CONTROLLING INTERSAMPLE ANALYTE VARIABILITY IN COMPLEX BIOLOGICAL MATRICES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority of US Provisional Application No. 62/849,212, filed May 17, 2019, which is incorporated by reference in its entirety for any purpose.

FIELD

The present disclosure relates generally to controlling variability of inter-sample analyte measurements from a biological matrix. In some embodiments, the present disclosure relates to methods for controlling the levels of one or more proteins from a urine sample as measured by an aptamer based assay.

BACKGROUND

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 exhibit high between-sample variability are a primary challenge of working with these sample types. Controlling for 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 controlling inter-sample analyte measurement variability in biological matrices. The present 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

In one aspect, the present disclosure provides a method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) generating a model based on the levels of the analyte of the first dilution series;

d) selecting a reference value, wherein the reference value is selected from:

(i) a second dilution series reference value that is an analyte level at a specific dilution of the second dilution series;

(ii) a model reference value that is an analyte level at a specific dilution of the model; and

(iii) an arbitrary reference value that is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the second dilution series or the model;

e) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the second dilution series reference value to a model value, wherein the model value is an analyte level at a specific dilution of the model, wherein the second dilution series reference value and the model value are equivalent or substantially equivalent;

(ii) a horizontal translation (ΔX) of the model reference value to a second dilution series value, wherein the second dilution series value is an analyte level at a specific dilution of the second dilution series, wherein the model reference value and the second dilution series value are equivalent or substantially equivalent; and

(iii) horizontal translations of a second dilution series value (ΔX) and a model value (ΔY) to the arbitrary reference value, wherein the model value is an analyte level at a specific dilution of the model, wherein the second dilution series value is an analyte level at a specific dilution of the second dilution series, and wherein the second dilution series value, the model value and the arbitrary reference value are equivalent or substantially equivalent;

f) performing the at least one horizontal translation of the remaining values in (i) the second dilution series, (ii) the model, or (iii) the second dilution series and the model, wherein the horizontal translations of the remaining values results in a lined composite translation; and

g) fitting a function to the lined composite translation series thereby forming a composite dilution model.

In another aspect, the present disclosure provides a method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) generating a model based on the levels of the analyte of the first dilution series;

d) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the second dilution series to the model;

(ii) a horizontal translation (ΔX) of the model to the second dilution series; and

(iii) horizontal translations of the second dilution series (ΔX) and the model (ΔY) to an arbitrary reference value, wherein the arbitrary reference value is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the second dilution series or the model,

wherein the at least one horizontal translation results in a lined composite translation series; and

e) fitting a function to the lined composite translation series thereby forming a composite dilution model.

In yet another aspect, the present disclosure provides a method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) selecting a reference value, wherein the reference value is selected from:

(i) a second dilution series reference value that is an analyte level at a specific dilution of the second dilution series; and

(ii) an arbitrary reference value that is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the first dilution series or the second dilution series;

d) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the second dilution series reference value to a first dilution series value, wherein the first dilution series value is an analyte level at a specific dilution of the first dilution series, wherein the second dilution series reference value and the first dilution series value are equivalent or substantially equivalent; and

(ii) horizontal translations of the first dilution series (ΔX) and the second dilution series (ΔY) to the arbitrary reference value;

wherein the at least one horizontal translation results in a lined composite translation series; and

e) fitting a function to the lined composite translation series thereby forming a composite dilution model.

In one aspect, the present disclosure provides a method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the second dilution series to the first dilution series; and

(ii) horizontal translations of the first dilution series (ΔX) and the second dilution series (ΔY) to an arbitrary reference value, wherein the arbitrary reference value is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the first dilution series or the second dilution series;

wherein the at least one horizontal translation results in a lined composite translation series; and

d) fitting a function to the lined composite translation series thereby forming a composite dilution model.

In another aspect, the present disclosure provides a method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) generating a first model based on the levels of the analyte of the first dilution series and a second model based on the levels of the analyte of the second dilution series;

d) selecting a reference value, wherein the reference value is selected from:

(i) a first model reference value that is an analyte level at a specific dilution of the first model; and

(ii) an arbitrary reference value that is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the first model or the second model;

d) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the first model to the second model; and

(ii) horizontal translations of the first model (ΔX) and the second model (ΔY) to the arbitrary reference value; and

wherein the horizontal translation result in a lined composite translation series; and

e) fitting a function to the lined composite translation series thereby forming a composite dilution model.

In yet another aspect, the present disclosure provides a method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) generating a first model based on the levels of the analyte of the first dilution series and a second model based on the levels of the analyte of the second dilution series;

d) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the first model to the second model; and

(ii) horizontal translations of the first model (ΔX) and the second model (ΔY) to an arbitrary reference value, wherein the analyte level at that specific dilution is not found in the first model or the second model; and

wherein the horizontal translation result in a lined composite translation series; and

e) fitting a function to the lined composite translation series thereby forming a composite dilution model.

In one aspect, the present disclosure provides a method for determining a relative dilution of a biological test sample from a subject, the method comprising horizontally translating the level of at least one analyte from a biological test sample to a composite dilution model developed for the at least one analyte, thereby determining the relative dilution of the biological test sample from the subject, wherein the composite dilution model was developed using the method as disclosed herein.

Example 1. A method for generating a composite dilution model, the method comprising:

a) determining the level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

LISTING OF CLAIMS

1. A method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) generating a model based on the levels of the analyte of the first dilution series;

d) selecting a reference value, wherein the reference value is selected from:

(i) a second dilution series reference value that is an analyte level at a specific dilution of the second dilution series;

(ii) a model reference value that is an analyte level at a specific dilution of the model; and

(iii) an arbitrary reference value that is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the second dilution series or the model;

e) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the second dilution series reference value to a model value, wherein the model value is an analyte level at a specific dilution of the model, wherein the second dilution series reference value and the model value are equivalent or substantially equivalent;

(ii) a horizontal translation (ΔX) of the model reference value to a second dilution series value, wherein the second dilution series value is an analyte level at a specific dilution of the second dilution series, wherein the model reference value and the second dilution series value are equivalent or substantially equivalent; and

(iii) horizontal translations of a second dilution series value (ΔX) and a model value (ΔY) to the arbitrary reference value, wherein the model value is an analyte level at a specific dilution of the model, wherein the second dilution series value is an analyte level at a specific dilution of the second dilution series, and wherein the second dilution

series value, the model value and the arbitrary reference value are equivalent or substantially equivalent;

f) performing the at least one horizontal translation of the remaining values in (i) the second dilution series, (ii) the model, or (iii) the second dilution series and the model, wherein the horizontal translations of the remaining values results in a lined composite translation; and

g) fitting a function to the lined composite translation series thereby forming a composite dilution model.

2. The method of claim 1, wherein the method comprises:

d) selecting a second dilution series reference value that is an analyte level at a specific dilution of the second dilution series;

e) performing a horizontal translation (ΔX) of the second dilution series reference value to a model value, wherein the model value is an analyte level at a specific dilution of the model, wherein the second dilution series reference value and the model value are equivalent or substantially equivalent; and

f) performing the horizontal translation of the remaining values in the second dilution series, wherein the horizontal translation of the remaining values results in a lined composite translation.

3. The method of claim 1, wherein the method comprises:

d) selecting a model reference value that is an analyte level at a specific dilution of the model;

e) performing a horizontal translation (ΔX) of the model reference value to a second dilution series value, wherein the second dilution series value is an analyte level at a specific dilution of the second dilution series, wherein the model reference value and the second dilution series value are equivalent or substantially equivalent; and

f) performing the horizontal translation of the remaining values in the model, wherein the horizontal translation of the remaining values results in a lined composite translation.

4. The method of claim 1, wherein the method comprises:

d) selecting an arbitrary reference value that is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the second dilution series or the model;

e) performing a horizontal translation of a second dilution series value (ΔX) and a model value (ΔY) to the arbitrary reference value, wherein the model value is an analyte level at a specific dilution of the model, wherein the second dilution series value is an analyte level at a specific dilution of the second dilution series, and wherein the second dilution series value, the model value and the arbitrary reference value are equivalent or substantially equivalent; and

f) performing the horizontal translations of the remaining values in the second dilution series and the model, wherein the horizontal translations of the remaining values results in a lined composite translation.

5. A method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) generating a model based on the levels of the analyte of the first dilution series;

d) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the second dilution series to the model;

(ii) a horizontal translation (ΔX) of the model to the second dilution series; and

(iii) horizontal translations of the second dilution series (ΔX) and the model (ΔY) to an arbitrary reference value, wherein the arbitrary reference value is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the second dilution series or the model,

wherein the at least one horizontal translation results in a lined composite translation series; and

e) fitting a function to the lined composite translation series thereby forming a composite dilution model.

6. The method of claim 5, wherein the method comprises performing a horizontal translation (ΔX) of the second dilution series to the model.

7. The method of claim 5, wherein the method comprises performing a horizontal translation (ΔX) of the model to the second dilution series.

8. The method of claim 5, wherein the method comprises performing a horizontal translation of the second dilution series (ΔX) and the model (ΔY) to an arbitrary reference value that is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the second dilution series or the model.

9. A method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) selecting a reference value, wherein the reference value is selected from:

(i) a second dilution series reference value that is an analyte level at a specific dilution of the second dilution series; and

(ii) an arbitrary reference value that is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the first dilution series or the second dilution series;

d) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the second dilution series reference value to a first dilution series value, wherein the first dilution series value is an analyte level at a specific dilution of the first dilution series, wherein the second dilution series reference value and the first dilution series value are equivalent or substantially equivalent; and

(ii) horizontal translations of the first dilution series (ΔX) and the second dilution series (ΔY) to the arbitrary reference value;

wherein the at least one horizontal translation results in a lined composite translation series; and

e) fitting a function to the lined composite translation series thereby forming a composite dilution model.

10. The method of claim 9, wherein the method comprises performing a horizontal translation (ΔX) of the second dilution series to the first dilution series.

11. The method of claim 9, wherein the method comprises horizontal translations of the first dilution series (ΔX) and the second dilution series (ΔY) to an arbitrary reference value, wherein the arbitrary reference value is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the first dilution series or the second dilution series.

12. A method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the second dilution series to the first dilution series; and

(ii) horizontal translations of the first dilution series (ΔX) and the second dilution series (ΔY) to an arbitrary reference value, wherein the arbitrary reference value is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the first dilution series or the second dilution series;

wherein the at least one horizontal translation results in a lined composite translation series; and

d) fitting a function to the lined composite translation series thereby forming a composite dilution model.

13. The method of claim 12, wherein the method comprises performing a horizontal translation (ΔX) of the second dilution series to the first dilution series.

14. The method of claim 12, wherein the method comprises horizontal translations of the first dilution series (ΔX) and the second dilution series (ΔY) to an arbitrary reference value,

wherein the arbitrary reference value is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the first dilution series or the second dilution series.

15. A method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) generating a first model based on the levels of the analyte of the first dilution series and a second model based on the levels of the analyte of the second dilution series;

d) selecting a reference value, wherein the reference value is selected from:

(i) a first model reference value that is an analyte level at a specific dilution of the first model; and

(ii) an arbitrary reference value that is an analyte level at a specific dilution, wherein the analyte level at that specific dilution is not found in the first model or the second model;

d) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the first model to the second model; and

(ii) horizontal translations of the first model (ΔX) and the second model (ΔY) to the arbitrary reference value; and

wherein the horizontal translation result in a lined composite translation series; and

e) fitting a function to the lined composite translation series thereby forming a composite dilution model.

16. The method of claim 15, wherein the method comprises performing a horizontal translation (ΔX) of the first model to the second model.

17. The method of claim 15, wherein the method comprises horizontal translations of the first model (ΔX) and the second model (ΔY) to the arbitrary reference value.

18. A method for generating a composite dilution model, the method comprising:

a) determining a level of an analyte in a first dilution series of a first biological sample comprising the analyte, wherein the first dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

b) determining a level of the analyte in a second dilution series of a second biological sample comprising the analyte, wherein the second dilution series comprises at least three (3) different dilutions, and the level of the analyte is determined in each of the at least three different dilutions;

c) generating a first model based on the levels of the analyte of the first dilution series and a second model based on the levels of the analyte of the second dilution series;

d) performing at least one horizontal translation, wherein the at least one horizontal translation is selected from:

(i) a horizontal translation (ΔX) of the first model to the second model; and

(ii) horizontal translations of the first model (ΔX) and the second model (ΔY) to an arbitrary reference value, wherein the analyte level at that specific dilution is not found in the first model or the second model; and

wherein the horizontal translation result in a lined composite translation series; and

e) fitting a function to the lined composite translation series thereby forming a composite dilution model.

19. The method of claim 18, wherein the method comprises performing a horizontal translation (ΔX) of the first model to the second model.

20. The method of claim 18, wherein the method comprises horizontal translations of the first model (ΔX) and the second model (ΔY) to an arbitrary reference value.

21. The method of any one of the preceding claims, wherein the level of the analyte is determined in each of at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 different dilutions in the first dilution series.

22. The method of any one of the preceding claims, wherein the level of the analyte is determined in each of at least 8 different dilutions.

23. The method of any one of the preceding claims, wherein the level of the analyte is determined in each of at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 different dilutions in the second dilution series.

24. The method of any one of the preceding claims, wherein the level of the analyte is determined in each of at least 8 different dilutions in the second dilution series.

25. The method of any one of the preceding claims, wherein each model is independently selected from a linear regression model, a LOESS curve fitting model, a non-linear regression model, a spline fit model, a mixed effects regression model, a fixed effects regression model, a generalized linear model, a matrix decomposition model, and a four parameter logistic regression (4PL) model,

26. The method of any one of the previous claims, wherein the level of the analyte is the relative amount of the analyte or the analyte concentration.

27. The method of any one of the preceding claims, wherein the selected reference value is within the linear range of the dilution series or model.

28. The method of claim 27, wherein the selected reference value is the center point of the linear range.

29. The method of any one of the preceding claims, wherein the first and second biological samples comprise urine or are derived from urine.

30. The method of any one of the preceding claims, wherein the first and second biological samples are collected from the same subject.

31. The method of any one of the preceding claims, wherein the first and second biological samples are collected from different subjects.

32. The method of claim 31, wherein the first biological sample is collected at a first time point and the second biological sample is collected at a second time point.

33. The method of claim 32, wherein the first time point and the second time point differ by at least 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 36 hours, 48 hours, 60 hours or 72 hours.

34. The method of any one of the preceding claims, wherein the level of the analyte is measured by an assay that uses an aptamer, antibody, mass spectrophotometer or a combination thereof.

35. The method of any one of preceding claims, wherein the dilution factor for each of the first dilution series and second dilution series is a constant dilution factor.

36. The method of any one of any one of the preceding claims, wherein the dilution factor for each of the first dilution series and the second dilutions series is at least a two-fold, a three-fold, a four-fold, a five-fold, a six-fold, a seven-fold, an eight-fold, a nine-fold, a ten-fold dilution.

37. The method of any one of claims 1 to 35, wherein the dilution factor for each of the first dilution series and the second dilutions series is an exponential or logarithmic dilution factor.

38. The method of any one of the preceding claims, wherein the first biological sample and the second biological sample are the same type of biological sample.

39. The method of any one of claims 1 to 35, wherein the first dilution series and the second dilution series are each at least a 5-point serial titration at at least a 1:2 titration factor.

40. The method of any one of the preceding claims, wherein the method further comprises horizontally translating the level of at least one analyte from a biological test sample to the composite dilution model for the at least one analyte, thereby determining the relative dilution of the biological test sample.

41. The method of claim 40, wherein the biological test sample and the first and second biological samples used to form the composite dilution model are the same sample type.

42. The method of claim 40 or claim 41, wherein the biological test sample and the first and second biological samples used to form the composite dilution model are urine samples or are derived from urine samples.

43. A method for determining a relative dilution of a biological test sample from a subject, the method comprising horizontally translating the level of at least one analyte from a biological test sample to a composite dilution model developed for the at least one analyte, thereby determining the relative dilution of the biological test sample from the subject, wherein the composite dilution model was developed using the method of any one of claims 1 to 42.

44. The method of any one of claims 40 to 43, comprising horizontally translating the level of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 50, at least 75, at least 100, at least 150, or at least 200 different analytes from the biological test sample to the respective composite dilution model developed for each of the different analytes to determine the relative dilution of the biological test sample for each of the different analytes, and using the relative dilution for each of the different analytes to determine the relative dilution of the biological test sample.

45. The method of claim 44, wherein the relative dilution of the biological test sample is derived from the central tendency of the relative dilutions of each of the different analytes.

46. The method of claim 44 or claim 45, wherein the relative dilution of the biological test sample is derived from the median, mean or mode of the relative dilutions of each of the different analytes.

47. The method of any one of claims 40 to 46, further comprising calculating the relative dilution of the biological test sample with the derived relative dilution factor.

48. The method of any one of the preceding claims, wherein each analyte is a target protein.