METHODS AND SYSTEMS FOR INSTRUMENT VALIDATION

BACKGROUND

[0001] Installation and calibration of laboratory instrumentation can be a time consuming and expensive process. In many cases, engineers from the instrument supplier must be on site to perform these processes. This cost is generally passed on to the user. In some cases, experienced users can successfully calibrate properly manufactured instruments using multi-step procedures. During such calibration, physical standards and well plates may be used in combination with manual procedures. Manual calibration processing and data inspection is error prone and may rely on ad hoc or subjective measures. While a final system verification step may provide resilience against accepting suboptimal calibrations, automation offers improved objectivity and uniformity during such activities.

[0002] After installation and after several uses, it is important to validate that the instrument is working properly. Often, a user will manually run a known assay to validate an instrument, such as an RNase P assay.

[0003] In a traditional RNase P assay example, a standard curve is generated from the Ct (cycle threshold) values obtained from a set of replicate standards (1,250, 2,500, 5,000, 10,000 and 20,000 copies). The standard curve is then used to determine the copy number for two sets of unknown templates (5,000 and 10,000 replicate populations). The instrument is validated if it can demonstrate the ability to distinguish between 5,000 and 10,000 genomic equivalents with a 99.7% confidence level for a subsequent sample run in a single well.

SUMMARY

[0004] In one exemplary embodiment, a method for validating a PCR instrument is provided. The method includes receiving amplification data from a validation plate to generate a plurality of amplification curves. The validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region. The method further includes determining a set of fluorescence thresholds by evenly dividing by a predetermined number a range of fluorescence values within the exponential regions of the plurality of amplification curves and determining, for each fluorescence threshold of the set, a

first set of cycle threshold (C_t) values of amplification curves generated from the samples of the first quantity and a second set of C_t values of amplification curves generated from the samples of the second quantity. The method includes calculating if the first and second quantities are sufficiently distinguishable based on C_t values at each of the plurality of fluorescence thresholds.

[0005] In another exemplary embodiment, a computer-readable storage medium encoded with processor-executable instructions for validating a PCR instrument is provided. The instructions comprise instructions for receiving amplification data from a validation plate to generate a plurality of amplification curves. The validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region. The instructions further comprise instructions for determining a set of fluorescence thresholds by evenly dividing by a predetermined number a range of fluorescence values within the exponential regions of the plurality of amplification curves and determining, for each fluorescence threshold of the set, a first set of cycle threshold (C_t) values of amplification curves generated from the samples of the first quantity and a second set of C_t values of amplification curves generated from the samples of the second quantity. The instructions comprise instructions for calculating if the first and second quantities are sufficiently distinguishable based on C_t values at each of the plurality of fluorescence thresholds.

[0006] In another exemplary embodiment, a system for validating a PCR instrument is provided. The system includes a processor and a memory configured to store processor-executable instructions. The instructions include instructions for receiving amplification data from a validation plate to generate a plurality of amplification curves. The validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region. The instructions further include instructions for determining a set of fluorescence thresholds by evenly dividing by a predetermined number a range of fluorescence values within the exponential regions of the plurality of amplification curves and determining, for each fluorescence threshold of the set, a first set of cycle threshold (C_t) values of amplification curves generated from the samples of the first quantity and a second set of C_t values of amplification curves generated from the samples of the second quantity. The instructions include instructions for calculating if the first and second quantities are sufficiently distinguishable based on C_t values at each of the plurality of fluorescence thresholds.

[0007] In another exemplary embodiment, a system for validating a PCR instrument is provided. The system includes a PCR instrument interface configured to receive amplification data from a validation plate to generate a plurality of amplification curves. The validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region. The system further includes a (cycle threshold) C_t calculator configured to: determine a set of fluorescence thresholds by evenly dividing by a predetermined number a range of fluorescence values within the exponential regions of the plurality of amplification curves, and determine, for each fluorescence threshold of the set, a first set of C_t values of amplification curves generated from the samples of the first quantity and a second set of C_t values of amplification curves generated from the samples of the second quantity. The system includes a C_t database configured to store the first and second set of C_t values for each fluorescence threshold of the set. The system further includes a validator configured to calculate if the first and second quantities are sufficiently distinguishable based on C_t values at each of the plurality of fluorescence thresholds.

DESCRIPTION OF THE FIGURES

[0008] FIG. 1 illustrates an exemplary method for validating an instrument according to various embodiments described herein;

[0009] FIG. 2 illustrates another exemplary method for validation an instrument according to various embodiments described herein;

[0010] FIG. 3 illustrates determining a plurality of fluorescence thresholds from amplification data according to various embodiments described herein;

[0011] FIG. 4 illustrates a system for validation of an instrument according to various embodiments described herein;

[0012] FIG. 5 illustrates an exemplary computing system for implementing various embodiments described herein;

[0013] FIG. 6 illustrates an exemplary distributed network system according to various embodiments described herein;

CLAIMS

What is claimed is:

1. A method for validating a PCR instrument, the method comprising:

receiving amplification data from a validation plate to generate a plurality of amplification curves, wherein the validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region;

determining a set of fluorescence thresholds by evenly dividing by a predetermined number a range of fluorescence values within the exponential regions of the plurality of amplification curves;

determining, for each fluorescence threshold of the set, a first set of cycle threshold (C_t) values of amplification curves generated from the samples of the first quantity and a second set of C_t values of amplification curves generated from the samples of the second quantity; and

calculating if the first and second quantities are sufficiently distinguishable based on C_t values at each of the plurality of fluorescence thresholds.

2. The method of claim 1, further comprising:

displaying an indication the instrument is validated if the first and second quantities are sufficiently distinguishable.

3. The method of claim 1, wherein the calculating if the first and second quantities are sufficiently distinguishable includes determining a mean (μ) and a standard deviation (σ) of the first and second set of C_t values.

4. The method of claim 3, wherein the first and second quantities are sufficiently distinguishable if the following equation results in a positive number:

$$\left(\left(\mu C_{tquant1} - 3\sigma C_{tquant1}\right) - \left(\mu C_{tquant2} + 3\sigma C_{tquant2}\right)\right).$$

5. The method of claim 1, wherein the sample is a RNase P gene.

6. The method of claim 1, wherein the first and second quantity are different.

7. The method of claim 1, wherein there is a 2-fold difference between the second quantity and the first quantity.

8. The method of claim 4, wherein the first and second quantities are sufficiently distinguishable if the equation results in a positive number for at least one fluorescence threshold.

9. The method of claim 1, further comprising:

discarding a predetermined number of outliers from the first and second set of C_t values.

10. The method of claim 1, wherein the set of fluorescence thresholds includes 90 fluorescence thresholds.

11. A computer-readable storage medium encoded with processor-executable instructions for validating a PCR instrument, the instructions comprising instructions for:

receiving amplification data from a validation plate to generate a plurality of amplification curves, wherein the validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region;

determining a set of fluorescence thresholds by evenly dividing by a predetermined number a range of fluorescence values within the exponential regions of the plurality of amplification curves;

determining, for each fluorescence threshold of the set, a first set of cycle threshold (C_t) values of amplification curves generated from the samples of the first quantity and a second set of C_t values of amplification curves generated from the samples of the second quantity; and

calculating if the first and second quantities are sufficiently distinguishable based on C_t values at each of the plurality of fluorescence thresholds.

12. The computer-readable storage medium of claim 11, further comprising instructions for:

displaying an indication the instrument is validated if the first and second quantities are sufficiently distinguishable.

13. The computer-readable storage medium of claim 11, wherein the instruction for calculating if the first and second quantities are sufficiently distinguishable includes determining a mean (μ) and a standard deviation (σ) of the first and second set of C_t values.

14. The computer-readable storage medium of claim 13, wherein the first and second quantities are sufficiently distinguishable if the following equation results in a positive number:

$$\left(\left(\mu C_{tquant1} - 3\sigma C_{tquant1}\right) - \left(\mu C_{tquant2} + 3\sigma C_{tquant2}\right)\right).$$

15. The computer-readable storage medium of claim 11, wherein the sample is a RNase P gene.

16. The computer-readable storage medium of claim 11, wherein the first and second quantity are different.

17. The computer-readable storage medium of claim 11, wherein there is a 2-fold difference between the second quantity and the first quantity.

18. The computer-readable storage medium of claim 14, wherein the first and second quantities are sufficiently distinguishable if the equation results in a positive number for at least one fluorescence threshold.

19. The computer-readable storage medium of claim 11, further comprising instructions for:

discarding a predetermined number of outliers from the first and second set of C_t values.

20. The computer-readable storage medium of claim 11, wherein the set of fluorescence thresholds includes 90 fluorescence thresholds.

21. A system for validating a PCR instrument, the system comprising:

a processor; and

a memory configured to store processor-executable instructions for:

receiving amplification data from a validation plate to generate a plurality of amplification curves, wherein the validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region;

determining a set of fluorescence thresholds by evenly dividing by a predetermined number a range of fluorescence values within the exponential regions of the plurality of amplification curves;

determining, for each fluorescence threshold of the set, a first set of cycle threshold (C_t) values of amplification curves generated from the samples of the first

quantity and a second set of C_t values of amplification curves generated from the samples of the second quantity; and

calculating if the first and second quantities are sufficiently distinguishable based on C_t values at each of the plurality of fluorescence thresholds.

22. The system of claim 21, the memory further comprises instructions for:

displaying an indication the instrument is validated if the first and second quantities are sufficiently distinguishable.

23. The system of claim 21, wherein the instruction for calculating if the first and second quantities are sufficiently distinguishable includes determining a mean (μ) and a standard deviation (σ) of the first and second set of C_t values.

24. The system of claim 23, wherein the first and second quantities are sufficiently distinguishable if the following equation results in a positive number:

$$\left(\left(\mu C_{tquant1} - 3\sigma C_{tquant1}\right) - \left(\mu C_{tquant2} + 3\sigma C_{tquant2}\right)\right).$$

25. The system of claim 21, wherein the sample is a RNase P gene.

26. The system of claim 21, wherein the first and second quantity are different.

27. The system of claim 21, wherein there is a 2-fold difference between the second quantity and the first quantity.

28. The system of claim 24, wherein the first and second quantities are sufficiently distinguishable if the equation results in a positive number for at least one fluorescence threshold.

29. The system of claim 21, wherein the memory further comprises instructions for:

discarding a predetermined number of outliers from the first and second set of C_t values.

30. The system of claim 21, wherein the set of fluorescence thresholds includes 90 fluorescence thresholds.

31. A system for validating a PCR instrument, the system comprising:

a PCR instrument interface configured to receive amplification data from a validation plate to generate a plurality of amplification curves, wherein the validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region;

a (cycle threshold) C_t calculator configured to:

determine a set of fluorescence thresholds by evenly dividing by a predetermined number a range of fluorescence values within the exponential regions of the plurality of amplification curves, and

determine, for each fluorescence threshold of the set, a first set of C_t values of amplification curves generated from the samples of the first quantity and a second set of C_t values of amplification curves generated from the samples of the second quantity;

a C_t database configured to store the first and second set of C_t values for each fluorescence threshold of the set; and

a validator configured to calculate if the first and second quantities are sufficiently distinguishable based on C_t values at each of the plurality of fluorescence thresholds.

32. The system of claim 31, further comprising:

a display engine configured to display an indication the instrument is validated if the first and second quantities are sufficiently distinguishable.

33. The system of claim 31, wherein the validator is further configured to, for calculating if the first and second quantities are sufficiently distinguishable, determine a mean (μ) and a standard deviation (σ) of the first and second set of C_t values.

34. The system of claim 33, wherein the validator determines that first and second quantities are sufficiently distinguishable if the following equation results in a positive number:

$$\left(\left(\mu C_{tquant1} - 3\sigma C_{tquant1}\right) - \left(\mu C_{tquant2} + 3\sigma C_{tquant2}\right)\right).$$

35. The system of claim 31, wherein the sample is a RNase P gene.

36. The system of claim 31, wherein the first and second quantity are different.

37. The system of claim 31, wherein there is a 2-fold difference between the second quantity and the first quantity.