Challenges and proposed solutions

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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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Goal

- Some laboratories met challenges when implementing the PSPT assay
  - Due to relative experience with an immunology based *in vivo* potency assay
    - Particularly the mouse ELISA
  - Without experience they were unable to effectively assess outcomes
  - Some standards of practice were different for mature labs
- *In-study* challenges and their solutions, as well as challenges requiring *post-study* solutions have been catalogued and will be introduced into a revision of the *in vivo* and ELISA PSPT protocols
- Follow-up will also include guidance on assay validation

Catalogue of challenges

- PSPT ELISA (titers calibrated from a 4PL curve)
  - Design challenges
    - Dilution of conjugate
    - Initial dilutions of the positive control (PC)
  - Results challenges
    - Substandard PC curves
    - High negative control readings/low test sample readings
    - "Negative" responders

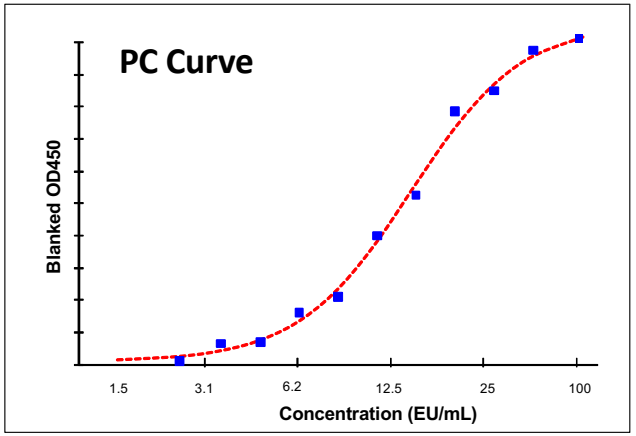
Solutions:
- *ELISA optimization*
- *Extension of dynamic range and in vivo optimization*

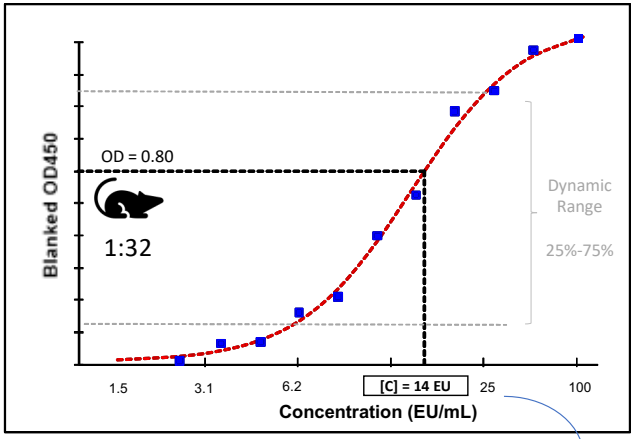
- PSPT *in vivo* (parallel line analysis/PLA from 4 inoculation levels of a test and a reference sample)
  - Design challenges
    - Doses of test vaccines (underdosing)
  - Results challenges
    - High proportions of negative mouse titers

Solution: in vivo optimization

Mouse immunogenicity ELISA

- Calibration of dilution series of mouse sera giving Ab titers
  - Based on a positive control (PC) “concentration response” curve
    - PC is assigned 100 EU/mL
    - PC curve is generated from a prescribed (by SOP) concentration series (initial dilution and 2-fold series)
  - PC readouts (OD) are fit to a four-parameter logistic (4PL) function (using EXCEL® Solver)
  - Mouse sera are diluted over a 2-fold series
    - Dilutions of a mouse serum that fall within the “dynamic range” (DR) are interpolated from the PC curve
    - Titers are “dilution corrected”
    - The final titer is the geometric mean of the “dilution corrected” titers that fall in the DR.
    - Titer assigned 2.5 EU/mL if below the curve (negative)

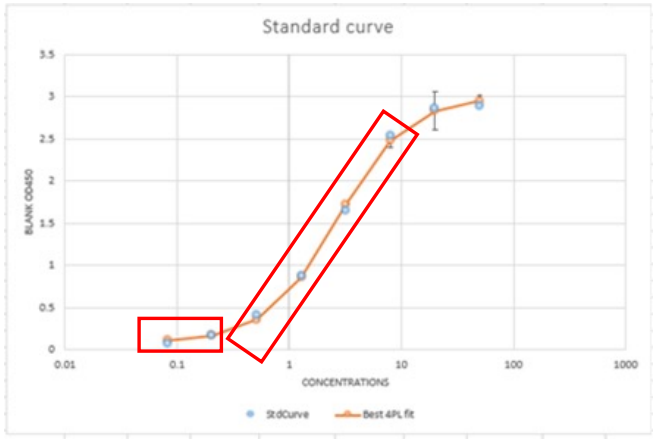




$$Dil\ Corrected\ Titer = dil \times EU = 32 \times 14 = 448$$

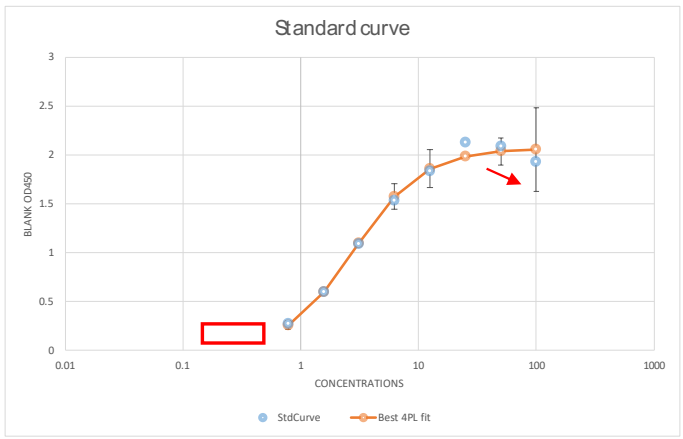
ELISA challenges and solutions (1/3)

- ELISA protocol conditions yielded substandard PC curves (also high negative controls)



	Min asymptote	slope	Intercept	Max asymptote
Curve fit	A	B	C	D
MsExcel Solver	0.09	1.39	2.68	3.00

- Ideal features
  - 2+ pts “on asymptotes”
  - 4+ pts in “dynamic range”
  - High maximum response



	Min asymptote	slope	Intercept	Max asymptote
Curve fit	A	B	C	D
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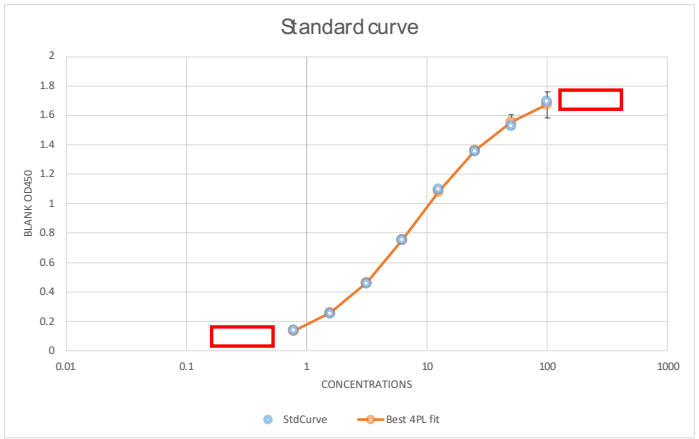
- Missing lower asymptote
- “Hook” effect
- Low maximum response

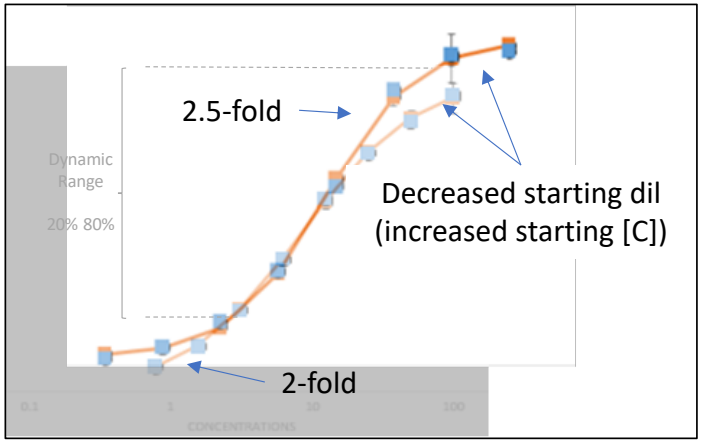


	Min asymptote	slope	Intercept	Max asymptote
Curve fit	A	B	C	D
MsExcel Solver	0.00	1.06	8.49	1.80

- Missing lower and upper asymptotes
- Shallow slope
- Low maximum response

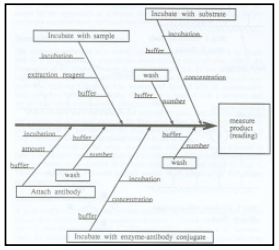
ELISA challenges and solutions (2/3)

- Consequences
  - Narrow dynamic range (DR) – coupled with high negative control values
  - High variability in mouse titers → wide confidence interval on RP estimate
  - Increased risk of false negative mouse titers
- *In-study* solutions
  - Reoptimized conjugate dilution ("checkerboard")
    - Generate curves at 1:2000, *1:4000*, 1:8000, 1:16000, and 1:32000
    - Select dilution that yielded ideal features in the positive control curve
  - Retest mouse sera after selective optimizations
    - Increase fold dilution of positive control from 2-fold to 2.5-fold
    - Change starting dilution
    - Extended DR from 25%-75% to 20%-80%

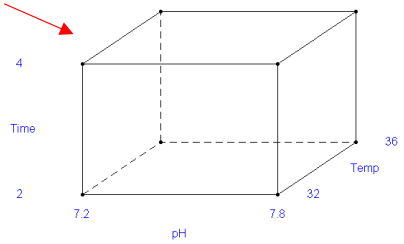


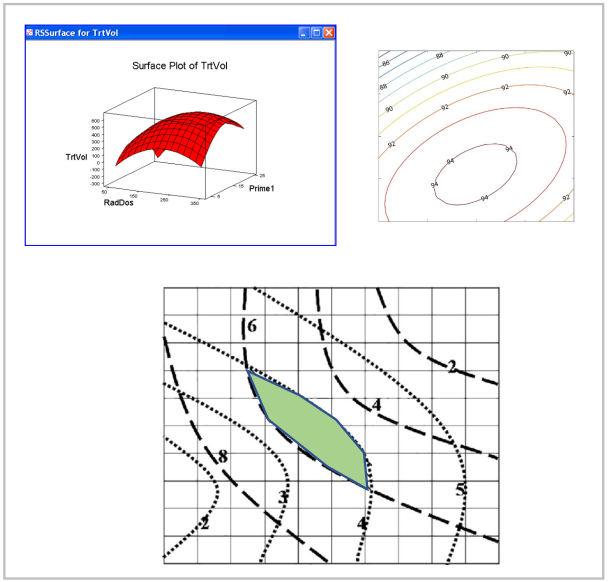
ELISA challenges and solutions (3/3)

- *Post study* ELISA optimization (*prototype*)
  - Multi-factor design of experiments (DOE)
    - Factors:
      - Conjugate dilution
      - Incubation time/temperature
      - Washing strength
      - …
    - Optimization responses
      - Negative control response/lower asymptote (⇓)
      - Upper asymptote (⇑)
      - Dynamic range/slope (⇔)
      - Visual
    - Design study,
      - Analyze data,
        - Identify region of optimal responses

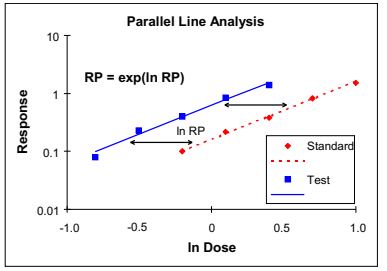


Run	pH	Time	Temperature
1	7.2	2-hrs	36°
2	7.2	2-hrs	32°
3	7.2	4-hrs	36°
4	7.2	4-hrs	32°
5	7.8	2-hrs	36°
6	7.8	2-hrs	32°
7	7.8	4-hrs	36°
8	7.8	4-hrs	32°





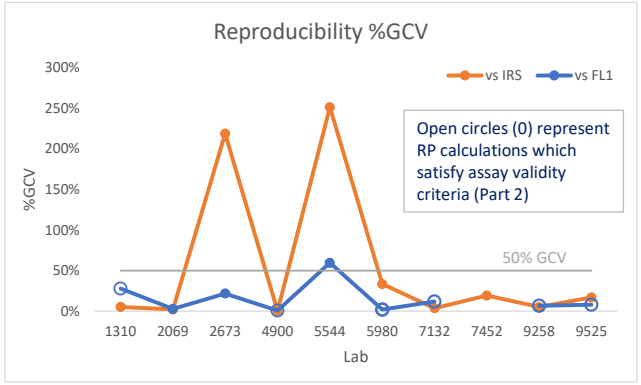
in vivo parallel line analysis

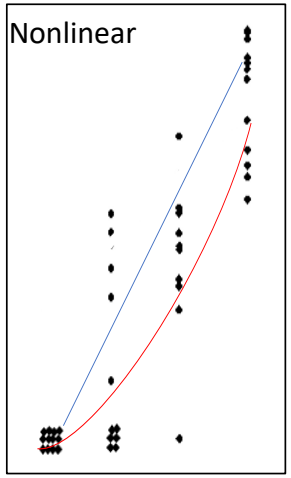


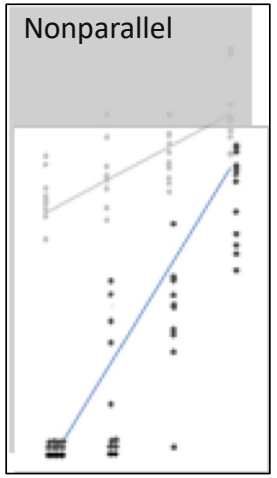
- Parallel line analysis (PLA) was performed on vaccine dose response series (4-doses) of each test lot and a Standard
  - Using *CombiStats* (EDQM)
  - Performed using both an IRS/NRS and a test lot (FL1) as the standard
- The relative potency (RP) of each test lot is determined if a collection of “system suitability” and “sample suitability” criteria are met (via ANOVA p-value)
  - System suitability:
    - *Linearity* of the standard response profile
  - Sample suitability:
    - *Linearity* of the test lot response profile
    - *Parallelism* of the test lot profile and the standard profile
    - The *95% confidence interval on RP* must fall within 50% to 200% of the estimated value

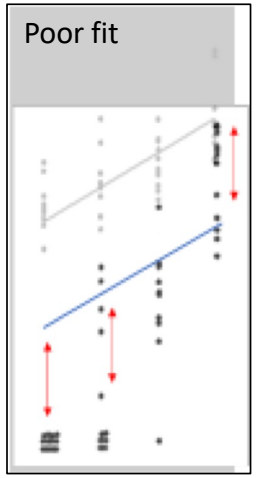
in vivo challenges and solutions (1/2)

- System and sample suitability criteria were satisfied for all lots in 6/10 labs when RP was calculated versus FL1; 0/10 labs against the IRS/NRS
- Test vaccines were collectively under-dosed in all laboratories (will be discussed during Q&A)
  - Resulting in numerous negatives (2.5) at lower doses
  - Causing:
    - Nonlinearity of concentration response
    - Nonparallelism between test and standard
    - Excess variability due to poor fit to “pooled curves” (yielding a wide confidence interval)









in vivo challenges and solutions (2/2)

- *In study* solutions
  - Elimination of data in PLA (CombiStats processing)
    - Dropped mice with responses at or below the NC
    - Dropped low doses that yielded a high proportion of negative responses
  - Note: this should not be required after *in vivo* and *in vitro* optimizations
- *Post study* solution
  - “Dose range” test and reference vaccines after ELISA optimization

Needs going forward

- Update SOP to reflect solutions to challenges
  - ELISA optimization
    - Conjugate dilution (or DOE)
    - Starting dilution of PC
    - Dilution increment (2-fold, 2.5-fold, 3-fold …)
    - Expectations for acceptable performance (e.g., PC pts. on asymptotes, DR, slope; NC response)
  - *in vivo* optimization
    - Dose-ranging of test vaccine(s)
    - Rules for data screening (no. negatives/missing mice, dose elimination, …)
    - Additional/alternative bases for validity criteria (e.g., USP equivalence approach; vs ANOVA)
- DCVMN support on other laboratory needs as well as assay qualification/validation

Thank you!

Questions?