes of reagent and analyte, and detecting the complexes. Examples of "reagents" include but are not limited to nucleic acid primers, peptides, small molecules, substrates for the enzyme, and antibodies.

Various methods to detect Steap3 levels are known in the art. For example, various PCR based assays such as TaqMan assays are known in the art and are commercially available.

The sequence of Steap3 can be determined using any of the methods for nucleic acid sequence determination known in the art, such as conventional Sanger sequencing or NextGen sequencing methods.

At the protein level, various antibodies directed against the Steap3 protein are known in the art and are commercially available. Steap3 ELISA kits are also commercially available.

Assays to measure Steap3 activity are also known. For example, an assay that measures the production of ferrous iron using 200 μ M ferrozine as an indicator and monitoring the increase in absorbance at $\lambda=562$ nm may be used. See, e.g., Ohgami et al., "The Steap proteins are metalloredutases", Blood, 108: 1388-1394 (2006).

In some embodiments, data on the level or activity or sequence of Steap3 may be combined with other indicators or biomarkers of blood storage, such as those disclosed in U.S. Patent Application No. 2014/0178904, to obtain a score which is indicative of blood storage quality.

The information from the assays above can be quantitative and sent to a computer system of the invention. The information can also be qualitative, such as observing patterns or fluorescence, which can be translated into a quantitative measure by a user or automatically by a reader or computer system. In an embodiment, the subject can also provide information other than assay information to a computer system, such as race, height, weight, age, gender, eye color, hair color, family medical history and any other information that may be useful to a user, such as a clinical factor described above.

Other embodiments of the present teachings comprise Steap3 detection reagents packaged together in the form of

a kit for conducting any of the assays of the present teachings. In certain embodiments, the kits comprise reagents for protein detection of Steap3 proteins, such as antibodies. For example, the kit may comprise antibodies or fragments thereof, specific for Steap3 (primary antibodies), along with one or more secondary antibodies that may incorporate a detectable label; such antibodies may be used in an assay such as an ELISA. Alternately, the antibodies or fragments thereof may be fixed to a solid surface, e.g. an antibody array. In certain embodiments, the kits comprise oligonucleotides that specifically identify one or more Steap3 nucleic acids based on homology and/or complementarity with Steap3 nucleic acids. The oligonucleotide sequences may correspond to fragments of Steap3 nucleic acids. For example, the oligonucleotides can be more than 200, 200, 150, 100, 50, 25, 10, or fewer than 10 nucleotides in length. In other embodiments, the kits comprise antibodies to proteins encoded by the Steap3 nucleic acids. The kits of the present teachings can also comprise aptamers. The kit can contain in separate containers a nucleic acid or antibody (the antibody either bound to a solid matrix, or packaged separately with reagents for binding to a matrix), control formulations (positive and/or negative), and/or a detectable label, such as but not limited to fluorescein, green fluorescent protein, rhodamine, cyanine dyes, Alexa dyes, luciferase, and radiolabels, among others. Instructions for carrying out the assay, including, optionally, instructions for generating a DAI score, can be included in the kit; e.g., written, tape, VCR, or CD-ROM. The assay can for example be in the form of a Northern hybridization or a sandwich ELISA as known in the art.

In some embodiments of the present teachings, Steap3 detection reagents can be immobilized on a solid matrix, such as a porous strip, to form at least one Steap3 detection site. In some embodiments, the measurement or detection region of the porous strip can include a plurality of sites containing a nucleic acid. In some embodiments, the test strip can also contain sites for negative and/or positive controls. Alternatively, control sites can be located on a separate strip from the test strip. Optionally, the different detection sites can contain different amounts of immobilized nucleic acids, e.g., a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of Steap3 in the sample. The detection sites can be configured in any suitably detectable shape and can be, e.g., in the shape of a bar or dot spanning the width of a test strip.

In other embodiments of the present teachings, the kit can contain a nucleic acid substrate array comprising one or more nucleic acid sequences. In some embodiments the substrate array can be on a solid substrate, such as what is known as a "chip." See, e.g., U.S. Pat. No. 5,744,305. In some embodiments the substrate array can be a solution array; e.g., xMAP (Luminex, Austin, Tex.), Cyvera (Illumina, San Diego, Calif.), RayBio Antibody Arrays (RayBiotech, Inc., Norcross, Ga.), CellCard (Vitra Bioscience, Mountain View, Calif.) and Quantum Dots' Mosaic (Invitrogen, Carlsbad, Calif.).

In some embodiments, the present invention is practiced using computer implementation. In one embodiment, a computer comprises at least one processor coupled to a chipset. Also coupled to the chipset are a memory, a storage device, a keyboard, a graphics adapter, a pointing device, and a network adapter. A display is coupled to the graphics adapter. In one embodiment, the functionality of the chipset is provided by a memory controller hub and an I/O controller

hub. In another embodiment, the memory is coupled directly to the processor instead of the chipset.

The storage device is any device capable of holding data, like a hard drive, compact disk read-only memory (CD-ROM), DVD, or a solid-state memory device. The memory holds instructions and data used by the processor. The pointing device may be a mouse, track ball, or other type of pointing device, and is used in combination with the keyboard to input data into the computer system. The graphics adapter displays images and other information on the display. The network adapter couples the computer system to a local or wide area network.

As is known in the art, a computer can have different and/or other components than those described previously. In addition, the computer can lack certain components. Moreover, the storage device can be local and/or remote from the computer (such as embodied within a storage area network (SAN)).

As is known in the art, the computer is adapted to execute computer program modules for providing functionality described herein. As used herein, the term "module" refers to computer program logic utilized to provide the specified functionality. Thus, a module can be implemented in hardware, firmware, and/or software. In one embodiment, program modules are stored on the storage device, loaded into the memory, and executed by the processor.

Embodiments of the entities described herein can include other and/or different modules than the ones described here. In addition, the functionality attributed to the modules can be performed by other or different modules in other embodiments. Moreover, this description occasionally omits the term "module" for purposes of clarity and convenience.

The following examples of specific aspects for carrying out the present invention are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

EXAMPLES

Example 1: Identification of Steap3

Background

There is substantial donor-to-donor variability in the quality of human RBC storage. Like humans, RBCs of different strains of inbred mice store differently; RBCs from C57BL/6 (B6) mice store well whereas RBCs from FVB mice store poorly (24-hr post-transfusion recoveries).

Methods and Materials

B6×FVB F1 mice were interbred to generate an F2 population that has a random assortment of chromosomes, including recombination events. F2 mice were phenotyped by storing RBCs for 7 days, followed by transfusion and calculation of 24 hour recoveries. DNA from each F2 mouse was analyzed using a panel of 1400 single nucleotide polymorphisms (SNPs). Genetic elements associated with RBC post-transfusion survival were identified by Quantitative Trait Loci (QTL) analysis. Additional resolution was obtained by backcrossing F2 mice with poor storage to B6 parents, and selecting poor-storing progeny to breed for each next generation.

Results

QTL analysis of 154 F2 mice revealed a peak signal on chromosome 1 at location rs4137908, with extreme statistical significance ($p=2.09E-31$). However, the peak was broad, and ranged over 149 Mb (using a false-discovery rate of 0.05). SNP analysis of the backcrossed pedigree was used

to further refine the boundaries to a 9.5 Mb region, containing 64 genes, 35 of which encoded proteins. Focusing on Non-Synonymous SNPs between B6 and FVB strains identified 5 genes (Gli2, Steap3, Ccdc93, Rab3gap1, and Tli). Based upon known biology, Steap3 was chosen as a leading candidate. Steap3 is the primary ferrireductase in erythroid cells, converting Fe³⁺ to Fe²⁺, both mitigating oxidative stress and allowing transferrin-dependent iron uptake. Mice lacking Steap3 have profound anemia. Moreover, a human family has been reported with a nonsense mutation in the human steap3 orthologue and a congenital hypochromic anemia. The SNP between B6 and FVB mice leads to an A-V mutation at position 350, which is within the conserved ferric reductase superfamily domain.

Example 2: Steap3 Activity and RBC Storage

To test the ability of measuring Steap3 activity as a predictor of RBC storage from genetically distinct donors, in a pre-clinical tractable animal model, a variety of different strains of mice was characterized for RBC storage properties. Each strain had slightly different RBC storage characteristics, as measured by post-transfusion 24-hour recoveries of stored RBCs (FIG. 1A). 6 particular strains, ranging in storage, were chosen for further analysis by ferrozine assay (measuring Steap3 activity) (see FIG. 1B). A Pearson's correlation coefficient of -0.7185 was observed between the ferrozine activity and the 24-hr RBC recoveries (FIG. 10). These data demonstrate, in a whole animal model, that measuring Steap3 activity predicts (as an inverse relationship) the post-transfusion circulation of RBCs (e.g. 24-hour recovery) for donors of different genetic composition.

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While specific aspects of the invention have been described and illustrated, such aspects should be considered illustrative of the invention only and not as limiting the invention as construed in accordance with the accompanying claims.

All publications and patent applications cited in this specification are herein incorporated by reference in their entirety for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference for all purposes.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

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The invention claimed is:

1. A method for determining red blood cell (RBC) storage quality of an RBC unit, the method comprising:

obtaining a test RBC sample from the RBC unit comprising Steap3 protein;

contacting the test RBC sample with a reagent that binds Steap3 protein to generate a complex of the reagent and Steap3 protein;

detecting the complex to obtain RBC storage quality data associated with the RBC unit, wherein the RBC storage quality data comprises a measured level of Steap3 protein or activity in the test RBC sample, as compared to the corresponding level in a control RBC sample suitable for transfusion; and

storing the RBC storage quality data in a storage memory,

wherein the RBC storage quality data indicates that the RBC unit is suitable for transfusion when the measured level of Steap3 protein or activity in the test RBC sample is lower than or equal to the corresponding level in the control RBC sample suitable for transfusion

or

wherein the RBC storage quality data indicates that the RBC unit is not suitable for transfusion when the measured level of Steap3 protein or activity in the test RBC sample is higher than the corresponding level in the control RBC sample suitable for transfusion.

2. The method of claim 1, wherein the RBC storage quality data is obtained before storage of the RBC unit.

3. The method of claim 1, wherein the RBC storage quality data is obtained during the time of storage of the RBC unit.

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4. The method of claim 1, wherein the RBC storage quality data is obtained by determining Steap3 ferric reductase activity.

5. The method of claim 1, further comprising administering the RBC unit to a subject if the RBC storage quality data indicates that the RBC unit is suitable for transfusion. 5

6. The method of claim 1, further comprising not administering the RBC unit to a subject if the RBC storage quality data indicates that the RBC unit is not suitable for transfusion. 10

7. The method of claim 1, wherein the reagent is ferrozine and the detecting the complex comprises monitoring absorbance of ferrous iron-ferrozine complex at $\lambda=562$ nm.

8. The method of claim 7, wherein the ferrozine is at 200 μ M. 15

9. The method of claim 1, wherein the RBC storage quality data for the Steap3 marker comprises Steap3 protein activity level in the test RBC sample, as compared to the corresponding level in the control RBC sample suitable for

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transfusion, and the method further comprises determining post-transfusion survival of RBCs by

generating data on a level of at least one additional biomarker or indicator of blood storage in the test RBC sample, as compared to the corresponding level in the control RBC sample suitable for transfusion; and

generating a score by mathematically combining the Steap3 protein activity level in the test RBC sample and the level of the at least one additional biomarker or indicator of blood storage in the test RBC sample, wherein the score is indicative of post-transfusion survival of RBCs in the RBC unit, as compared to post-transfusion survival of RBCs in the control RBC sample suitable for transfusion.

10. The method of claim 9, wherein the score is used to determine whether the RBC unit will be administered to a subject.

* * * * *

专利名称(译)	steap3作为红细胞储存和毒性的生化标记		
公开(公告)号	US10677781	公开(公告)日	2020-06-09
申请号	US16/360898	申请日	2019-03-21
[标]申请(专利权)人(译)	BLOODWORKS		
申请(专利权)人(译)	BLOODWORKS		
当前申请(专利权)人(译)	BLOODWORKS		
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CPC分类号	G01N33/5005 G01N33/53 C12Q1/6883 G01N33/80 G01N2800/7066 G01N2333/82 G01N2333/90209 C12Q2600/106 C12Q2600/156 C12Q2600/158 G01N33/48		
代理人(译)	NGUYEN , THU		
优先权	PCT/US2015/029552 2015-05-06 WO 61/989440 2014-05-06 US		
其他公开文献	US20190204298A1		
外部链接	Espacenet		

摘要(译)

提供了通过测量红细胞样品中一种或多种标志物的水平来确定红细胞的输血后存活或毒性以及红细胞单位是否适合输血的组合物和方法。

