W0930-03-020

Aegyris™ – The simple and efficient solution for the validation of immunogenicity assays to support vaccine clinical trials

Rafiqul Islam[1], Ivy Truong[2], Mohammed Moin[2]
[1]Celerion Inc., [2]Somru BioScience, Inc.

CONTACT INFORMATION: Rafiqul.Islam@Celerion.com

aaps PharmSci 360
ADVANCING PHARMACEUTICAL SCIENCES, CAREERS, AND COMMUNITY

PURPOSE

The foundation for any vaccine immunogenicity trial is the measurement of binding and neutralizing antibodies with well-designed bioassays. It is also crucial to recognize that the methods used to quantify assay endpoints (e.g., antibody concentration) require robust statistical approaches. The statistical methods used during method validation and in-study sample analysis can have significant effects on their accuracy and precision. In addition these methods must also be defined and uniformly applied within and across laboratories as, in most cases, multiple labs are involved in the measurement of antibodies to support vaccine studies.

This poster discusses a number of statistical methodological issues related to bioassay design and analysis that would ultimately lead to reliable measurement of antibodies with minimum variance that would be comparable across laboratories. In addition, it also demonstrates how these statistical methodologies can be implemented in a simple and efficient manner using a commercially available software - Aegyris™.

OBJECTIVE(S)

The objective of the poster is to present robust statistical approaches for the establishment of the following to support vaccine clinical studies:

- Comparison of curve fit algorithms
- Establishment of parallelism
- Comparison of data between laboratories
- Bayesian approach to study sample concentration determination

METHOD(S)

The intended purpose of vaccine immunogenicity trials is to identify one or more biological endpoints that may be used as surrogates for immunity. For most vaccine trials, the most accepted endpoints are related to antibody levels expressed in concentration units (e.g. µg/ml) or titers (1/serum dilution) for functional assays.

The key step in the implementation of such methods is the validation of the method to ensure that the most accurate estimate for antibody concentrations can be derived with minimal variability. This requires sophisticated statistical approaches to ensure that the assay is validated for the intended purpose. The statistical approaches include standard curve modeling, parallelism test, trending to identify variability, drift, degradation, etc. We have utilized a robust curve -fitting technique that identifies outliers in an iteratively reweighted least squares algorithm to improve assay accuracy and precision. In addition, robust statistical analysis should be performed to define the following in regards to in-study validation (i.e. sample analysis).

- Define robust statistical approaches to establishing parallelism between the reference standard and sample dilution curves
- Define a robust approach to on calculating antibody concentrations when samples are run in multiple dilutions

We have also developed a statistical approach to compare data between laboratories using a series of serum specimens with known or assigned antibody concentrations. The results are then evaluated to determine the extent of agreement or disagreement.

All statistical analysis was performed using a web-based software platform, Aegyris™. The web-based front-end of the software provides rich user interface for data analysis and visualization. The back-end integrates with a fully functional on-demand R statistical analytic engine. The application is massively scalable and supports real-time analytics by storing data in a NoSQL database. A built-in open interface module allows seamless importing and exporting of data from various third-party laboratory instrument software or LIMS. Report generation, user authentication/authorization, security polices (set in the Administration Console) and audit trails are some of the few core features of the software.

RESULT(S)

Comparison of Curve Fit Algorithm

There are a wide variety of mathematical functions and curve-fitting methods available to model serially diluted standard reference serum. We have evaluated seven different models using an evaluation index calculated from the deviations of the predicted concentrations of the standards from their known values to determine the model that provides least deviation. While 4- or 5-parameter logistics curve is the most widely used in the industry as “default”, we demonstrate that no one model will be optimal for all experimental situations and a statistical measure should be used to identify the right model on a case by case basis. We have also demonstrated that the selection of the mathematical function used to model standard curves can have a significant impact on within-assay and between-assay variability. In some cases the differences in data processing techniques account for a significant portion of between-assay variability (Figure 1).

While most commercial software allows for utilizing various curve fit approaches, they do not allow for direct comparison of these curve-fitting methods. Aegyris™ offers multiple approaches to compare different curve-fitting algorithms (Table 1).

Evaluation of Parallelism

It is important to establish parallelism between the serially diluted standard reference serum and serum sample curves to support the assumption that the antibody-binding characteristics are similar enough to allow the determination of antibody levels in the diluted serum sample. One of the common approaches used in the laboratory is to evaluate the % CV of dilution adjusted concentrations in conjunction with recovery trend to determine parallelism (Table 2). While this approach provides some indication of parallelism, it is not sufficient to determine parallelism to support vaccine studies. Robust off-the-shelf statistical methods are required to improve the accuracy and reliability of the parallelism metric. There are two statistical approaches commonly used to evaluate parallelism: response comparison methods and parameter comparison methods. Parameter comparison methods compare some or all of the parameters of independently fitted curve models. Aegyris™ allows for slope ration and model parameter confidence interval approach for the evaluation of parallelism. Response comparison method compares the predicted responses of the simultaneously fitted curves using a constrained model or free model.

Aegyris™ allows for full curve parallelism evaluation using well referenced chi-square statistics. This is more reliable than traditional F-test based method. The F-test is too sensitive to high-quality curve fits and overly insensitive to poor curve fits of the free models. When the quality of fit of the free models is high, the F-test metric may fail assays that are known to be parallel. Conversely, when the quality of fit of the free models is poor, the F-test metric may pass assays as parallel that are known to be non-parallel. The chi-square test applied directly on the chi-square–distributed extra-sum-of squares statistic is overcomes this limitation and more reliable for the evaluation of parallelism.

Comparison of Data Between Laboratories

Very often, more than one laboratory is involved in the analysis of immunogenicity samples to support vaccine studies. It is critical that these laboratories standardize their processes so that data is reliable and accurate for the intended use. Most commercial immunoassay software lacks visualization tools to compare data between laboratories. We have utilized box plot analysis in Aegyris™ to compare between laboratories (Figure 3). The antibody concentrations for quality control concentrations over 11 runs were used to compare inter-laboratory performance. In addition software allows for measurement of constant bias, proportional bias, correlation coefficient, concordance coefficient, gold standard correlation and paired t-test to compare method performance between laboratories.

Bayesian Approach to Study Sample Concentration Determination

In most cases samples from vaccine immunogenicity studies are run in multiple dilutions and the concentration of the antibody is estimated by combining measurements of several different dilutions of an unknown sample. The standard approach implemented in commercial software discards measurements that are below and above the quantitation limits and assumes the weight of each measurement within reportable range is equal. The assumption of equal weight is inaccurate due to the fact that the relation between concentration and measurement is nonlinear and heteroscedastic. The Aegyris™ software allows for calculating the sample concentrations using a Bayesian approach which employs the following weights: 1) measurements with higher variance have lower weights; 2) measurements at the upper asymptote of the curve have higher weights; 3) smaller dilutions are assigned smaller weights due to the fact that the variance is magnified when the low-dilution estimates are scaled back up.

When we used a Bayesian approach on various experimental data, it consistently provided better measurements compared to a routine inverse method. It performed significantly better when the data is noisy. In addition, Bayesian approach allows for extending the reportable range of the assay.

Figure 1: Comparison of three Curve-fit models

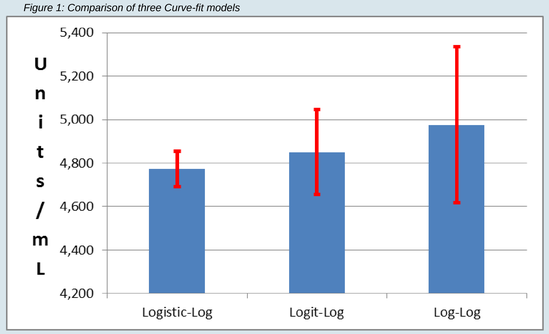


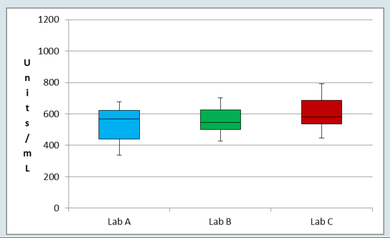
Table 1: Approaches to comparison of curve fitting algorithms

Parameter	Description
AdObs	How far on average does the fitted line deviate from the observed points
VStd	The standard points replicate variance
Ratio (dObs/VStd)	Ratio of the deviation from observed points and variance of standard point replicate. Lower the ratio is better the curve fit
Sum-of-squares	Sum of squares of the vertical distances of the data points from the curve
F-test (SS)	Extra Sum of Square F-test is based on the difference between sum of squares of the two model
Chi-square	The sum of the square of the ratio of the distance of a point from the curve divided by the predicted standard deviation at that value of X.

Table 2: Evaluation of parallelism- Recovery approach

%CV	Dilution adjusted concentration increase or decrease	
	Yes	No
Less than or equal to 30%	Lines are not parallel; but within acceptable limits	Lines are parallel
Greater than 30%	Lines are not parallel	Unacceptable variability; repeat assay and/or re-optimize immunoassay

Figure 2: Box plots showing the distribution of concentration for Quality Control (QC) sample



CONCLUSION(S)

The successful implementation of vaccine immunogenicity assays requires robust statistical approaches. Due to lack of easy-to-use software tools, analytical scientists often revert back to “routine” statistical methods. We demonstrate that the selection of statistical techniques used to calculate antibody levels have a significant impact on their accuracy and precision. While there are numerous software systems available for bioanalytical laboratories, they mostly focus on supporting “routine” immunoassay data analysis with minimal statistical procedures and not suitable for vaccine immunogenicity analysis. The Aegyris™ software can be a valuable tool for bioanalytical scientists looking to develop statistically robust immunogenicity methods to support vaccine clinical trials.

REFERENCES

- Christensen,R. et al. (2008) Parametric nonparameteric statistics. Am. Stat, 62, 296–306
- Finney, D. J. (1978). Parallel line assays. In: Statistical Methods in Biological Assays. 3rd ed. London: Charles Griffin, pp. 69–132.
- Gelman, A. et al. (2004a) Bayesian Data Analysis. Chapman&Hall/CRC, Boca Raton,FL, USA.
- Gelman,A. et al. (2004b) Bayesian analysis of serial dilution assays. Biometrics, 60,407–417.
- Higgins,K.M. et al. (1998) The effect of serial dilution error on calibration inference in immunoassay. Biometrics, 54, 19–32
- Passing, H. and Bablok, W. “A New Biometrical Method Procedure for Testing the Equality of Measurements from Two Different Analytical Methods,” J. Clin. Chem. Clin. Biochem. 21, 709–720 (1983)
- Magari, R.T. “A Statistical Approach for Hematology Comparison Studies,” Lab.Hematol. 4,199–203 (1998)

celerion Translating Science to Medicine

Somru BioScience