### 4.6.2 USING SOFTWARE TO ESTIMATE RELATIVE POTENCY

Relevant references and guidance documents:

- Veterinary Services Memorandum (VSM):  800.112, Appendix III, Section 2.2.9.
- Center for Veterinary Biologics (CVB) Notice: 13-18.
- CVBSOP0102, current version.

Introduction

CVB Notice 13-18 announced that CVB has discontinued use of RelPot software (described in Supplemental Assay Method 318, now obsolete) as of Jan. 1, 2014.  The CVB laboratory has transitioned to using commercial software for estimating relative potency. The notice provides a framework for firms to gradually transition to commercial software for their outlines that currently include RelPot.  CVB does not endorse any particular commercial software, and equivalent calculations can be carried out in *several* available software systems.  *What follows is purely for illustrative purposes.*  The discussion below applies both to new assays and transition proposals for legacy assays.

Examples of relative potency calculations

The CVB Statistics Section has provided *examples* for estimating relative potency using SoftMax Pro (SMP) 6.2.1 (or later).  These files are simplified versions of those used by the CVB laboratory, and they serve to *illustrate* calculations that could readily be carried out in *several other* software systems.  The example files may be found at the Molecular Devices Community website for SoftMax Pro:

- SMP:: Analyzing partially linear data for serial release testing:
  http://www.softmaxpro.org/protocol/show/58
- SMP:: Analyzing full dose response data for serial release testing:
  http://www.softmaxpro.org/protocol/show/60

Examples for using Gen5 to calculate estimates of relative potency may be found on BioTek's tech resource page.  Several files are available, allowing selection by curve fit type, horizontal axis units, and competitive vs. immunometric.  These files perform the same activity as in the above example files and are another *illustration* of calculations that could be carried out in *several other* software systems:

- Gen5::  Estimating Relative Potency sample files:
  http://www.biotek.com/resources/articles/estimating-relative-potency-sample-file.html

Firms may use the above links to access the examples.  Firms (including those not using either of the mentioned software) may consult CVBSOP0102, posted to the CVB website at
http://www.aphis.usda.gov/animal_health/vet_biologics/publications/CVBSOP0102.pdf

This document describes the calculations implemented in the above files as applied in a general way to situations where the procedure has not been fully specified in an outline. Ordinarily every assay is expected to have a fully specified calculation procedure as outlined below.

Expectations for proposals for estimating relative potency

Proposals for estimating relative potency, either for transitioning legacy assays or for new assays, should be routed to the Statistics Section for comment. For legacy assays, a full validation report is *not* expected. Rather, data from at least ten serial release tests may be used to support the proposal. However, it is *not* acceptable for a firm to simply submit Outline revisions without a transition proposal. For new assays, use of the proposed method should be incorporated in the assay validation report that is normally expected per VSM 800.112.

Key items to look for include the following, and the Statistics Section will provide feedback with respect to these points. The data model should be specified, e.g., parallel line analysis, or nonlinear curves such as three-parameter logistic functions, with additional details specified according to the table on the following page. The procedure should be fully specified, so that an independent laboratory (such as CVB's), possibly using different software, would be able to reproduce the calculation. Revisions to the Special Outline or Outline of Production should reflect the level of detail needed by an independent laboratory to carry out the procedure.

| Parallel Line | Full Dose Response |
|---|---|
| Both a (nonzero) minimum slope and an acceptance range for the ratio of the slopes of the serial to the reference.<br>• Minimum slopes less than 0.10 in minimum absolute value should not be entertained.<br>• For legacy assays only, an acceptance range for the slope ratio of 0.80 to 1.25, inclusive, will be allowed for consistency with RelPot.<br>• For new assays, an acceptance range tied to data, which includes 1.0 and whose limits do not exceed 0.80 to 1.25, should be specified.[1] | Acceptance ranges for the ratios of upper asymptote and scale factor between the serial and the reference. Preferably these ranges should be based on data, and should include 1.0. |
| Specify the procedure for identifying the pseudo-linear region. Specific guidance for this option is given in the next section. | If an assay has a demonstrated "hook effect" (an initial deviation from the upper asymptote), it is acceptable to exclude the dilutions involved in the hook effect |

Acceptance ranges for positive and negative controls and reagent blanks

Software version number, and any settings and options that need to be specified.
- It is usually helpful for the firm to submit an electronic file illustrating the intended calculations in the native format of the chosen software. This will help the Statistics Section evaluate the suitability of the proposed method
- The phrase "version XXX or later" may be used for common curve fitting software with transparent algorithms, such as SoftMax or Gen5. It does not apply to software based on undocumented proprietary algorithms that may change at vendor discretion or software used for other purposes.

Auxiliary files, such as template files, should be version controlled and specified.

Specifying a pseudo-linear region

Relative potency assays evaluated by the parallel line method must identify the pseudo-linear regions of the two response curves that will be used for calculating the RP. To properly do that, the assay must be optimized well enough to perform consistently. If so, it is usually easy to specify a method for identifying the correct regions. The definition of relative potency as the ratio of equipotent dilutions leads naturally to

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[1] One option for designing such an interval would be to calculate the mean ± two standard deviations of slope ratios from assay validation data. Of the two resulting limits, use the one furthest from 1.0, and reciprocate it to form the corresponding other limit. This method created an interval symmetric about the ideal slope ratio of 1.0 but tied to the variability of the observed data.

a method based on the response (OD), but the structure of a serial release test may lead to a method that utilizes the dilutions, or both OD and dilutions. Examples:

OD. Choose an OD response range based on supporting data to select the region where the responses of reference and test serials are expected to behave linearly. Use all dilutions within that range.

OD and dilutions. Choose a dilution range for the reference based on supporting data to select the region where the response of the reference behaves linearly. Note the OD range spanned by those dilutions. Then select the dilutions of the test serial that fall within that OD range.

Dilutions. Choose dilution ranges based on supporting data to select the regions where the responses of reference and test serials are expected to behave linearly. Because this method is not primarily based on the response, it may be less consistent in capturing the appropriate regions, so optionally a small amount of leeway in adjusting the selected dilution subset would be acceptable.

- A single dilution shift of a subset within the candidate range is acceptable. For example, if a candidate range for a given preparation is defined as a set of $n$ consecutive dilutions, then segments of length $n$-1 or $n$ within that range may be considered for the final choice.[2]
- The procedure for choosing the subsets from within the candidate range must be fully specified and reproducible.[3]
- The pseudo-linear regions for the reference and serial need not use the same dilutions. However, the OD response of the two preparations should overlap. A lack of such overlap should result in declaring the test invalid.

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[2] When this criterion is applied to a reference and test serial together, up to 9 possible combinations of segments may be considered. For instance, choose at least 3 consecutive dilutions between dilutions 2 through 5. The allowed segments are dilutions 2-4, 3-5, and 2-5. Avoid specifying that *only* segments of exactly $n$-1 dilutions (excluding segments of length $n$) would be considered.

[3] For instance, select the combination whose slope ratio is closest to 1.0.