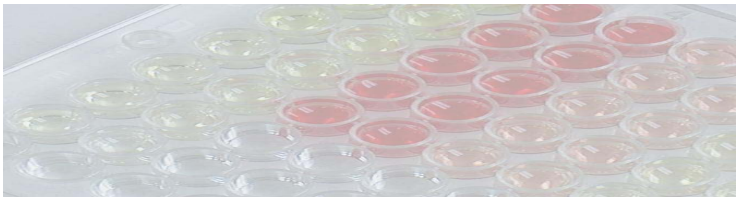
StatLIA® from Brendan



StatLIA® SmartQC™ Statistical Assay Analysis: Take the guesswork out of assay performance and result analysis.

- Bioassays
- Immunoassays
- Quantitative Tests
- Qualitative Tests
- Multiplexed Assays
- Parallelism
- Immunogenicity

Traditional QC methods do not allow one to assess assay performance in terms of concentration measurement precision.

StatLIA's SmartQC™ Statistical Assay Analysis provides at-a-glance assay performance evaluation, troubleshooting and result reliability.

The accuracy of a result from an immunoassay or bioassay is dependent on the assay performance quality. Therefore, a set of tools and metrics is required that allow one to objectively evaluate the performance of that assay. Although remarkable improvements in immunoassay and bioassay sensitivity, throughput and automation have been made in recent years, advances in the assay quality control and quality assurance methods have not progressed proportionately. The following examples demonstrate obvious flaws in existing methods routinely applied to assay QC.

Figure 1A shows a typical dose/response curve. Visual inspection of this curve suggests that the computed curve fits the standard data points well. While applying traditionally used assay quality control methods, using known concentrations of the analyte to interrogate the performance of the assay at distinct low, medium and high concentration regions of the dose/response curve, one would conclude that the computed dose/response relationship is able to accurately measure the concentration of samples from their measured response. However, this method does not allow one to assess the assay performance in terms of concentration measurement precision. In order to determine if quantitative measurements are reliable, some additional information regarding the quality of this assay is required. In Figure 1B, the same dose/response relationship is plotted together with the 95% confidence interval limits computed from a set of dose response curves from previously run reference assays. In addition, the concentration error profiles that StatLIA computes for every assay are also compared. This shows how the present assay has performed compared to its expected (normal) performance predicted based on historical data. One can easily determine that the present assay is significantly dif-

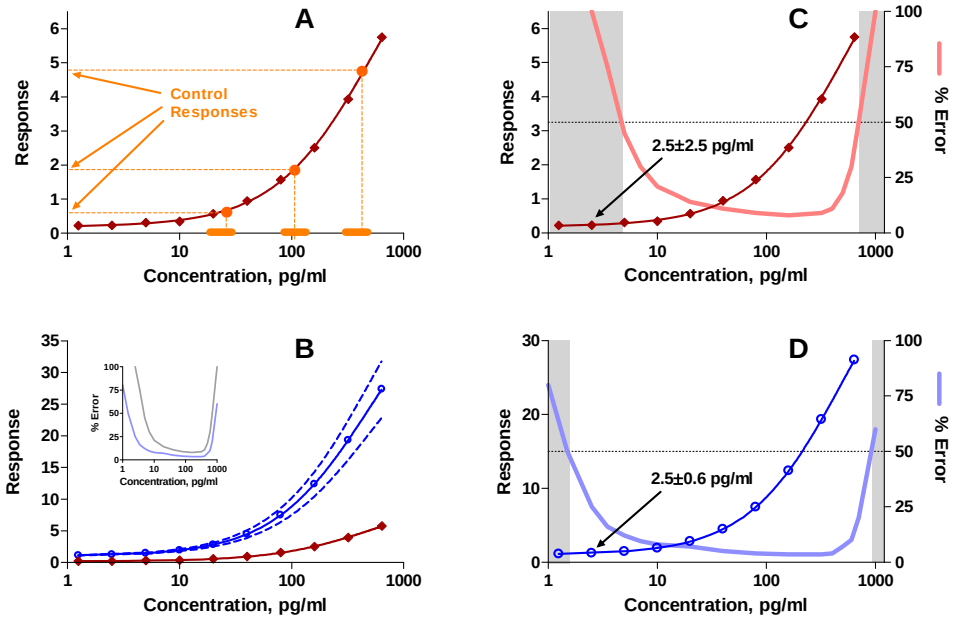


Figure 1– The dose/response curve from the present assay (A) can be assessed for quality using typical high, medium, and low concentration controls, but the most effective assessment compares the present assay to the performance of a historical pool of assays (B). While traditional QC method would not have flagged the present assay, StatLIA identifies its poor performance (C) greatly impacting the results as compared to a normally performing reference assay (D).

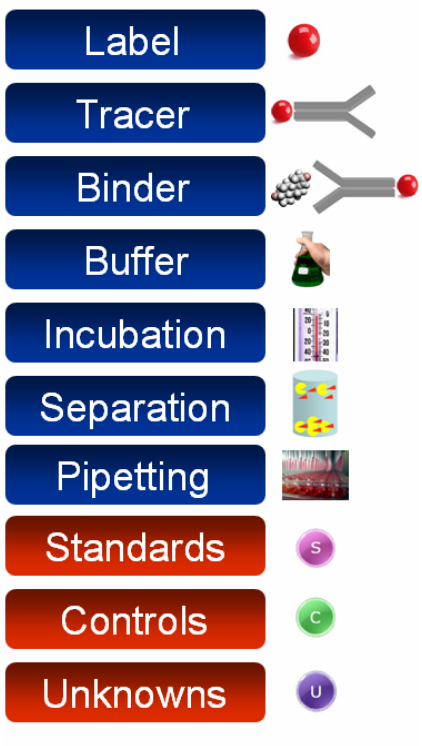


Figure 2– Components of an assay

ferent from what is historically observed. (In fact, in the present assay, incubation temperature was lower than that required by the assay protocol, because the incubator power was left off). As a result, the analyte concentration in all the samples can be determined in the present assay with much larger error, as is illustrated in Figures 1C and 1D, in which the dose/response curves and the concentration error profiles are overlaid. The reliability of the concentration measurement is severely affected at the edges of the concentration range (e.g. 2.5 ± 2.5 pg/ml in the present assay versus 2.5 ± 0.6 pg/ml for the same specimen in a normally performing assay from the reference set). Thus, being able to compare the performance of the present assay to a historical set of assays has allowed us to determine that this assay is not performing to its required capability, and the resulting measurements would have reduced accuracy and reliability.

Given the impact of assay performance on the accuracy and reliability of the result, effective assay quality control methods are required. This report will focus on the visual tools and metrics offered by the StatLIA® SmartQC™ Statistical Assay Analysis, that allow the end user to apply the StatLIA's powerful and effective QC tools as well as their own quality control criteria to gauge the performance of an assay based on objective measurements [1].

The ideal assay quality control system should:

- Effectively determine the reliability and acceptability of the assay
- Immediately identify errant results
- Determine the cause of the error
- Provide informative feedback on the assay's performance
- Assess the impact of the error on the results
- Definitively determine between a bad control specimen and a bad assay to avoid needlessly repeating a good assay
- Satisfy regulatory requirements

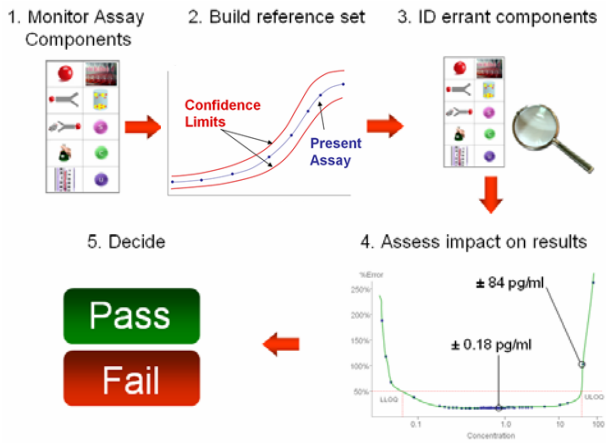
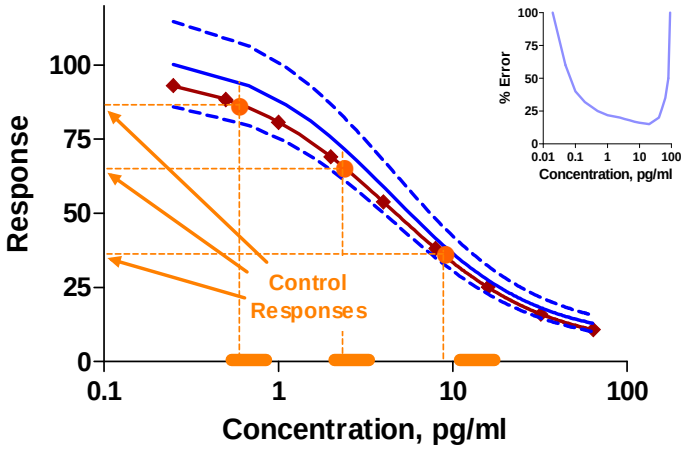


Figure 3– SmartQC™ workflow

The most effective way to assess assay performance is to compare that assay against a historical set of previously run assays with characterized intra-assay and inter-assay variance. (A discussion of intra-assay variance can be found in Brendan Technologies Tech Note 'Improved quantitative assay accuracy through StatLIA® TrueFit™ Data Reduction System… *Where the biology dictates the curve fit*'). This set of previously run assays also provides a benchmark against which to make improvements to the test method.

Figure 4– Traditional QC methods that rely on high, medium and low concentration controls would have failed the present assay simply because the observed response of the low concentration control is higher than expected. SmartQC™ evaluates the present assay based on how it performs relative to reference assays. This example demonstrates that the results computed from this standard curve (inset shows the concentration error profile) remain unaffected by this errant control.



The potential source of inter-assay variation can be broken down by analyzing the components of an immunoassay. These components (Figure 2) are monitored using a set of measured parameters so that a pool of assays performed under the same conditions can be compared (Figure 3). This pool of assays is referred to as the reference assay set. The intra-assay variance associated with the reference set assays is modeled using the weighting feature of StatLIA's TrueFit™ System. Confidence intervals can be determined for the reference assay by plotting the 99% confidence region surrounding the reference assay. The performance of assay components for the present assay are statistically compared with the assay components for the reference set. If an assay component is performing differently from the reference set, and if the difference is statistically significant, that assay and component are flagged. Further, the impact on the result by this errant component is assessed. StatLIA provides effective metrics and visual tools that allow objective decisions to be made regarding the impact of errant parameters on assay results.

Figure 4 illustrates how StatLIA provides metrics for the evaluation of assay quality control. In this example, a dose/response relationship is shown for an inhibitory assay where the present assay is being compared to a reference assay set. Traditional controls (High, Medium and Low) are represented on the graph. The Low control does not fall within the quality control criteria specific to this assay (shown as the red blocks on the x and y axes). By the QC protocol established for this assay using traditional methods, this laboratory would not be able to report measurements using this assay. However, comparing to the reference set through StatLIA, it is apparent that the present assay is performing adequately. Also, the assay quality control metrics used to determine the performance of the assay show that the assay is acceptable. The only metric StatLIA has flagged is the Control component of this assay. Since the standard curve responses, Fit Probability (FitProb™), limits of quantitation and other metrics are acceptable, it is easy to determine that the assay itself is good, whereas the control is bad. However, because traditional quality control methods do not provide sufficient information, this laboratory would have no choice but to repeat an otherwise acceptable assay.

StatLIA also provides visual tools for the evaluation of assay quality control. The precision profile (a graph that maps the error associated with a concentration estimate) is an extremely powerful feature,

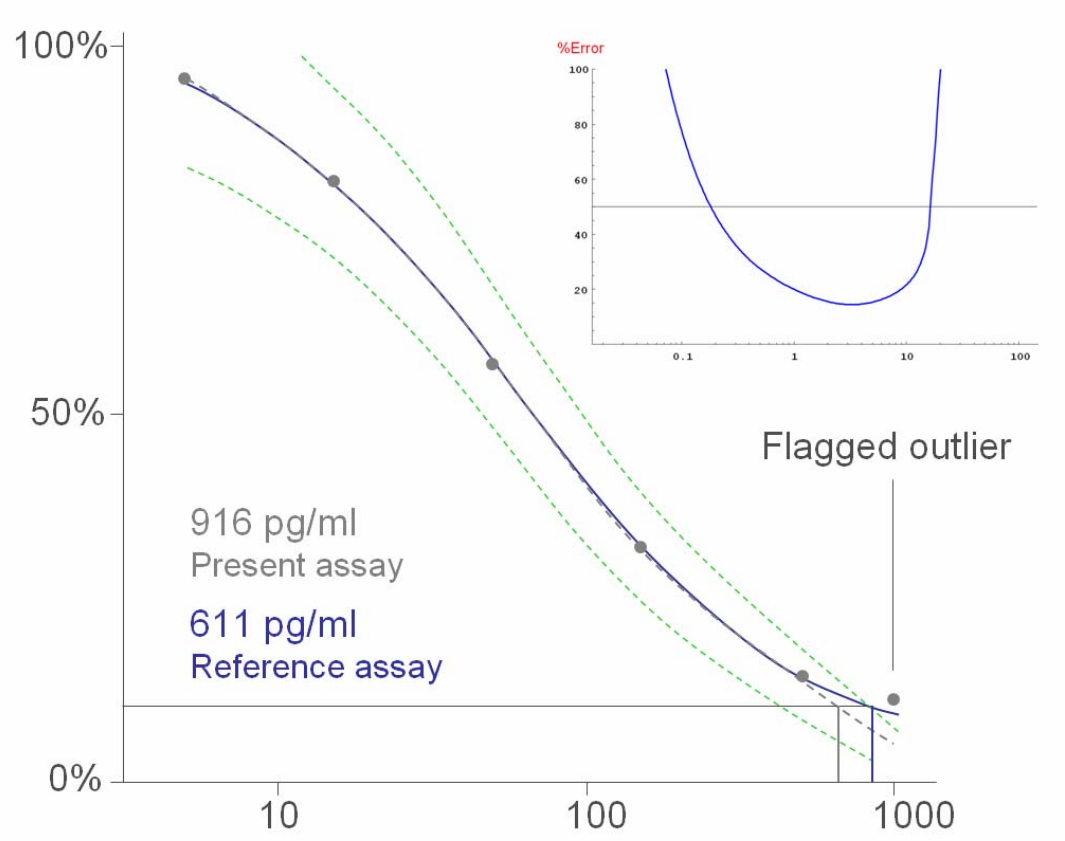


Figure 5– Inclusion of an outlier can have significant impact on the accuracy of the computed results.

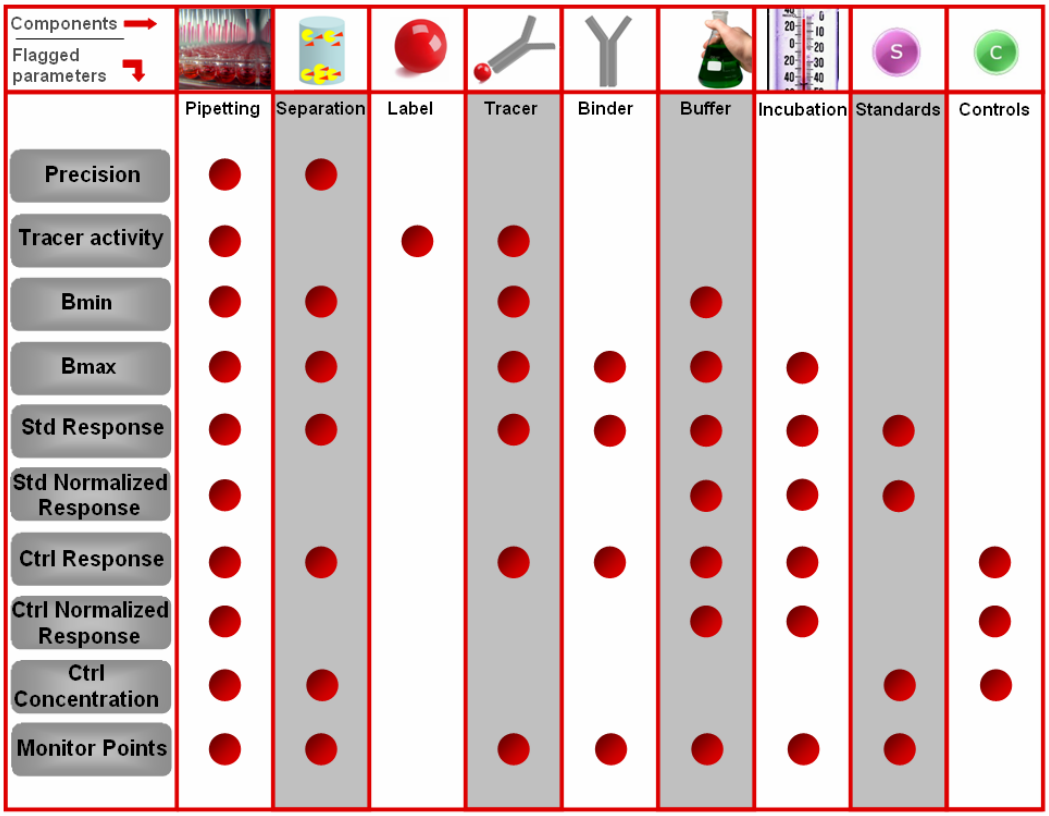
showing how the assay performs over the entire range of measured concentrations. For the assay in Figure 4, the precision profile shows less than 50% error over three logs of concentration. Hence, the Low Control error detected by StatLIA's quality control is an independent event that has not impacted the quality of the assay or the measurements based on that assay's results.

Figure 5 shows another example of how StatLIA's visual tools can be used to assess the quality of an assay's performance. In this example, a dose response for the present assay is compared to the reference assay set. StatLIA has flagged an errant standard with a response outside the confidence interval limits that has significant impact on the shape of the present assay curve. Traditional quality control methods might have identified this problem only if the Low Control would fall in this particular region of the curve. Comparing the present assay with the confidence interval, it is easy to identify the errant standard. The implication of not identifying this error is significant. Figure 5 illustrates a concentration estimate in this region using the present assay as well as the dose/response curve for the reference set. The affected curve shape would have shifted a measurement in this region from 611 pg/ml using the reference set to 916 pg/ml using the present assay.

The precision profile shown in Figure 5 clearly displays how the errant component identified for this assay impacts the assay measurements. Specifically, the measured concentration range that falls within the less than 50% error interval has been reduced to less than two logs. Thus, only a fraction of the concentration range can be used reliably.

StatLIA monitors several parameters associated with assay performance that can be used, in combination with flagged components, to troubleshoot errant assays. Fig-

Figure 6– SmartQC™ trouble-shooting guide



ure 6 shows a chart that illustrates how StatLIA troubleshoots assays based on matching the flagged parameters with the errant assay component(s). In the example assay illustrated in Figure 4, StatLIA flagged the “Control Adjusted Response”, “Control Normalized Response” and the ”Control Concentration” assay parameters, which can be found on the left column of Figure 6. The only assay component listed on the top row of Figure 6 that shows a combination of those flagged parameters, and no other parameters, is the “Controls” assay component.

StatLIA’s SmartQC™ Statistical Assay Analysis provides powerful, yet simple at-a-glance feedback on the performance of your assay and your results. This ensures that only results that meet your criteria for accuracy are reported. On the other hand, such a smart approach to the assessment of the assay QC metrics eliminates the need to discard many assays that would have otherwise failed traditional QC methods. SmartQC™ also provides you with troubleshooting tools that automatically identify errant assay components, which is critically important for test method design and development.

References

(1) Gottschalk, P.G.; Dunn, J.R. Determining the Error of Dose Estimates and Minimum and Maximum Acceptable Concentrations from Assay with Nonlinear Dose-Response Curves. *Computer Methods and Programs in Biomedicine*, **2005**; 80:204-215.

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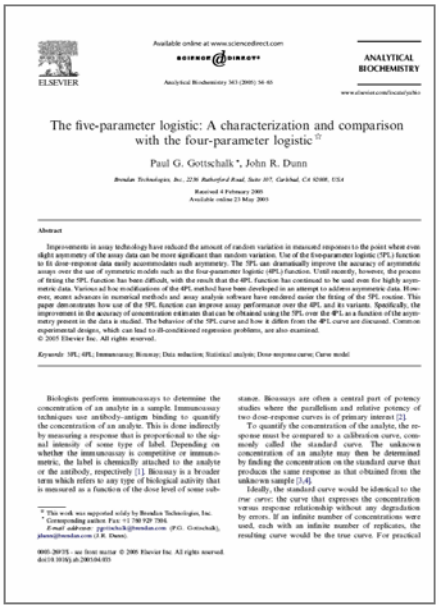
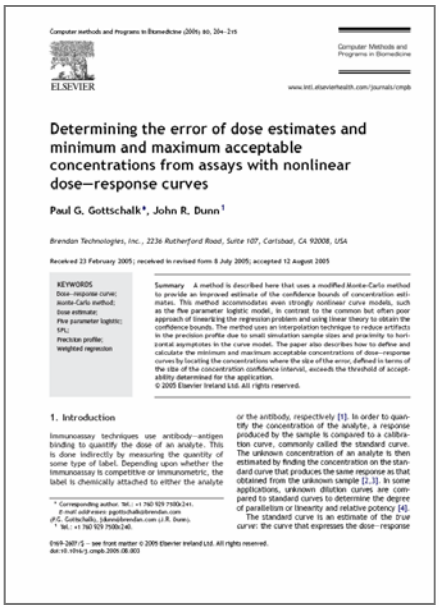


The five-parameter logistic: A characterization and comparison with the four-parameter logistic ☆

Paul G. Gottschalk *, John R. Dunn



Determining the error of dose estimates and minimum and maximum acceptable concentrations from assays with nonlinear dose–response curves

Paul G. Gottschalk*, John R. Dunn[1]

MEASURING PARALLELISM, LINEARITY, AND RELATIVE POTENCY IN BIOASSAY AND IMMUNOASSAY DATA

Paul G. Gottschalk and John R. Dunn

Brendan Technologies, Inc., Carlsbad, California, USA

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