In silico simulation applied to bioassay development

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Introduction

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  - could be a complex process
  - usually requires a great amount of historical data
- To compile a sufficient amount of data may be costly and time consuming
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How could we proceed saving time and reducing laboratory costs?

By re-creating experimental conditions in silico
Goal

- Already in 2011, in the session of USP Science & Standard Symposium, it was assessed:

  “the use of simulations to make decisions about analysis strategy, and how proper choices for design and analysis can reduce the amount of work required in the laboratory to establish bioassay validation”

Highlight usefulness of in silico simulations applied to bioassay development by means of a real case study
Case study: MDRP Test for in vivo-potency assay

- Case study goal: improvement and optimization, by means of in silico simulation study, of the mathematical model used for relative potency in-vivo immunogenicity test for a new vaccine.

- Multiple Dilution Relative Potency assay (MDRP): tested and reference (a clinically qualified batch) vaccines are given to different groups of mice at 6 different concentrations of the vaccine in order to estimate potency on the basis of the dose-response curves. Standard and the test vaccines must have the same slope-factor and the same maximum and minimum response level at extremes parts. The horizontal displacement between the two curves measures the relative potency (RP)
Mathematical model:
Four parameter logistic model (4PL)

- Mathematical model to be improved and optimized with the simulation study:

\[ y = D + \frac{A - D}{1 + e^{-B(\ln(dose - C))}} + e \]

where:

- \( y \) denotes the response value
- \( D \) denotes the pre-defined lower asymptote of the curve
- \( A \) denotes the upper asymptote
- \( B \) denotes the slope-factor
- \( C \) denotes the horizontal location
MDRP Test – available data

- In order to re-create experimental conditions in silico a limited amount of data was at disposal:
  - 4 independent runs
  - In each of the 4 runs two operators performed two repetitions of the same batch for a total of 2 repetitions x 8 mice x 2 operators x 4 runs = 128 observations for each dilution
MDPR Test – Steps of the simulation study

- 1\textsuperscript{st} step – System learning:
  - Choice of the dose-response model: four parameters logistic model with pre-defined lower asymptote (4PL)
  - Identification of the variance components: between assay variability and within assay variability

- 2\textsuperscript{nd} step – Study of the calibration data:
  - Estimation of the dose-response model parameters
  - Estimation of the variance components by an advanced two steps variance decomposition

- 3\textsuperscript{rd} step – Assay generation:
  - 20000 ref-ref assays sharing similar characteristics with the real one were generated

- 4\textsuperscript{th} step – Evaluation of the similarity between simulated and real assays
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3rd step – Assay generation

- For assay $k$, repetition $i$, dose $h$ and mouse $m$, the response value was generated as

$$y_{kihm} = D + \frac{\hat{A} - D}{1 + e^{B(\ln dose - \hat{C})}} + \Delta_{kihm}$$

where:
- $D$ represents the pre-defined lower asymptote
- $\hat{A}$, $\hat{B}$ and $\hat{C}$ represent the parameters estimates
- $\Delta_{kihm} = \varepsilon_k + \varepsilon_{ki} + \varepsilon_{kih} + \varepsilon_{kihm}$ represents the error associated with the $k$-th assay, $i$-th replicate, $h$-th dose and $m$-th mouse and $\varepsilon_k$, $\varepsilon_{ki}$, $\varepsilon_{kih}$, $\varepsilon_{kihm}$ are values sampled from Normal probability distributions with variances equal to the estimated variance components and mean equal to 0
- $\varepsilon_k$ is the same for each response value within the $k$-th assay
- $\varepsilon_{ki}$ is the same for each response value within the $i$-th replicate in the $k$-th assay
- $\varepsilon_{kih}$ is the same for each response value within the $h$-th dose in the $i$-th replicate in the $k$-th assay
- $\varepsilon_{kihm}$ is different for each mouse
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MDPR Test - Taking advantage of simulated assays

1) Improve MDRP with 4PL mathematical model

2) Optimization MDRP by means of a different mathematical model
Goal: to improve MDRP with 4PL mathematical model

Before the simulation study, in the MDRP, deviations from parallelism and linearity were determined by means of the significance testing approach.

Significance testing approach is a widely accepted method to test bioassay validity in terms of departures from linearity and parallelism. Nevertheless:

- it could give rise to more frequent rejections when either sample size or precision of an assay increase (e.g. Yang, H. et al., Implementation of Parallelism testing for Four-parameter Logistic Model in Bioassays, PDA J Pharm Sci and tech 2012, 66 262-269)
- it could happen that non-parallel curves may pass the parallelism test due to poor assay precision.
1) Improve MDRP with 4PL math. model (2/3)

- **Equivalence testing approach as a remedy**
  - In this framework, it consists of choosing a measure of non-linearity and non-parallelism and to specify a range of acceptable values, typically called ‘indifference zone’, for the chosen measures
  - **Challenge**: the setting of appropriate equivalence bounds for the chosen measures involves the compiling of historical data

- As it often occurs, not enough historical data were at disposal.
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- **Equivalence testing approach as a remedy**
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- Simulated reference vs reference (ref-ref) assays were used to set acceptable regions.
1) Improve MDRP with 4PL math. model (3/3)

- The 20000 ref-ref simulated assays reflect situations in which a vaccine is compared to itself. In all the simulated assays, the two curves are actually parallel and linear and consequently, the deviations observed from parallelism and linearity are due only to the system variability.

- Measures of departures from non-linearity and non-parallelism were chosen (respectively non-linearity and non-parallelism sum of squares) and computed for each simulated assay.

- For an assay, if one of the chosen measures is greater than the 99th percentile obtained from the in silico simulations, the assay has to be considered invalid.
2) Optimization MDRP by a different math. model

**Goal:** to reduce assay variability by investigating an alternative approach to analyse mice data titres

- Comparing several mathematical models
- Study strengths and weakness of the more promising mathematical model
- Overcome the statistical drawbacks of the method
- Set needed limits
2) Optimization MDRP by a different math. model: Comparing several mathematical models

- The dose-response curve theoretically follows a four parameters logistic model with pre-defined lower asymptote.
  - The simulated assays were also used to investigate on other possible mathematical models
    - 1-dilution model
    - 2-dilutions model
    - Parallel Line model (PLA)

- By comparing the previous mathematical models, PLA has been selected as the model to be optimized
2) Optimization MDRP by a different math. model: Strengths and weakness of the math model

- PLA was selected as the more promising model. However the concerns on which of using the classical PLA for this particular assay were
  - the difficulty to obtain assays satisfying the requirements for a statistically valid fit
  - the poor precision on estimates obtained for some assays
2) Optimization MDRP by a different math. model:
Strengths and weakness of the math model

- The PLA optimization was performed by using simulated assays to investigate on the reason of the weakness of PLA
  - the poor precision of the estimates
  - the high number of invalids (for linearity and parallelism) with the significance approach
By considering the RP estimates and their corresponding 95% CI computed for the simulated assays

- A linkage between the regression p-value and the poor precision of the assays was confirmed and consequently a very stringent discriminability criterion on the regression p-value was proposed.

- The better dose ranging was defined on the basis of assay precision and number of “false” invalids with the significance approach.

- Measure of departures from linearity and parallelism were chosen to apply equivalence approach instead of the significance one.
2) Optimization MDRP by a different math. model: Set needed limits

- Equivalence limits for departure from
  - linearity
  - parallelism

were set on the basis of the Monte Carlo distributions of the chosen measures obtained by means of in silico simulations
How simulated assays helped the optimization

- The 20000 generated ref-ref assays were used during the optimization of the MDRP test for:
  - choosing the best mathematical model to use (e.g. 4 parameter logistic, parallel line model exc.)
  - choosing the best format (number of repetitions)
  - choosing the dilution range
  - setting the limits to introduce the equivalence approach to evaluate the validity of the assay
  - comparing the equivalence approach with the significance one (commonly used)
How simulated assays helped the optimization

- The simulation studies allowed to achieve the following results:
  - Improvement in the precision of the assay by about 40%
  - Large Reduction of invalid assays (about 95%)
  - Statistically meaningful comparison between different analysis models, validity criteria etc.

- The 20000 ref-ref assays are available for future investigations and test improvements
Conclusion

- The simulation instrument enables to generate "infinity" data from a calibration sample.

- Introduce simulation technique gives the advantage to minimize the use of animals and get more information from a limited number of tests.

- Introduce simulation technique gives the advantage to reduce the time and the costs of a test optimization.

- It is possible to perform simulation studies of different complexity. The complexity depends on the aim of the simulation study and on the different applications planned for the generated data.
Backup
In silico simulation - Steps

- System learning
- Study of calibration data
- Assays generation
- Evaluation of similarity between the generated assays and the available ones
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The adopted statistical model used for the representation of the dose-response curve is the 4 parameter logistic method described in the Eur.Ph. chapter 5.3 with pre-defined lower asymptote (named 4PL)

\[ y = D + \frac{A - D}{1 + e^{-B(Dose-C)}} \]

where:
- \( y \) denotes the response value
- \( D \) denotes the pre-defined lower asymptote of the curve
- \( A \) denotes the upper asymptote
- \( B \) denotes the slope-factor
- \( C \) denotes the horizontal location
System learning: variance components

- Identification of system variance components:
  - **Between assays variability**
    - Different runs (e.g. different lot of mice)
  - **Within assay variability**:
    - Within repetitions
      - Ref1 vs Ref2 (e.g. independent preparations)
    - Within doses
      - Independent errors (e.g. error in the single step dilution)
    - Animal variability
      - Different mice (e.g. individual behavior)
In silico simulation - Steps

- System learning

- Study of calibration data

- Assays generation

- Evaluation of similarity between the generated assays and the available ones
Simulation study – Dose – response model

- The adopted mathematical representation of the dose – response model is forced to the adopted model for the in-vivo immunogenicity test

Four parameters logistic curve (with pre-defineslower asymptote)

\[ y = D + \frac{A - D}{1 + e^{-B(Dose - C)}} \]

where:

- \( y \) denotes the response value in logarithmic scale
- \( D \) denotes the pre-defined lower asymptote of the curve.
- \( A \) denotes the upper asymptote
- \( B \) denotes the slope-factor
- \( C \) denotes the horizontal location
Simulation study – Checking the homoscedasticity assumption on the data

- Investigation on homoscedasticity assumption on the subset of available data by means of:
  - Graphical representations of the standard deviations of the 8 mice groups for each dose and for each antigen
  - Levene’s test for the homogeneity of the variances among doses
Simulation study – Estimation of variance components - models

- Given the chosen dose – response model, the residuals variance components are assumed to be:
  - Independent
  - Normally distributed
  - Additive

- Two different variance decomposition were performed:
  1) Residuals variance decomposition by assuming homoscedasticity within doses
  2) Residuals variance decomposition by collapsing homoscedasticity within doses
In silico simulation - Steps

- System learning
- Study of calibration data
- Assays generation
- Evaluation of similarity between the generated assays and the available ones
1) Residuals variance decomposition by assuming homoscedasticity within doses

- Once model parameters are estimated, a mixed procedure was performed in order to decompose the variance of the residuals considering as random effects:
  - Operator(Run)*
  - Replicate(Operator(Run))
  - Class_Dose(Replicate(Operator(Run)))

* It was assumed that the between assays variability could be comparable to the variability between Run*Operator observed in the available data
Generating assays by assuming homoscedasticity within doses

- Response values are then generated as

\[ y_{kihm} = D + \hat{A} - D \left( 1 + e^{-\hat{B}(\ln d - \hat{C})} \right) + \Delta_{kihm} \]

where:

- \( \hat{A}, \hat{B} \) and \( \hat{C} \) denote the parameters estimates of the nonlinear model
- \( \Delta_{kihm} = \varepsilon_k + \varepsilon_{ki} + \varepsilon_{khi} + \varepsilon_{kihm} \) represent the error associated with the \( k \)-th assay, \( i \)-th replicate, \( h \)-th dose and \( m \)-th mouse
- \( \varepsilon_k, \varepsilon_{ki}, \varepsilon_{khi}, \varepsilon_{kihm} \) are values sampled from a Normal probability distribution with a variance equal to the estimated variance component
- \( \varepsilon_k \) are the same for each response value within the \( k \)-th assay
- \( \varepsilon_{ki} \) are the same for each response value within the \( i \)-th replicate in the \( k \)-th assay
- \( \varepsilon_{khi} \) are the same for each response value within the \( h \)-th dose in the \( i \)-th replicate in the \( k \)-th assay
2) Residual variance decomposition by collapsing homoscedasticity within doses (1° Step)

- Once model parameters are estimated, a mixed procedure was performed in order to decompose the variance of the residuals considering as random effects the components constant among doses
  - Operator(Run)*
  - Replicate(Operator(Run))

*It was assumed that the between assays variability could be comparable to the variability between Run*Operator observed in the available data
Residual variance decomposition by collapsing homoscedasticity within doses (2° Step)

- The variance of the residuals of the mixed procedure was further decomposed by dose considering as random effects the component considered different at each dose
  - Replicate(Operator(Run)) → variability between groups of mice at the same dose
1) Residuals variance decomposition by collapsing homoscedasticity within doses (3/3)

Response values are then generated as

\[ y_{kihm} = D + \frac{\hat{A} - D}{1 + e^{-\hat{B}(\ln dose - \hat{c})}} + \Delta_{ki} + \Gamma_{kihm} \]

where:

- \( \Delta_{ki} = \varepsilon_k + \varepsilon_{ki} \) represent the error associated with the \( k \)-th run, \( i \)-th replicate
- \( \varepsilon_k, \varepsilon_{ki} \) are values sampled from a Normal probability distribution with a variance equal to the estimated variance component of residuals
- \( \varepsilon_k \) are the same for response value within the \( k \)-th assay
- \( \varepsilon_{ki} \) are the same for response value within the \( i \)-th replicate in the \( k \)-th assay
- \( \Gamma_{kihm} = \delta_{kih} + \delta_{kihm} \) represent the error associated with the \( k \)-th run, \( i \)-th replicate, \( h \)-th dose and \( m \)-th mouse, \( \delta_{kih}, \delta_{kihm} \) sampled from a Normal probability distribution with a variance equal to the variance component of residuals estimated by dose
- \( \delta_{kih} \) are the same for response value within the \( h \)-th dose in the \( i \)-th replicate in the \( k \)-th assay
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Evaluation of similarity between the generated assays and the available ones

- Comparison of the percentages of invalid assays for parallelism and linearity
  - Percentages of invalid assays for linearity and parallelism on the available data are inside the 99% C.I. build with the percentages observed on the simulated assays

- Comparison of average and variability of RPs
  - The bias for the RP is less than 1%, while taking into account the limited number of available assays, the CV%s from simulated assays appear to be in line with the CV%s from available data

- Comparison of variance components
  - Very similar variance components estimates

- Similarity between generated assays and the available ones