

REDUCING INTERSAMPLE ANALYTE VARIABILITY IN COMPLEX BIOLOGICAL MATRICES

FIELD

5 The present disclosure relates generally to reducing variability of inter-sample analyte measurements from a biological sample. In some embodiments, the present disclosure relates to compositions and methods for reducing the variability in the inter-sample levels of one or more proteins as measured by a proteomic assay.

BACKGROUND

10 Inter-sample variability of analyte measurement in biological samples is a problem for biomarker discovery, metabolic analyses, gene expression analysis, protein pathway analysis, and diagnostic and prognostic tools, particularly when the outcome relies on quantitative biological signals that differ by a relatively small magnitude. Biological sample types that
15 exhibit high between-sample variability are a primary challenge of working with these sample types. Reducing such variability would provide for more consistent and meaningful datasets for experimental and clinical applications.

 Therefore, there continues to be a need for alternative compositions and methods for reducing inter-sample analyte measurement variability in biological matrices. The present
20 disclosure meets such needs by providing novel compositions and methods for normalizing biological signals in complex matrices, which reduce, minimize, or remove such variability.

SUMMARY

25 Provided herein are buffer-exchanged biological samples and methods of preparing biological samples for detecting a protein comprising buffer exchange. In some examples, there is reduced inter-sample variability of analyte levels measured in test samples comprising or generated from the buffer-exchanged biological samples relative to samples comprising or generated from non-buffer-exchanged biological samples. In some examples, there is reduced inter-sample variability of analyte levels in test samples generated using the methods comprising
30 buffer exchange relative to methods that do not comprise buffer exchange. In some examples, the linear range of measurement for analytes in the test samples comprising or generated from buffer-exchanged biological samples is greater than the linear range of measurement for analytes in test samples comprising or generated from non-buffer-exchanged biological samples. In some examples, the analyte is a protein, such as a target protein. In some examples, the biological
35 sample is a urine sample. In some examples, the total

protein concentration in the test sample is from 2 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$. In some examples, the total protein concentration in the test sample is from 2 $\mu\text{g/mL}$ to 60 $\mu\text{g/mL}$. In some such examples, the total protein concentration of the test sample or of the biological sample is adjusted to the range of from about 2 $\mu\text{g/mL}$ to about 60 $\mu\text{g/mL}$ following determination that the total protein concentration is not in the range. In some examples, the total protein concentration of the test sample is in the range of from about 2 $\mu\text{g/mL}$ to about 60 $\mu\text{g/mL}$ without an adjustment of total protein concentration. In some such examples, the total protein concentration is measured using a fluorescence readout assay. In some examples, the total protein concentration in the test sample is from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$. In some such examples, the total protein concentration of the test sample is adjusted to the range of from about 70 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$ following determination that the total protein concentration is not in the range. In some examples, the total protein concentration of the test sample is in the range of from about 70 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$ without an adjustment of total protein concentration. In some such examples, the total protein concentration is measured using a BCA assay. In some examples, the total protein concentration of each of a plurality of test samples or of a plurality of biological samples is adjusted to be the same.

In one aspect, the present disclosure refers to a method for preparing a plurality of biological samples for detecting a protein comprising:

- generating a plurality of test samples by performing a buffer exchange on the plurality of biological samples using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant, and
- generating a plurality of adjusted test samples by adjusting the total protein concentrations of a plurality of test samples, wherein the total protein concentration of each adjusted test sample is about the same;

wherein the method does not comprise concentrating the total protein of the plurality of biological samples prior to performing the buffer exchange.

In one aspect, the present disclosure refers to a method for preparing a biological sample for detecting a protein comprising:

- generating a test sample by performing a buffer exchange on the biological sample using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant; and
- generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from about 70 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$.

In one aspect, the present disclosure refers to a method for preparing a biological sample for detecting a protein comprising:

generating a test sample by performing a buffer exchange on the biological sample using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic
5 surfactant;

determining the total protein concentration of the test sample; and

generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from about 70 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$.

10 In one aspect, the present disclosure refers to a method of preparing a biological sample for detecting a protein comprising:

generating a test sample by performing a buffer exchange on the biological sample using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant;

15 determining the protein concentration of the test sample; and

if the total protein concentration of the test sample is not in the range of from about 70 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$, generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from about 70 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$.

20 In one aspect, the present disclosure refers to a test sample comprising a buffer-exchanged biological sample, wherein the test sample has a total protein concentration from about 70 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$, and wherein the buffer-exchanged biological sample comprises a buffering agent, one or more salts, a chelating agent and a nonionic surfactant.

25 In one aspect, the present disclosure refers to a method for detecting a target protein in a test sample comprising:

a) contacting the test sample with at least one capture reagent, wherein the at least one capture reagent is capable of binding to the target protein to form a complex;

b) incubating the test sample with the at least one capture reagent under conditions that allow for the complex to form; and

30 c) determining the level of the target protein in the test sample by measuring the level of the at least one capture reagent, the complex, or the protein; wherein the level of the at least one capture reagent or the complex is a surrogate for the level of the target protein;

wherein, the test sample is generated by performing a buffer exchange of a biological sample with a formulation comprising a buffering agent, a salt, a chelating agent and a nonionic surfactant; and

wherein, the total protein concentration of the test sample is about 70 $\mu\text{g/mL}$ to about less than 100 $\mu\text{g/mL}$

In one aspect, the present disclosure refers to a method for preparing a biological sample for detecting a protein comprising:

generating a test sample by performing a buffer exchange on the biological sample using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant; and

determining the total protein concentration of the biological sample or of the test sample; and

adjusting the total protein concentration of the biological sample or of the test sample, wherein the method does not comprise concentrating the total protein of the biological sample prior to performing the buffer exchange,

optionally wherein (i) the total protein concentration is adjusted before performing the buffer exchange or (ii) the total protein concentration is adjusted after performing the buffer exchange.

Certain examples of embodiments provided herein are listed below.

Embodiment 1. A method for preparing a biological sample for detecting a protein comprising:

a) generating a test sample by performing a buffer exchange on the biological sample using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant; and

b) generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from about 2 $\mu\text{g/mL}$ to about 60 $\mu\text{g/mL}$.

Embodiment 2. The method of embodiment 1 comprising determining the protein concentration of the test sample.

Embodiment 3. A method for preparing a biological sample for detecting a protein comprising:

a) generating a test sample by performing a buffer exchange on the biological sample using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant;

b) determining the total protein concentration of the test sample; and

which the total protein adjustment (and measurement if applicable) is performed before or after buffer exchange, unless the language clearly requires otherwise.

Certain methods

5 In some examples, methods disclosed herein facilitate standardization of total protein concentrations in biological samples or test samples while limiting inter-sample variability in analyte measurements and expanding the linear ranges of the analytes in the samples. In some examples, the methods comprise performing buffer exchange. In some such examples, the buffer exchange allows both adjustment of the total protein concentration of the biological sample and control of salt concentrations of the biological sample. Variation in salt concentrations in a biological sample may cause significant variation in measurement of analytes within the sample. The ability to adjust the total protein concentration and salt concentrations limits such inter-sample variability. In some examples, the methods herein expand the linear range of analyte concentrations, allowing more reliable measurement of analytes compared to measurement of analytes in biological samples or test samples that are not buffer-exchanged.

15 In some examples, methods herein comprise determining total protein concentration of the biological sample. In some such examples, the total protein concentration of the biological sample is determined prior to buffer exchange of the biological sample. In some examples, methods herein comprise determining total protein concentration of the test sample. In some such examples, the total protein concentration of the test sample is determined after buffer exchange of the biological sample. In some examples, the total protein concentration is determined using a fluorescence readout assay. In some examples, the total protein concentration is determined using a BCA assay. In some examples, the total protein concentration of the test sample is determined to be a certain value or within a certain range and is not adjusted. In some examples, the total protein concentration is determined to not be a certain value or is outside a certain range and is adjusted. In some examples, the range is not the same for every type of assay used to determine the total protein concentration. In some examples, methods herein comprise generating a plurality of test samples from a plurality of biological samples, wherein the total protein concentration of some test samples or of some biological samples is adjusted and the total protein concentration of other test samples or of other biological samples is not adjusted.

30 In some examples, the methods herein do not comprise ultrafiltration or concentration of the total protein of a biological sample. In some such examples, buffer exchange is performed using gel filtration chromatography.

Certain test samples

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In some examples, buffer-exchanged test samples disclosed herein comprise a total protein concentration less than about 60 µg/mL, between 60 and 100 µg/mL, or between 70 and 100 µg/mL. Some such examples comprise HEPES, NaCl, KCl, MgCl₂, Tween, and EDTA.

Inter-sample variability in biological samples

- 5 Variability in total protein concentration from biological samples, such as random untimed ‘spot’ urine specimens, represents a challenge for proteomic analyses. The normal range for total protein in a spot urine sample is from about 1 to 14 mg/dL with from about 50 to 80 mg excreted per day when at rest. Urinary protein concentration tends to increase with age, exercise, and standing posture. In nephrotic syndromes total excretion of protein may exceed
- 10 3.5 grams per day.
- Variability in protein concentrations between urine specimens was reported by Thomas et al. (Thomas, C.E., Sexton W., Benson K., Sutphen R., and J. Koomen. (2010). Urine collection and processing for protein biomarker discovery and quantification. *Cancer Epidemiol Biomarkers Prev* 19(4):953-959). In this study, all voids were collected from one individual over 10
- 15 consecutive days. The total protein concentration was determined for each and was found to vary by over 100-fold across specimens. The coefficient of variation (CV) was lowest for 24-hour pools (39%) and for first morning voids (41%). The greatest CV was observed for the second void of the day (54%) and for all spot collections (61%).
- Composition and concentrative properties of human urine. Prepared for the National
- 20 Aeronautics and Space Administration by McDonnell Douglas Astronautics Company Huntington Beach, CA) showed that 68 small molecule constituents make up 99% of the dissolved solids in urine. These 68 molecules add up to approximately 36.8 grams per liter in a typical urine sample. The top 10 molecules, ranked from highest to lowest by mass per unit volume, are urea, chloride, sodium, potassium, creatinine, inorganic sulfur, hippuric acid,
- 25 phosphorus, citric acid, and glucuronic acid. However, between urine specimens, the absolute concentration of any of these 68 components varies considerably. Table 1 below summarizes some of the observed differences among several urine samples.

Table 1. Variability in the Characteristics and Composition of Urine Samples

Characteristic/Composition	Urine
pH	4.5-8.0
Sodium	117-840 mg/dL
Chloride	187-840 mg/dL
Magnesium	2-20.5 mg/dL

Calcium	3-39 mg/dL
Phosphorus	47-107 mg/dL
Potassium	75-261 mg/dL

The variability in urine total protein concentrations from inter-subject comparisons and inter-sample comparison from the same subject, in addition to the variability in pH, salt concentration and other components make urine a challenging matrix for proteomic measurements in biomarker discovery and diagnostics because the measurement of protein levels may be impacted by the presence, specifically the concentration of these components (e.g., salts and other solids) in urine. Effectively, a sample with different levels of these components yet the same concentration of protein, assayed for protein levels, will give different protein measurements based on the levels of the different components of the sample.

The foregoing and other objects, features, and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

DESCRIPTION OF THE FIGURES

Figures 1A and 1B show the change in analyte measurements as a function of NaCl concentration. Both Figures 1A and 1B show the same data presented differently. Figure 1A shows on the x-axis the additional NaCl (mM) concentration to the SB17 buffer formulations, which starts with 102 mM NaCl, and the y-axis is the Log2-fold change in RFU (relative fluorescent units), which correlates with the amount of analyte present in the sample, relative to the control buffer condition (SB17 buffer). Figure 1B shows on the x-axis the additional NaCl (mM) concentration to the SB17 buffer, which starts with 102 mM NaCl, and the y-axis is the percent of analytes that are increased or decreased as a function of NaCl concentration.

Figures 2A and 2B show the change in analyte measurements as a function of MgCl₂ concentration. Both figures 2A and 2B are the same data presented differently. Figure 2A shows on the x-axis the additional MgCl₂ (mM) concentration to the SB17 buffer formulations, which starts with 5 mM MgCl₂, and the y-axis is the Log2-fold change in RFU (relative fluorescent units), which correlates with the amount of analyte present in the sample, relative to the control buffer condition (SB17 buffer). Figure 2B shows on the x-axis the additional MgCl₂ (mM) concentration to the SB17 buffer, which starts with 5 mM MgCl₂, and the y-axis is the percent of analytes that are increased or decreased as a function of MgCl₂ concentration.