USDA Center for Veterinary Biologics

Statistics Section

Work Instructions

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Analysis of Parallelism in RP ELISA Validation

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1 Introduction

Veterinary Services Memorandum 800.112, Appendix III, provides guidance on assay validation studies for ELISA relative potency assays. (USDA 2011) Section 2.2.5 outlines the design of the parallelism study conducted during assay validation and gives expectations for satisfactory parallelism. This note describes a method commonly used for the statistical analysis of data from such a study. This document is intended for personnel with a substantial background in mathematical statistics, including familiarity with nonlinear mixed effects models. (e.g. Pinheiro and Bates 2000, Littell et al. (2006))

2 Preliminaries

2.1 Data Visualization

The first step should always be to visualize the optical density (OD) profiles for suitability of the data and plausibility of the intended model fit. In particular, verify the following.

- *Full curve.* The full curves of both preparations from saturation through extinction must be present.
- *Parallelism.* Their profiles of the two curves should appear to have the same sigmoid shape and differ only by a horizontal shift.
- *Zero lower asymptote.* The ODs are typically corrected by subtracting the mean OD of a reagent blank or negative control. When this is done, we generally assume that the lower asymptote is zero. (For some assays, such as some competitive ELISAs, this assumption may not hold.)

2.2 Data Reduction

Next, for this analysis OD values within each dilution of a preparation on a plate are averaged prior to analysis. As a result, the data set will now have one mean OD value for each dilution of a preparation on a plate. This is done for convenience in Section 2.2.5 parallelism studies only, so that the appropriate random components are used to evaluate the curve fits, without including the within-plate/prep/dilution residual.

2.3 Model Parameterization

Finally, the analyst must choose a parameterization of the 3-parameter logistic (3PL) curve. (Ratkowsky and Reedy 1986) Let y denote the (blank corrected) optical density, and x the dilution. The three parameters of the curve are the upper asymptote, A; a scale factor, B; and a location parameter, C.

For assay validation studies, use the following parameterization.

$$y = \frac{A}{1 + \exp\left[(C - \log_d x)/B\right]} \tag{1}$$

The covariate is conventionally expressed in base d logarithms, where d is the dilution factor.[1]

Commercial software packages distributed with plate reading equipment may use another parameterization, which is acceptable for serial (lot) release testing.

$$y = \frac{A}{1 + (\frac{x}{C})^B} \tag{2}$$

The A parameter will have the same numerical value in both parameterizations, but B and C will have different numerical values depending on which parameterization is selected.[2]

3 Nonlinear mixed effects model

Using the parameterization of Eq.(1), the nonlinear mixed effects model can be formulated by decomposing each parameter into a fixed effect that depends on preparation (reference or test serial) and a random effect that depends on plate. (For simplicity we illustrate just a single random effect. Include others as necessary.) Mathematically, this is written as follows:

$$y_{(ij)k} = \frac{A_i + a_j}{1 + \exp\left[(C_i + c_j - \log_d x_{(ij)k})/(B_i + b_j)\right]} + \epsilon_{(ij)k} \tag{3}$$

[1] Any base will work, but the choice will naturally affect the location and scale parameter estimates.

[2] For additional details about parameterizations see STATWI0005.0#.

where i indexes the preparations (reference or test serial), j indexes the plates, and k indexes the dilutions. The fixed effects are denoted by capital letters, and the random effects by lower case letters.

The random effects are a for the upper asymptote, b for the scale factor, and c for the location parameter. Thus each parameter (A, B, and C) depends on one fixed effect (the preparation) and one random effect (the plate). A residual error is represented by ϵ. The random effects are typically modeled as follows:

$$\epsilon_{(ij)k} \sim N(0, \sigma_\epsilon^2) \tag{4}$$

$$\begin{bmatrix} a \\ b \\ c \end{bmatrix} \sim MVN\left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_a^2 & 0 & 0 \\ 0 & \sigma_b^2 & 0 \\ 0 & 0 & \sigma_c^2 \end{bmatrix}\right) \tag{5}$$

Here N is the normal distribution and MVN is the multivariate normal distribution. A diagonal covariance matrix is assumed for the random effects, since it is unlikely there will be enough data to fit an unstructured one. Sometimes, one or more of the random effect variance components is estimated to be negligibly small. By convention, when this happens we have chosen *not* to remove the random effect from the model. Finally, note that regarding Eq.(4), it assumes homogeneity and independence of residuals, which may not always be the case.

Once the model is fitted and parameter estimates are available, parameter ratios and their confidence intervals may be obtained by the delta method. This requires the estimated covariance matrix of the parameter estimates from the model fit. Critical values from the t-distribution should be used; the degrees of freedom will be the number of plates minus three, which is the number of random effects parameters (a, b, c).

References

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