

# *Qualitative Method Validation Studies for Quantal Data: LOD, dPOD, PRE = RLOD and $\omega$*

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# *Summary*

- Example POD vs. Concentration curves
- Ideal POD vs. Concentration curve
- Transition range models
- Method performance requirements I
- Method performance requirements II
- Limit of Detection ('LOD')
- The 'Concentration Fallacy' for micro

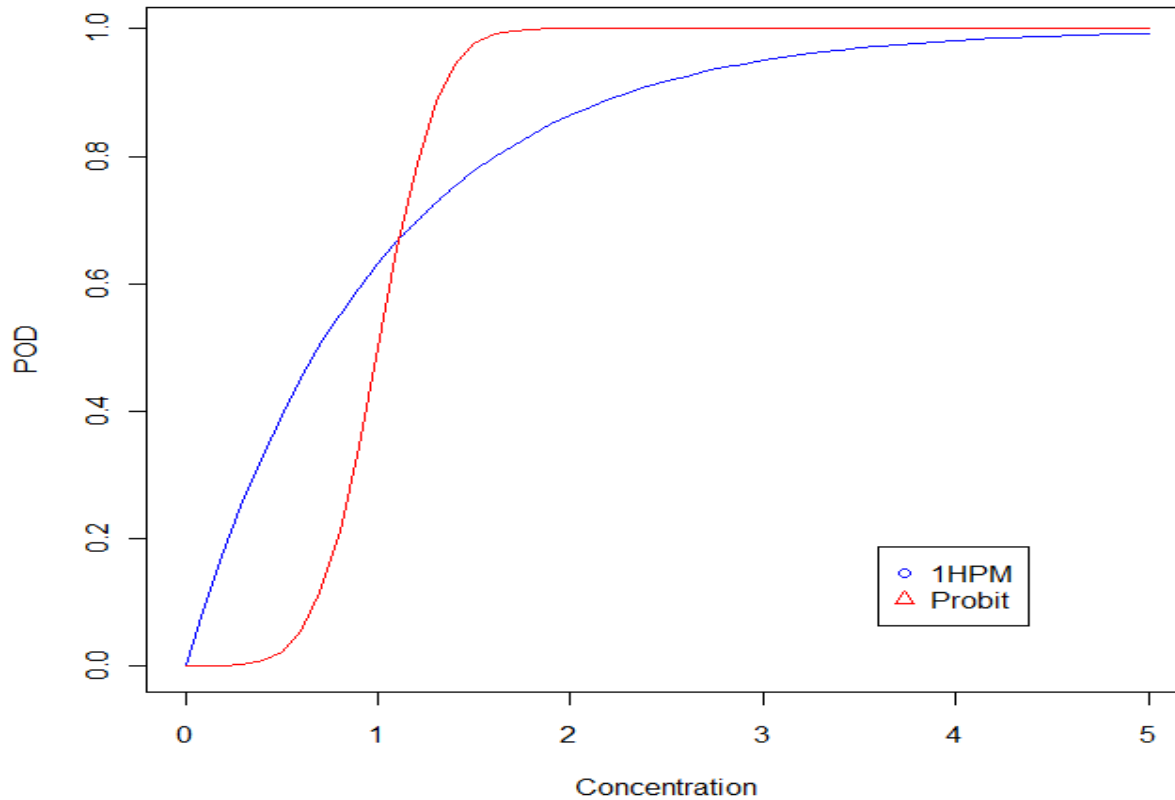
# *Summary (cont'd)*

- Method Performance Parameters III
- Difference in POD:  $dPOD(C,R)$
- Odds ratio  $\omega$
- Poisson Efficiency Ratio ('PRE')
- Relative LOD ('RLOD')
- Examples: Micro

# *Summary (cont'd.)*

- Non-micro methods
- Choice of metamer
- Warning for micro studies
- Conclusions & Recommendations

# *Example POD vs. Concentration curves*



# *Ideal Response vs. Concentration curve*

- POD = ‘Probability of Detection’  
= # Positive / # Trials  
= mean of 0 or 1 data
- The ideal test method gives  $\text{POD} = 0$  at Concentration = 0, and  $\text{POD} = 1$  for all concentrations  $> 0$ .
- For real methods, there is a transition from  $\text{POD} = 0$  to  $\text{POD} = 1$  over a range of Concentration.

# *Transition range models*

- True shape of transition curve depends upon underlying model of what happens in test method.
- Symmetric distribution threshold crossing: Probit and Logit (historically these have been most commonly used).
- Asymmetric distribution threshold crossing: can be concave or convex shape.
- ‘Hormesis’: drop-off at high concentrations.

# *Transition range models*

## *(cont'd)*

- There are a dozen or more possible model forms in common use.
- Choice of a model form is subjective and subject to controversy.
- Some curves convex, some curves concave, some symmetric.
- Logit and probit are traditionally used as middle ground when true shape is unknown.

# *Transition range models*

## *(cont'd)*

- Advantage over individual POD values *may* be improved precision by pooling across concentrations.
- If model form is incorrect, may have *worse* precision than individual POD values.
- Generally requires Concentration be known accurately.

# *Method Performance*

## *Requirements I: Confirmation*

- At the most basic level, a qualitative method is meant to discriminate between the presence and absence of an analyte.
- At zero concentration,  $POD < POD_{\max}$  with 95% confidence. (Control false positive fraction.)
- At moderate concentration,  $POD > POD_{\min}$  with 95% confidence. (Control false negative fraction.)
- Attainment of these two requirements validates the method as a ‘confirmation’ or ‘identification’ method in testing.

# *Method Performance Requirements I (cont'd)*

- No real method, despite claims, has  $POD = 0$  at zero concentration or  $POD = 1.0$  even at high concentration, due to various error sources, including human-in-the loop .
- One method is better than another if it has lower  $POD$  (FPF) at zero concentration and higher  $POD$  (lower FNF) at moderate concentration.

# *Method Performance Requirements*

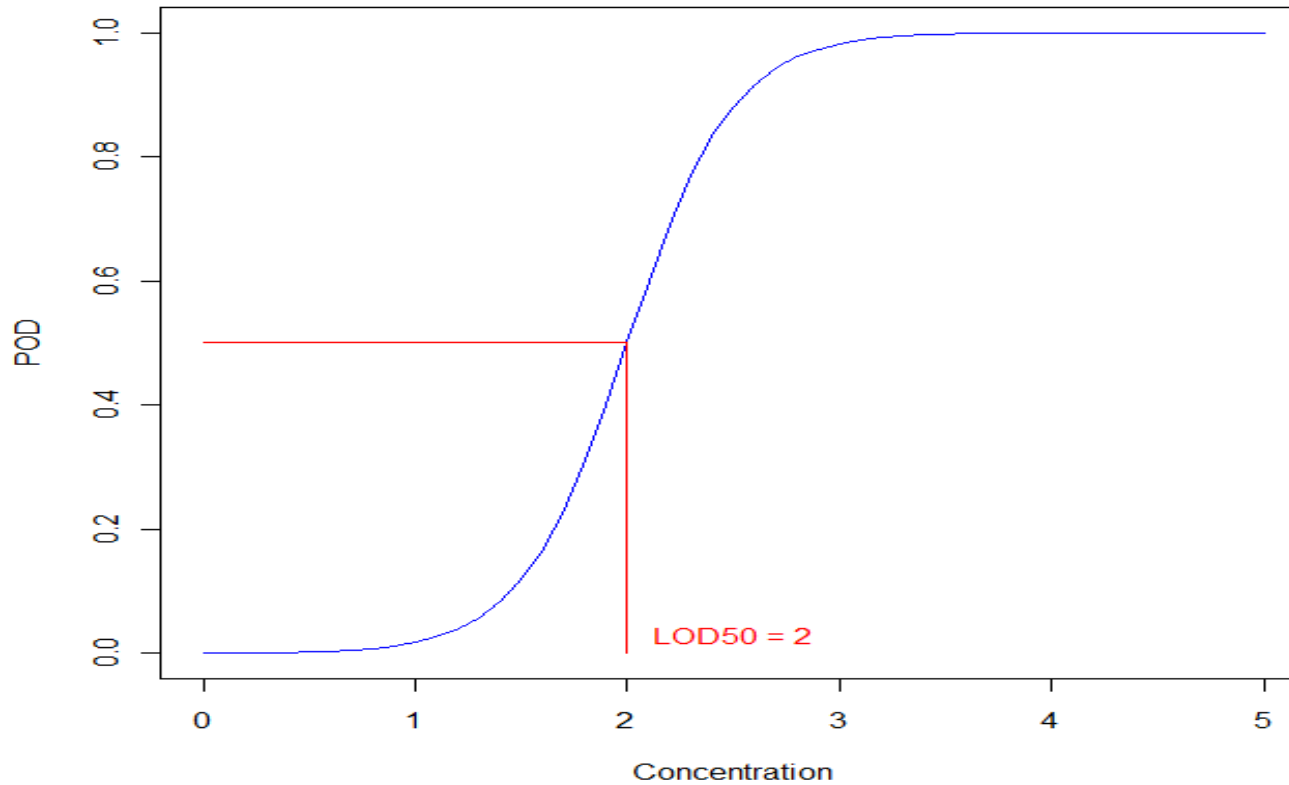
## *II: Transition region*

- The 'I' set of requirements does not speak to the transition range of the POD vs. Concentration curve.
- A method which satisfies the  $POD_{\min}$  performance requirement at lower Concentration is 'better' than another method does so at higher Concentration.
- A method which has  $POD < 1$  may still be useful in repeated testing if no better method is available (e.g., outbreak investigations for micro).

# *Limit of Detection 'LOD'*

- One way commonly used to characterize a method in the transitional range is to estimate the concentration at which a particular POD is attained.
- 'LOD50': Concentration for which  $POD = 0.50$
- 'LOD90': Concentration for which  $POD = 0.90$
- Various techniques for estimation, including non-parametric ones, such as linear interpolation and Spearman-Kaerber (POD-based), or assumed models.
- Requires several points (at least 2, preferably more) in the transitional or 'fractional' range.
- Requires accurately known concentrations!

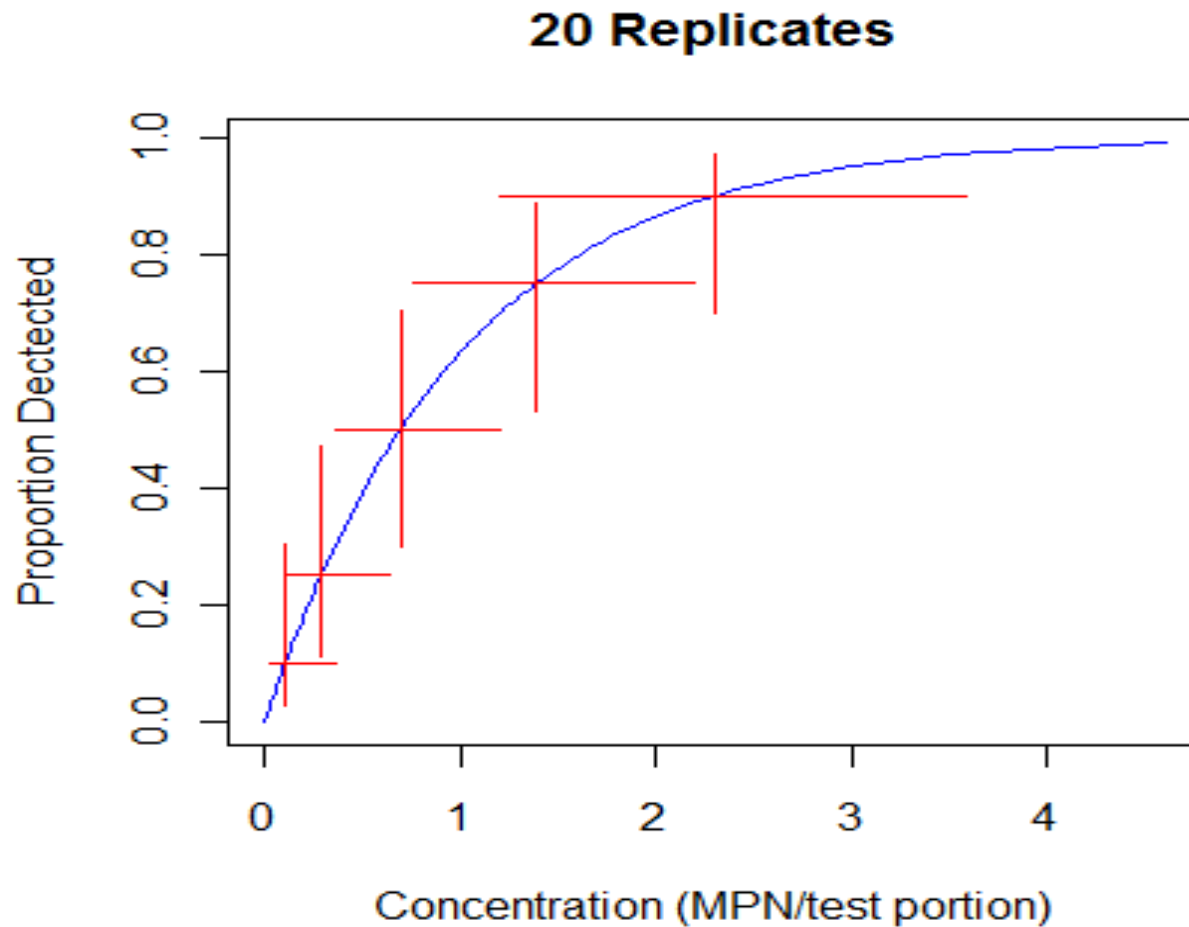
# LOD50



# The 'Concentration Fallacy' for Micro Methods

- The transition region for qualitative methods for micro testing typically occurs below 10 CFU/test portion and so cannot be quantified by plate count methods, particularly with other flora present. Instead a 'MPN' method is used, based on a reference qualitative method.
- So Concentration is determined from POD (not v.v.), and typically has large error limits (e.g.,  $\pm 60\%$  or much worse). POD is known more accurately than Concentration.
- Models fitting POD using Concentration as a predictor are *invalid*.
- LOD50 will be imprecise and unknown to a multiplicative factor (bias) due to clumping of cells.

# Micro: 1-Hit-Poisson Model



# *Method Performance Requirements*

## *III: Comparison of Methods*

- Two methods which both satisfy the ‘I’ requirements equally can only be discriminated if one or the other has data in its transition range.
- There are a number of measures of effect in common use to compare a candidate method ‘C’ to a reference method ‘R’ (or any two methods) to each other, based on measured POD values at different concentrations in the transition range (i.e., fractional POD range).

# *Difference in POD:* $dPOD(C,R)$

- The most basic comparison between a reference method 'R' and a candidate method 'C' is the difference in their POD values at a fixed concentration

$$dPOD(C,R) = POD(C) - POD(R)$$

- Non-constant for difference concentrations.
- Expected difference in # positives easily estimated as  $n \times dPOD(C,R)$ .
- Requires no assumptions, applicable in all cases.

# Odds ratio $\omega$

- The most common measure of effect used to compare two binary methods in scientific research is the ‘odds ratio’ or ‘ $\omega$ ’

$$\omega = \frac{\text{POD(R)}/[1-\text{POD(R)}]}{\text{POD(C)}/[1-\text{POD(C)}]}$$

- If a Logit model is appropriate, the ‘odds ratio’ is a constant across concentrations.

# *Poisson Relative Efficiency*

## *'PRE' or 'R'*

- LaBudde, R.A. (2006). Statistical analysis of interlaboratory validation studies. X. Poisson-plot and Poisson relative efficiency to compare the dose-response curves of two presence-absence methods. TR239. Least Cost Formulations, Ltd., Virginia Beach, VA.

$$\bullet \quad R = \frac{\ln [ 1 - \text{POD}(C) ]}{\ln [ 1 - \text{POD}(R) ]} = \frac{\gamma_R}{\gamma_C}$$

where the one-hit Poisson model '1HPM' is assumed to hold.

# *PRE (cont'd)*

- If both the reference and candidate methods obey the 1HPM model with cluster sizes  $\gamma_R$  and  $\gamma_C$ , resp., the  $R = \gamma_R / \gamma_C$  is the ratio of the two cluster sizes needed. If the reference method is 'better' (has a lower  $\gamma$  or LOD50), then  $R < 1.0$ .
- If the 1HPM is valid,  $R$  will be constant across different concentrations.
- Generally applicable to micro studies only.

# *Relative LOD 'RLOD'*

- Anon. (2008). ISO 16140.
- If a 1HPM model assumption is made for the mathematical form of POD, and  $\log(\text{Concentration})$  is used as the metamer in the model, a 'complementary-log-log' model results.
- For the complementary-log-log model,  $RLOD = R$ , and  $\gamma_R$  and  $\gamma_C$  are the factor coefficients for the 'Method' term in the regression model.
- 'RLOD' is the same value as 'PRE' = 'R'.

# *R vs. $\omega$ vs. POD*

- If  $\text{POD}(C)$  and  $\text{POD}(R)$  are both small,  
$$R \sim \omega \sim \text{POD}(C) / \text{POD}(R)$$
- If  $\text{POD}(C)$  and  $\text{POD}(R)$  are both large,  
$$R \sim \omega \sim \text{POD}(C) / \text{POD}(R)$$
- If  $\text{POD}(C) \sim \text{POD}(R)$ ,  
$$R \sim \omega \sim \text{POD}(C) / \text{POD}(R) \sim 1$$
- Differ more otherwise.

# Examples: Micro

Analyte	Matrix	Study	Level	MPN(R)	dPOD(C,R)	w(C,R)	R(C,R)
<i>Salmonella</i>	Raw ground turkey	Unpaired	H	3.66	-0.04	0.38	0.75
<i>Salmonella</i>	Raw ground beef #1	Unpaired	H	2.18	-0.13	0.40	0.65
			H	2.33	-0.10	0.45	0.70
<i>Salmonella</i>	Raw ground beef #2	Unpaired	H	2.11	-0.11	0.47	0.70
			L	0.58	-0.23	0.34	0.41
<i>Salmonella</i>	Dried whole egg	Paired	H	2.58	-0.05	0.59	0.82
			L	1.05	-0.03	0.88	0.92
<i>Salmonella</i>	Milk chocolate #2	Paired	H	1.55	-0.03	0.84	0.91
			L	0.48	-0.03	0.88	0.90
<i>Salmonella</i>	Dry dog food	Paired	H	1.10	-0.03	0.86	0.91
			L	0.27	-0.02	0.91	0.92
<i>E. coli</i> O157:H7	Raw ground beef	Unpaired	H	3.18	0.72	74.41	11.80
			L	0.84	0.46	11.61	7.93

# *Method Performance Requirements III*

Possible performance requirements:

$|dPOD(C,R)| < dPOD_{\max}$  with 95% confidence

$\omega(R,C) > \omega_0$  with 95% confidence

$R(C,R) > R_{\min}$  with 95% confidence

# *Non-Micro Methods*

- Toxins, residues, chemicals, allergens, botanicals.
- There is a large literature associated with POD vs. Concentration modeling and fitting for toxicology.
- Complementary-log-log is not typically a good match for non-micro methods, typically logit and probit have been used successfully.
- *None* of the standard regression models work for botanical identification methods where complex thresholding occurs.

# *Choice of metamer*

- Most models use either Concentration directly or  $\log(\text{Concentration})$  as a predictor.
- The transform of Concentration to a new independent variable is called the ‘metamer’ of Concentration.
- It should be noted that linear models using Concentration as metamer and linear models using  $\log(\text{Concentration})$  as metamer cannot both be correct.

# *Warning for micro studies*

- In the case of micro methods, cells are indivisible, and CFUs finitely divisible, so sampling error dominates at low concentrations. Method differences are obscured, if they have low LOD50's.
- The 1HPM or complementary-log-log model will *appear* to fit the data very well, and this is fallacious because Concentration is determined assuming 1HPM in the MPN method. Micro study modeling should not use numerical values of Concentration!

# *Conclusions*

- Models involving Concentration are flawed at inception for micro methods. (This applies to Method Performance Requirements III in the transition region and to LOD50.)
- Serial dilution should be considered as a possible method to achieve a POD-independent Concentration estimate.
- Many alternative models exist, each with their own history and literature.
- PRE (aka RLOD or R) depends on the assumption that the 1HPM is correct, which it often is not.

# *Conclusions (cont'd)*

- LOD50 requires Concentration be known reasonably accurately, problematic for micro.
- LOD50 can be nonparametrically estimated from POD, with no model assumptions.
- The choice of statistic used to characterize the transition region of POD vs. Concentration should be made based on the scientific validity of the model assumptions and the ease and usefulness of interpretation of the result.
- Chemical-based methods have accurate Concentrations; Micro studies do not.

# *Conclusions (cont'd)*

- Use of the wrong model form will may give poorer results than using the POD vs. Concentration curve directly, and comparing methods by POD difference ('dPOD').
- Model forms that work for one analyte or matrix may not be appropriate for another, even in the same scientific method area.
- Nonparametric methods (POD included) that are distribution and model assumption-free are preferred to unvalidated model assumptions.