Statistical Analysis

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International assessment of the PSPT in mice to replace the intracerebral-challenge Mouse Protection Test (MPT) for whole-cell Pertussis (wP)

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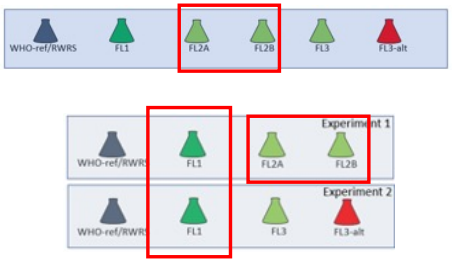
Planned analyses

- Goals
  1. *Reproducibility*
  2. *Consistency*
  3. *Stability indication*

Planned analyses

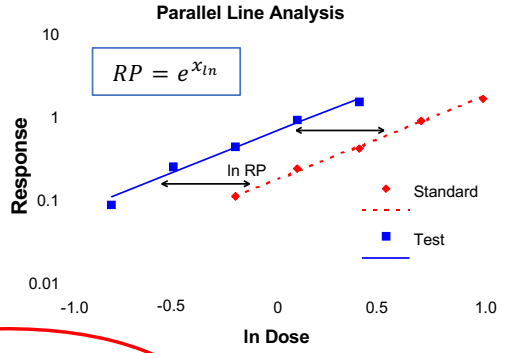
1. *Reproducibility* (ICH repeatability)
   - Replication in two optional designs was used to calculate *Reproducibility*
     - Option 1: 2 replicate immunization series of one lot (FL2A and FL2B)
     - Option 2: 2 replicate immunization series of one lot (FL2A and FL2B) in one experiment; 1 immunization series of another lot in two experiments

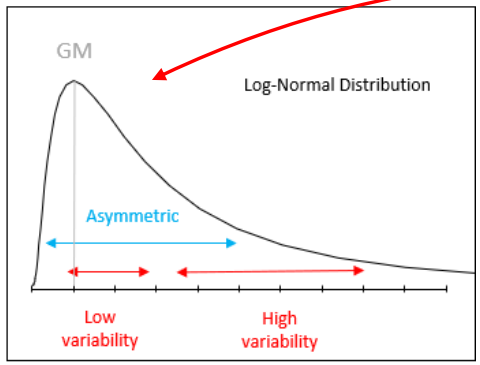


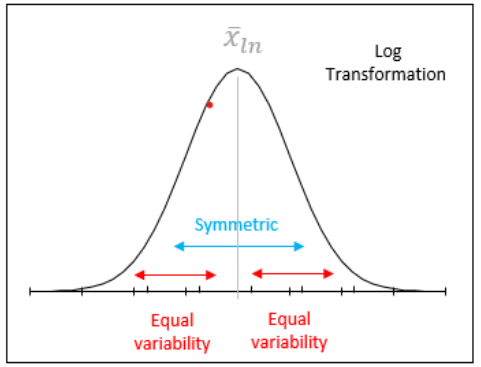
   - Standard deviations were calculated using natural log relative potency (ln RP)
     - Relative potencies were calculated versus an international/national reference standard (IRS/NRS) and versus FL1
   - *Reproducibility* is reported as percent geometric coefficient of variation (%GCV) per USP General Information Chapter <1033> *Biological Assay Validation*

Geometric mean, %GCV, and confidence intervals

- Parallel line analysis (PLA) yields potencies which have a skewed distribution (log normal-distribution)
  - Due to data processing – ln RP (shift) gives RP = *exp(ln RP)*
- The log-normal distribution can be "normalized" using log transformation
  - *Geometric mean (GM)* is the middle of the log-normal distribution ($GM = e^{\bar{x}_{ln}}$)
  - *Percent geometric coefficient of variation* is the variability ($\%GCV = 100[e^{s_{ln}} - 1]\%$)
- Leads to asymmetric confidence intervals (e.g., criterion of 50% to 200%)
- Note: the criterion CI represents variability of the "system" (e.g., mouse variability) not the repeatability or intermediate precision variability of the assay





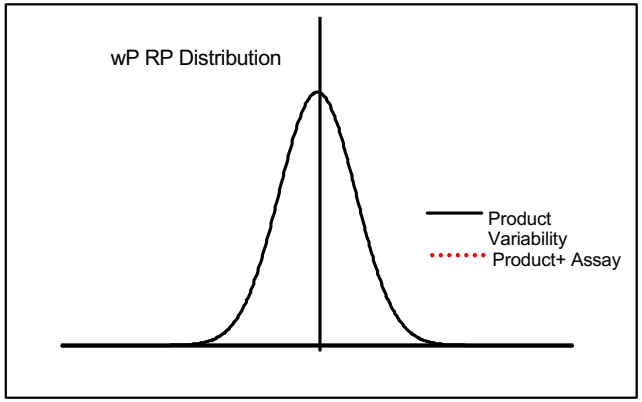


Planned analyses (cont.)

2. *Consistency* (variability of RP among lots within a manufacturer)
   - Three lots tested by each laboratory (FL1, FL2, and FL3)
     - Lots are from the same production for manufacturers (n = 7)
     - Lots are from different productions for NCL's (n = 3)
   - Variance component analysis (VCA) was performed on ln RP for each manufacturer
     - Relative potencies were calculated both versus an international/national reference standard (IRS/NRS) and versus FL1
     - VCA gives estimates of variabilities for Lots (n = 3) and replicates (n = 2 for one lot; see *Reproducibility*)
     - VC's were combined to estimate manufacturing variability (*Consistency*)
   - *Consistency* was reported as percent geometric coefficient of variation (%GCV) per USP General Information Chapter <1033> *Biological Assay Validation*

Manufacturing variability (Consistency) and variance components

- Manufacturing variability is the sum of product plus assay variabilities
- A dataset with RP measurements for multiple lots (e.g., FL1, FL2, FL3) and replicate measurements on one or more lots (e.g., FL2A and FL2B) can be analyzed by variance component analysis (VCA) to estimate individual product $\left(s^2_{Product}\right)$ and assay $\left(s^2_{Assay}\right)$ variabilities
- *Consistency* SD $(s_{Consistency})$ is calculated based on "propagation of variabilities" and expressed as %GCV$_{Consistency}$



$$s_{Consistency} = \sqrt{s^2_{Product} + s^2_{Assay}}$$

$$\%GCV_{Consistency} = 100 \cdot (e^{s_{Consistency}} - 1)\%$$

Planned analyses (cont.)

3. Stability indication
   - One lot (FL3) was artificially degraded in each laboratory yielding a lot designated FL3-alt (target RP ≈ 0.30)
   - A decrease in RP was calculated as $Fold\ Difference = RP_{FL3-alt}/RP_{FL3}$

Results

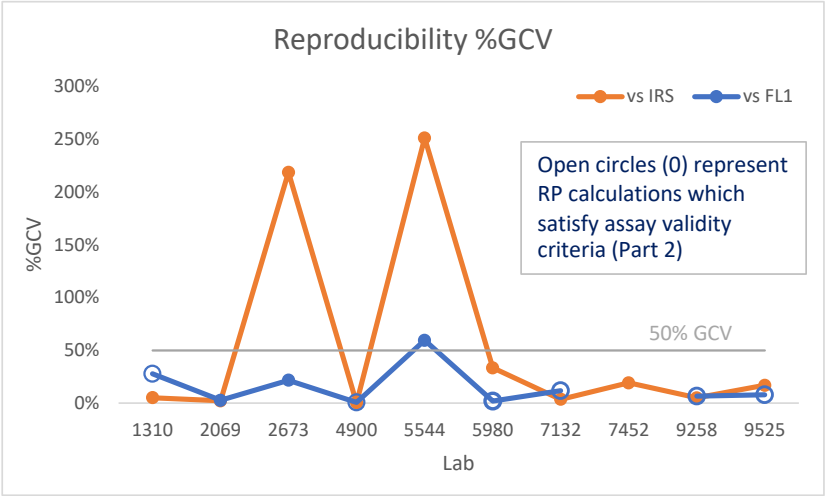
1. *Reproducibility*

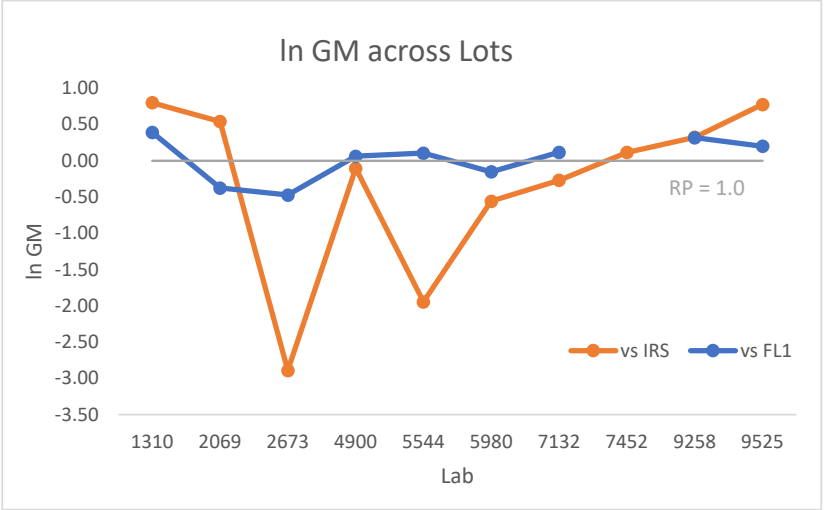
- Most %GCV results are less than 50% GCV
  - The average *Reproducibility %GCV* for valid results (in open circles) tested against FL1 is equal to *~12% GCV*
  - Note: the calculation does not include the "intermediate precision" component (between-run) of variability

$$s_{IP} = \sqrt{s^2_{Between-Run} + s^2_{Within-Run}}$$

- High %GCV results are due to low GM potencies across test lots (relative to the IRS/NRS) in 2 labs

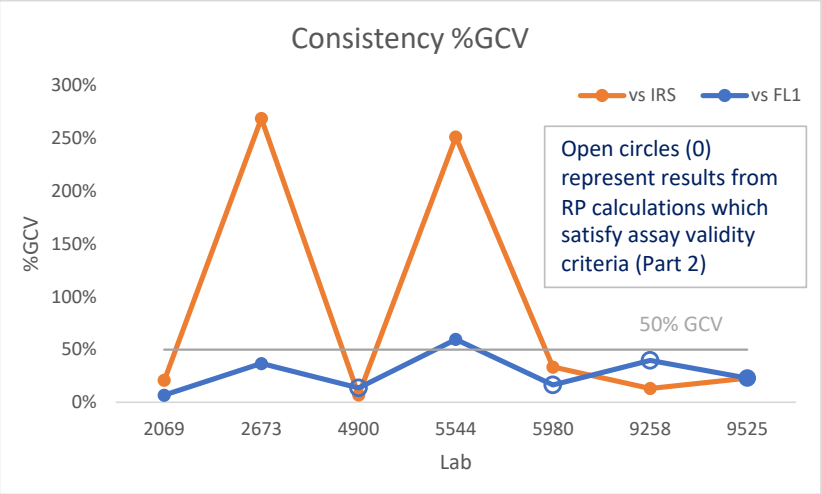
$$GCV \approx CV \approx \frac{s}{GM}$$

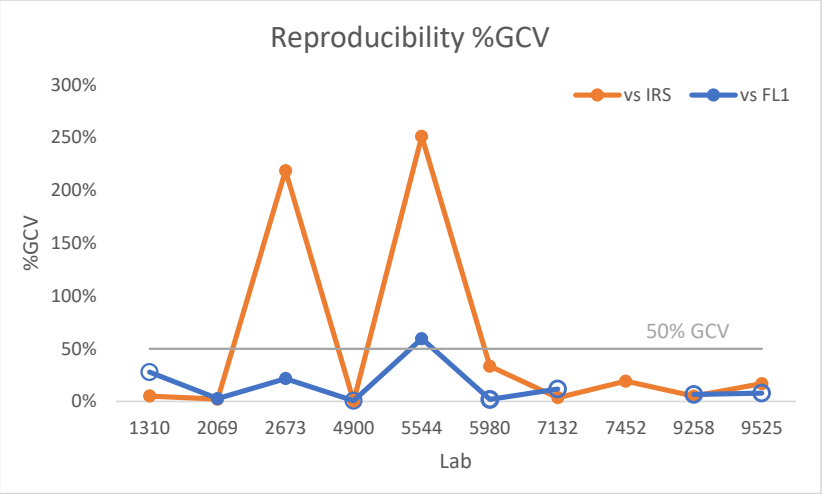




2. *Consistency*

- The average *Consistency %GCV* for valid results (in open circles) tested against FL1 is equal to *~30% GCV*
- The pattern of manufacturing variability (*Consistency*) is similar to the pattern in *Reproducibility*
  - *Consistency* is highly impacted by *Reproducibility*
- Points to an opportunity to improve *Consistency* – assay optimization
  - Subject of the next talk



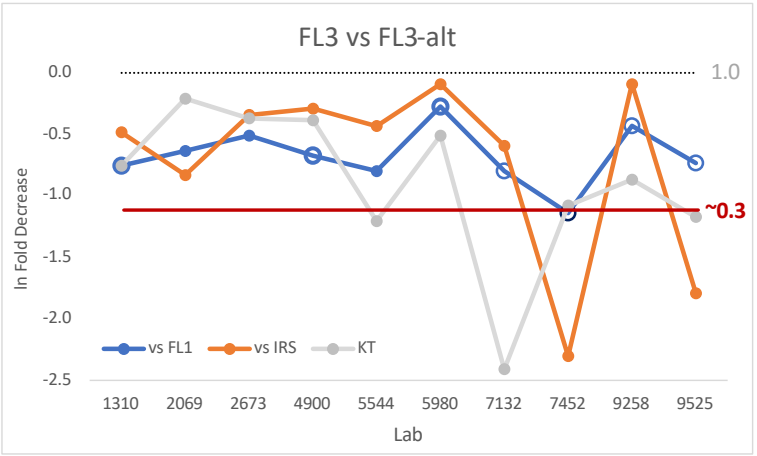


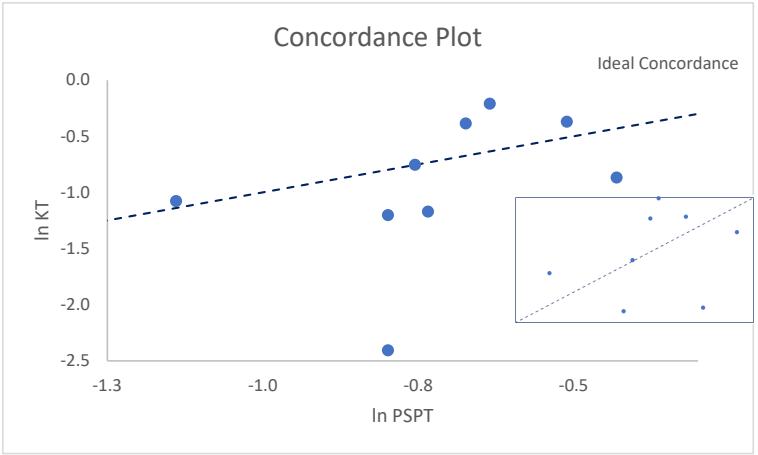
3. *Stability indication*

Days at 46°C	Potency (%)
0	100%
7	64%
14	40%
21	26%
28	16%

Based on WHO report *Temperature Sensitivity of Vaccines* (WHO/IVB/06.10, August 2006)

- A decrease in potency was observed in all labs
  - Though not a study criterion, a target potency (~30%) was observed for valid results in only 1 lab (7542, comparing FL3-alt to FL3 directly)
- PSPT showed reasonable concordance versus KT for all but one lab (7132)
  - Though hard to verify due to KT and PSPT assay variabilities





Overall Conclusions

- The PSPT study objectives were generally achieved with some provisos
  - Test lots yielded invalid results when tested against *IRS/NRS* in the assay
  - *Reproducibility* was satisfactory for 6 of the 10 labs that obtained valid results for all lots versus FL1 *(%GCV =12% < 50%)*
  - Manufacturing variability (*Consistency; %GCV = 30%*) can be improved through improvements in the assay
  - The PSPT was confirmed to be "*stability indicating*" in all laboratories
  - Failures to meet validity criterion and PSPT variability are due to several non-optimized factors in assay - subject of next talk

Questions?