

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.

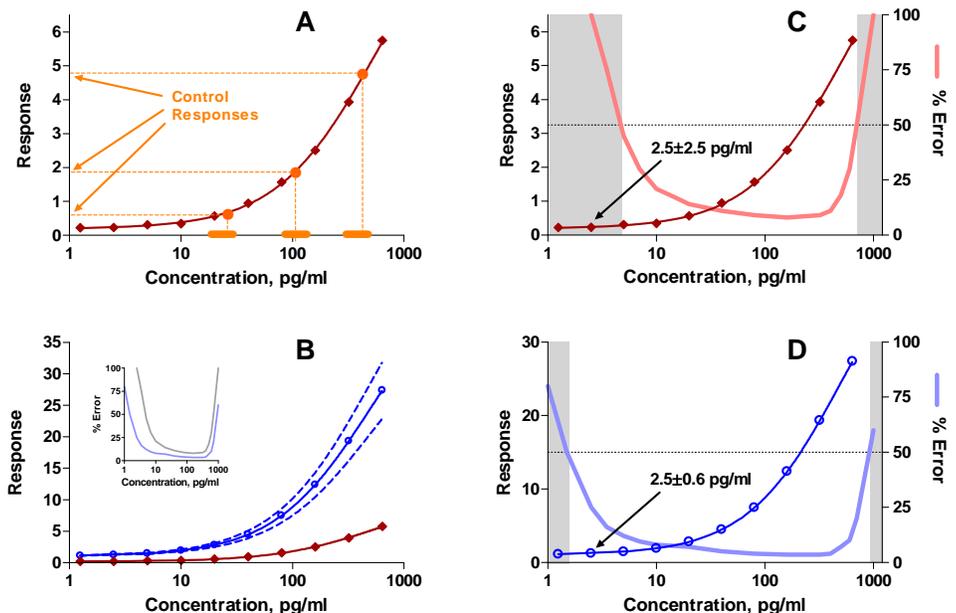
StatLIA's SmartQC[™] Statistical Assay Analysis provides at-a-glance assay performance evaluation, troubleshooting and result reliability.

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. 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).



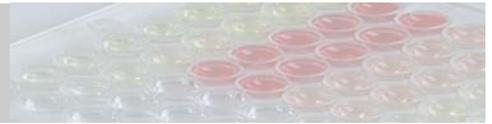
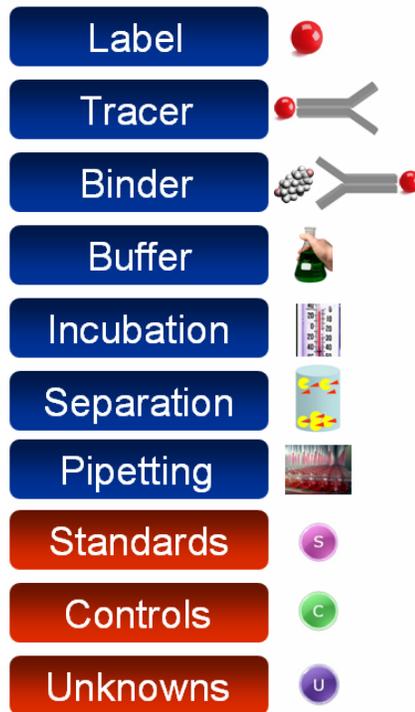


Figure 2– Components of an assay



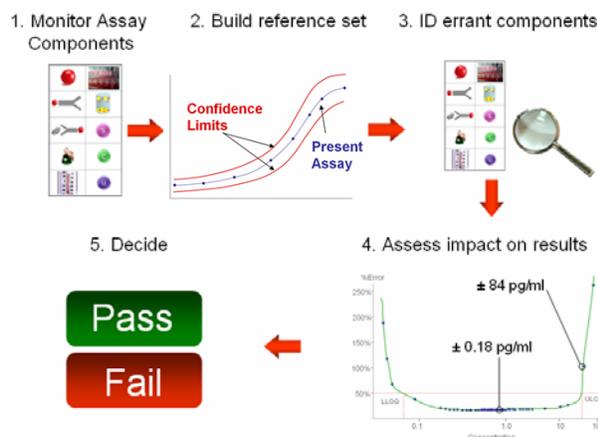
± 0.6 pg/ml for the same specimen in a normally performing assay from the reference set). Thus, being able to compare the performance of the present assay to a historical set of assays has allowed us to determine that this assay is not performing to its required capability, and the resulting measurements 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 measurements¹.

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

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



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 3– SmartQC™ workflow

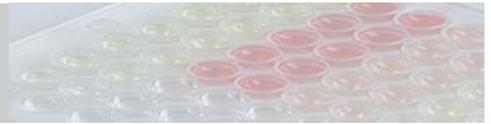
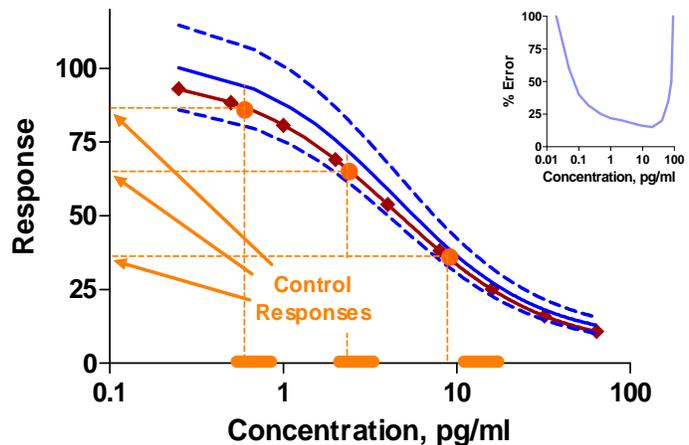


Figure 4— Traditional QC methods that rely on high, medium and low concentration controls would have failed the present assay simply because the observed response of the low concentration control is higher than expected. SmartQC™ evaluates the present assay based on how it performs relative to reference 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.

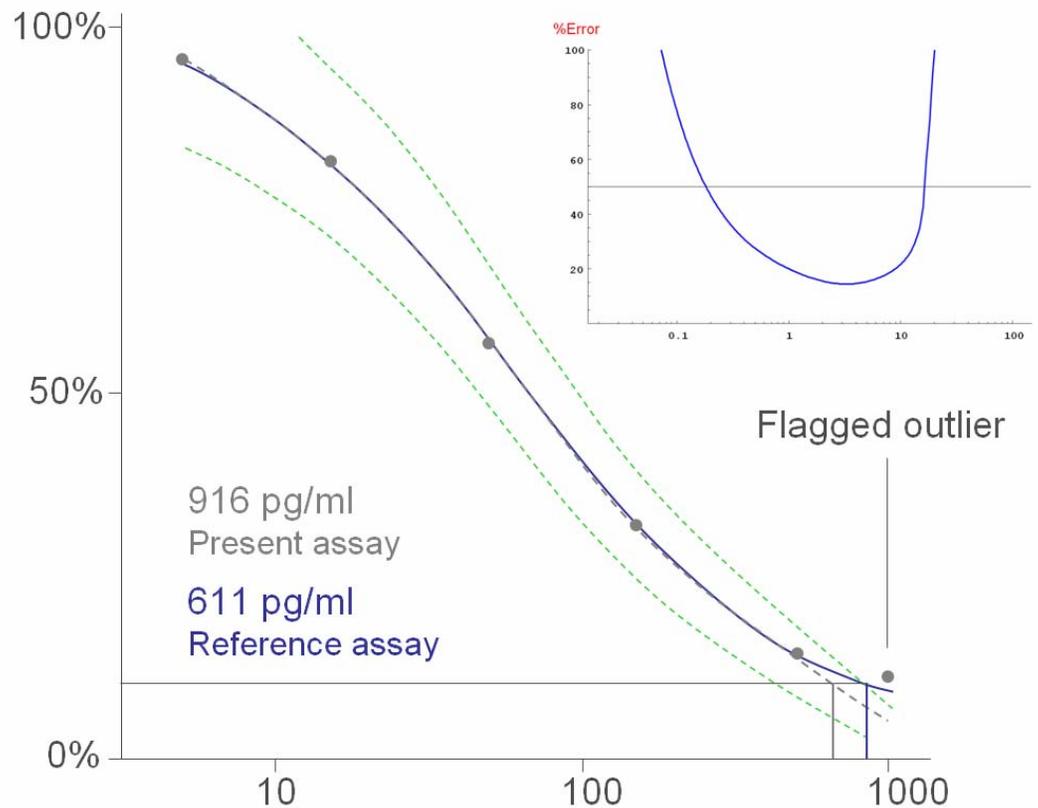
Figure 4 illustrates how StatLIA provides metrics for the evaluation of assay quality control. In this example, a dose/response relationship is shown for an inhibitory as-

say 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 in-

terval, 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-

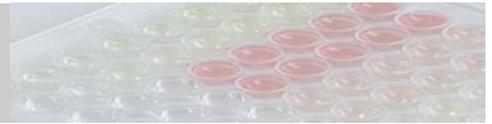


Figure 6– SmartQC™ trouble-shooting guide

Components →									
Flagged parameters ↓	Pipetting	Separation	Label	Tracer	Binder	Buffer	Incubation	Standards	Controls
Precision	●	●							
Tracer activity	●		●	●					
Bmin	●	●		●		●			
Bmax	●	●		●	●	●	●		
Std Response	●	●		●	●	●	●	●	
Std Normalized Response	●					●	●	●	
Ctrl Response	●	●		●	●	●	●		●
Ctrl Normalized Response	●					●	●		●
Ctrl Concentration	●	●						●	●
Monitor Points	●	●		●	●	●	●	●	

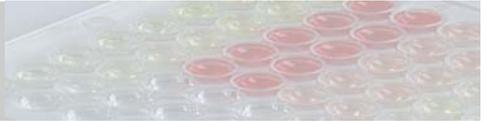
Figure 6 shows a chart that illustrates how StatLIA troubleshoots assays based on matching the flagged parameters with the errant assay component(s). In the example assay illustrated in Figure 4, StatLIA flagged the “Control Adjusted Response”, “Control Normalized Response” and the “Control Concentration” 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.

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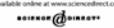
(1) Gottschalk, P.G.; Dunn, J.R. Determining the Error of Dose Estimates and Minimum and Maximum Acceptable Concentrations from Assay with Nonlinear Dose-Response Curves. *Computer Methods and Programs in Biomedicine*, **2005**; 80:204-215.



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The five-parameter logistic: A characterization and comparison with the four-parameter logistic^{1,2}

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Abstract

Improvements in assay technology have reduced the amount of random variation in measured responses to the point where non-stochastic asymmetry of the assay data can be more significant than random variation. Use of the five-parameter logistic (FPL) function to fit dose response data nearly accomplishes such asymmetry. The FPL can dramatically improve the accuracy of asymmetric assays over the use of symmetric models such as the four-parameter logistic (4PL) function. Until recently, however, the process of fitting the FPL function has been difficult, with the result that the 4PL function has continued to be used even for highly asymmetric data. Various ad hoc modifications of the 4PL method have been developed in an attempt to address asymmetric data. However, most advances in statistical methods for assay analysis software have centered around the fitting of the FPL curve. This paper demonstrates how use of the FPL function can improve assay performance over the 4PL, and its variants. Specifically, the improvement in the accuracy of concentration estimations that can be obtained using the FPL over the 4PL as a function of the asymmetry present in the data is modeled. The behavior of the FPL curve and how it differs from the 4PL curve are discussed. Common experimental designs, which can lead to ill-posed regression problems, are also examined.

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Keywords: FPL; 4PL; Immunoassay; Bioassay; Data reduction; Statistical analysis; Dose response curve; Curve model

Bioassays perform immunoassays to determine the concentration of an analyte in a sample. Immunoassay techniques use antibody-antigen binding to quantify the concentration of an analyte. This is done indirectly by measuring a response that is proportional to the signal intensity of some type of label. Depending on whether the immunoassay is competitive or immunometric, the label is chemically attached to the analyte or the antibody, respectively [1]. Bioassay is a broader term which refers to any type of biological activity that is measured as a function of the dose level of some substance. Bioassays are often a central part of potency studies where the parallelism and relative potency of two dose-response curves is of primary interest [2]. To quantify the concentration of the analyte, the response must be compared to a calibration curve, commonly called the standard curve. The unknown concentration of an analyte must then be determined by finding the concentration on the standard curve that produces the same response as that obtained from the unknown sample [2,3]. Ideally, the standard curve would be identical to the true curve; the curve that expresses the concentration versus response relationship without any degradation by errors. If an infinite number of concentrations were used, each with an infinite number of replicates, the resulting curve would be the true curve. For practical

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

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Keywords: Dose response curve; Monte Carlo method; Error estimate; Five parameter logistic; FPL; Fractional profile; Weighted regression

Summary: A method is described here that uses a modified Monte Carlo method to provide an improved estimate of the confidence bounds of concentration estimates. This method accommodates even strongly nonlinear curve models, such as the five parameter logistic model. In contrast to the common but often poor approach of linearizing the regression problem and using linear theory to obtain the confidence bounds, the method uses an interpolation technique to reduce artifacts in the precision profile due to small simulation sample sizes and proximity to horizontal asymptotes in the curve model. The paper also describes how to define and calculate the minimum and maximum acceptable concentrations of dose-response curves by finding the concentration where the size of the error, defined in terms of the size of the concentration confidence interval, exceeds the threshold of acceptability determined by the application.

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1. Introduction

Immunoassay techniques use antibody-antigen binding to quantify the dose of an analyte. This is done indirectly by measuring the quantity of some type of label. Depending upon whether the immunoassay is competitive or immunometric, the label is chemically attached to either the analyte or the antibody, respectively [1]. In order to quantify the concentration of the analyte, a response produced by the sample is compared to a calibration curve, commonly called the standard curve. The unknown concentration of an analyte is then estimated by finding the concentration on the standard curve that produces the same response as that obtained from the unknown sample [2,3]. In some applications, unknown dilution curves are compared to standard curves to determine the degree of parallelism or linearity and relative potency [4]. The standard curve is an estimate of the true curve; the curve that expresses the dose-response

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

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There is often a need to determine parallelism or linearity between pairs of dose-response data sets for various biological applications. This article describes a technique based on a modification of the well-known extra-sum-of-squares principle of statistical regression. The standard extra-sum-of-squares method uses an F-distribution ratio as a statistic, and as a consequence this statistic on the parallelism test. It is shown here that this metric does not directly measure the parallelism between the two curves, and can often vary in opposition to actual parallelism. To overcome this problem, a metric based on a chi-square test applied directly on the chi-square-distributed extra-sum-of-squares statistic is developed, which is shown to correspond directly to parallelism. This parallelism metric does not suffer from the shortcomings of the conventional F-ratio-based metric, and is a more reliable and appropriate measure of parallelism. The article also shows that the choice of curve model has a large effect on the sensitivity of relative potency, and that using an asymmetric model, such as the symmetric five-parameter logistic function, a generalization of the commonly used symmetric four-parameter logistic function, is necessary when working with asymmetric dose-response data. The effect of errors, as well as the importance of correct weighting on the parallelism metric and the relative potency, is also studied.

Key Words: Bioassay; Five parameter logistic; Four parameter logistic; Immunoassay; Linearity; Nonlinear weighted regression; Parallelism; Relative potency

1. INTRODUCTION

The determination of parallelism or linearity between sets of dose-response data plays an important role in a number of biological applications. Such applications include drug comparison, analyte confirmation, cross-reactivity, interfering substances, matrix compensation, concentration estimation, and stability studies. These applications can be reduced to two purposes: to determine if the biological responses to two substances are similar (indicating the similarity of the substances), or to determine whether two different biological environments will give similar dose-response curves to the same substance. The difference in the biological environments are often different matrices, but can be differences between any component of the reaction mixture or the incubation condition. These latter applications are generally referred to as linearity or recovery studies (Kilifi et al., 2000; Krieger and Schain, 1993; Thelen, 1992). They are the

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