Basic principle of stability study design and analysis for product licensure

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WORKSHOP ON
STATISTICAL ANALYSIS OF STABILITY TESTING

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Some notes on vaccine stability

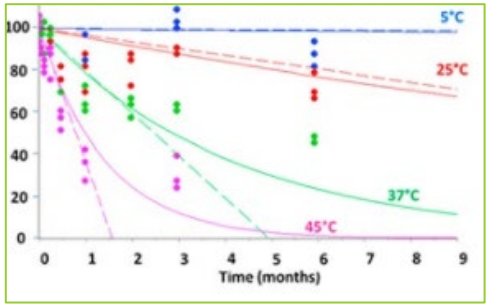
- Stability study goals differ throughout the product lifecycle
- The goal of a vaccine stability study is to estimate an important stability parameter (e.g., slope) rather than demonstrate conformance to specifications
- Stability study design and analysis should be effective at meeting the study goal (e.g., reducing uncertainty)
- Vaccine properties such as potency should remain within appropriate minimum and maximum requirements throughout shelf life
- The goal of lot release is to demonstrate that a manufactured lot will be effective throughout shelf life

Outline

- Some basis concepts
- Studies supporting product licensure
- Example of shelf life determination for a measles vaccine
- Determination of release potency
- Accelerated stability and its use in the release model
- Managing the release limit through design
- Summary

Some basic concepts

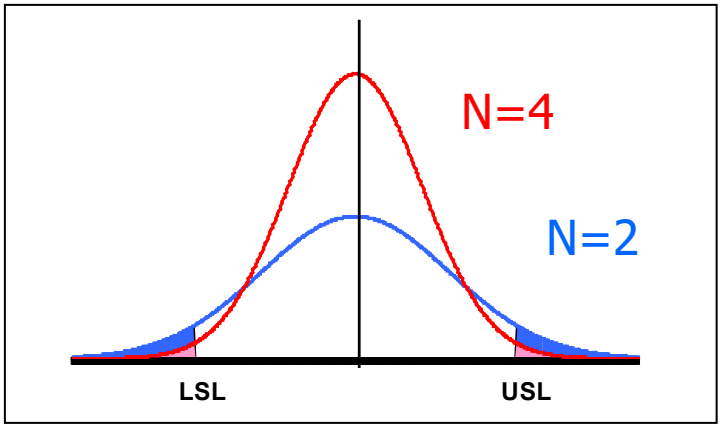
- Most vaccines are unstable
  - Require storage from 2-8°C (protein and conjugated polysaccharide vaccines) to -70 °C (mRNA vaccines)
- Kinetics are often 1st order (esp. potency)
  - $y = a \cdot e^{-bt}$ or $\ln(y) = \ln(a) - bt$
  - Revealed in early accelerated stability studies



- Most meaningful attributes of vaccines are measured using highly variable assays
  - e.g., potency – *in vivo* for older vaccines (>50% GCV)

Some basic concepts (cont.)

- Stability studies should be designed to provide a suitable fit to statistical (e.g., kinetics) models
- Statistical design is aimed at controlling (minimizing) the uncertainty associated with the statistical model
  - Uncertainty = z · Variability (e.g., 2-sigma)
  - Lower uncertainty results in lower risk
    - Risk (e.g., probability of an OOS) can be visualized as the area under a normal curve
  - Stability design elements include replication, time points, and ranges between time points



Studies supporting product licensure

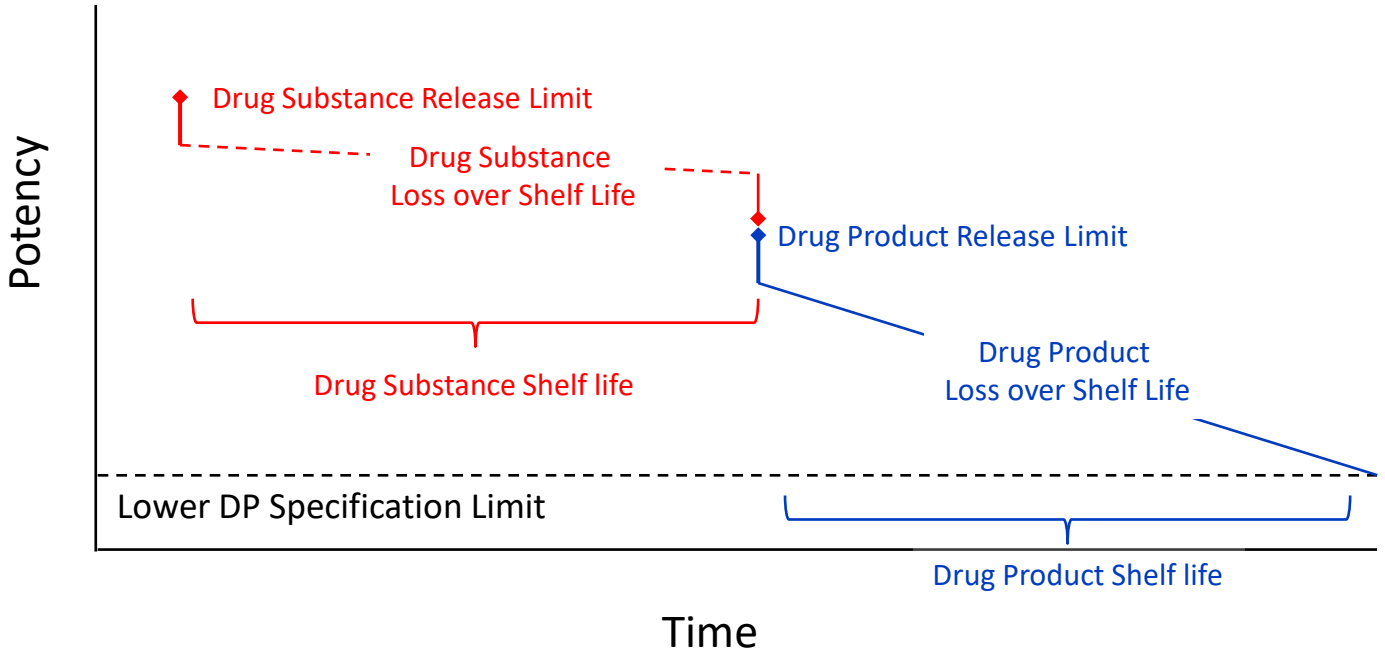
- Studies supporting product licensure include
  - Long term stability of drug substance (DS)
  - Long term stability of drug product (DP)
  - Accelerated stability at conditions of handling, excursion, and use

Studies supporting product licensure
Long term stability of drug substance

- Study goal:
  - Establish that DS can be stored at a specified storage condition for a specified period of time without impact on final product quality
- Quality criteria:
  - DS is suitable for filling into final container throughout DS shelf life
    - ***Adequate potency to ensure meeting target DP potency***
    - Note: this is a business risk

Studies supporting product licensure
Long term stability of drug substance (cont.)

- ***Adequate potency to ensure meeting target DP potency***



Studies supporting product licensure
Long term stability of drug substance (cont.)

- Cumulative age studies
  - Difficult to simulate the total age (DS + DP) of an antigen during development
    - Some DS's are stored 5 to 10-years
    - DP undergoes multiple temperature transitions
  - "Modeling" cumulative age is preferred to "studying" cumulative age
  - Accelerated stability studies may be performed to experimentally demonstrate cumulative age can be modeled rather than studied
    - e.g., Compare stability of DP which has been filled after accelerated aging of DS and not
  - Can be verified as part of ongoing monitoring of vaccine stability
    - Assess relationship of DP stability to age of DS

Studies supporting product licensure
Long term stability of final container product

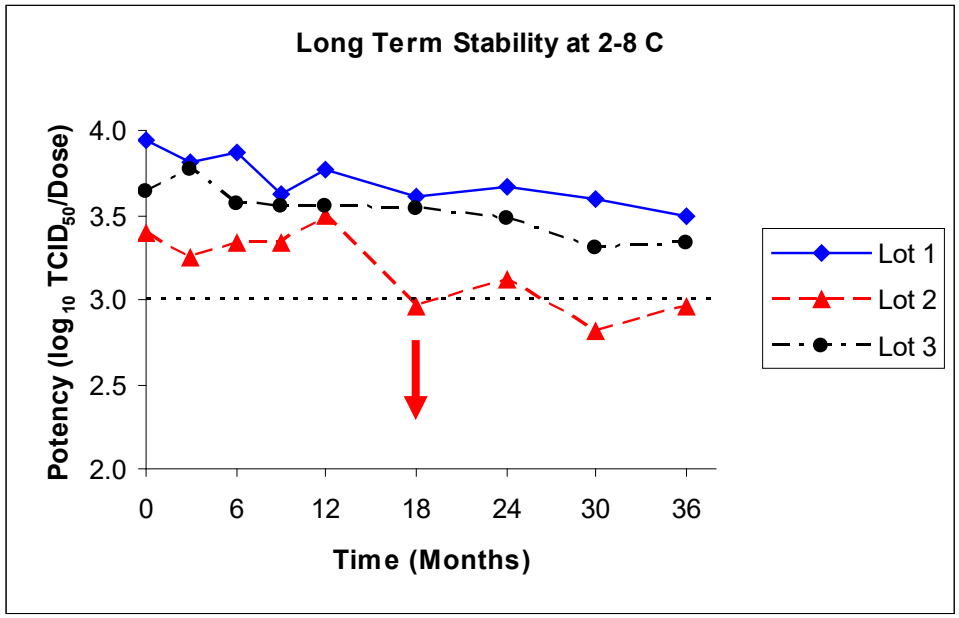
- Goal:
  - Establish shelf-life of DP, or
  - Develop a release model for DP
- Quality criteria:
  - Satisfactory potency through product shelf-life
  - Maintenance of levels of factors which might impact stability
    - Moisture of a lyophilized vaccine
    - pH of an aluminum adjuvanted vaccine

Studies supporting product licensure
Example of shelf life determination for a measles vaccine

- Typical design of 3 lots tested at ICH timepoints
  - Lower specification limit is 3.0 $\log_{10}$ $TCID_{50}$/mL

	Potency ($\log_{10}$ $TCID_{50}$/mL)		
Time (Months)	Lot 1	Lot 2	Lot 3
0	3.94	3.39	3.63
3	3.81	3.24	3.76
6	3.87	3.33	3.56
9	3.62	3.33	3.56
12	3.77	3.49	3.56
18	3.61	2.95	3.53
24	3.67	3.11	3.47
30	3.59	2.81	3.30
36	3.49	2.96	3.33

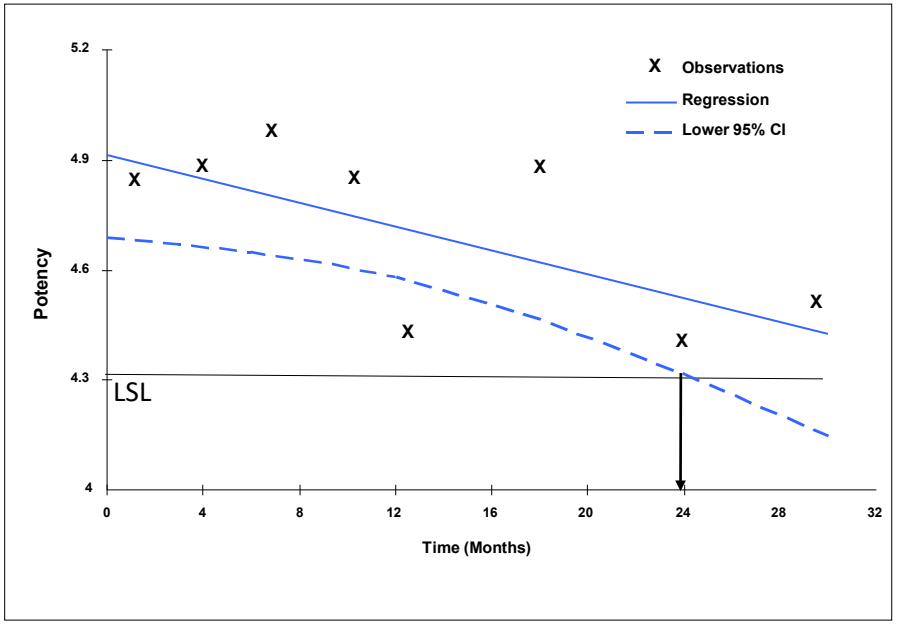
Studies supporting product licensure
Example of shelf life determination for a measles vaccine (cont.)



- Is shelf-life 12-months due to a measured value for Lot 2 below the lower specification limit at 18-months?
- Individual measurements do not effectively address the stability of the lot

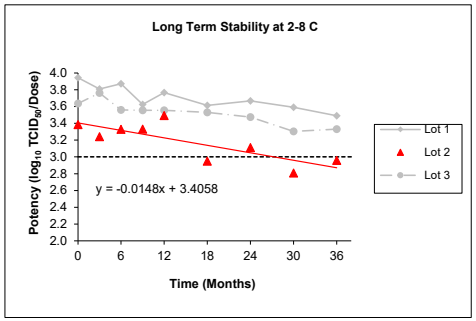
Studies supporting product licensure
Example of shelf life determination for a measles vaccine (cont.)

- ICH analysis (Q1E)
  - Goal: establish shelf-life
  - 3-lots (ICH Guidance)
  - Fit a statistical model
    - Tests parallelism and level (poolability)
    - Pooled or worst case
  - Shelf life is the intersection of the lower specification limit (LSL) and the lower 1-sided 95% confidence bound on the regression line



Studies supporting product licensure

Example of shelf life determination for a measles vaccine (cont.)

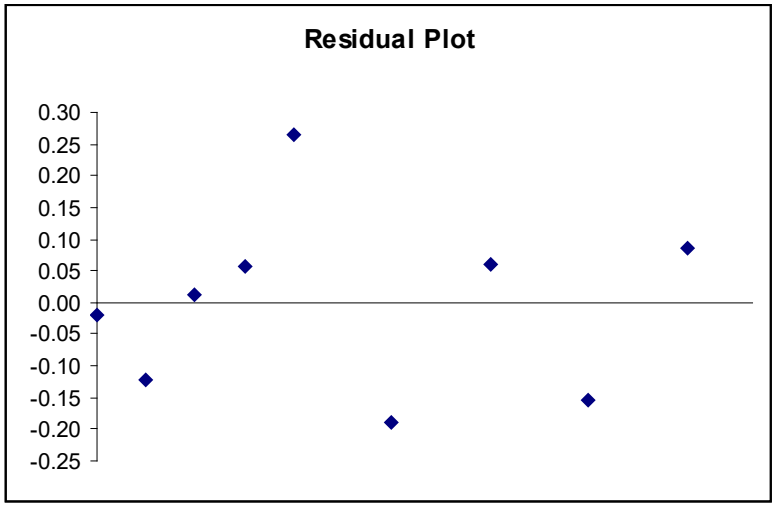


Example – live attenuated measles vaccine (cont.)

- Evaluation of Lot 2 alone

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	3.40627	0.08192	41.58	<.0001
month	1	-0.01483	0.00424	-3.50	0.0100

Root MSE	0.14976	R-Square	0.6365



- No pattern in the residuals (deviations of the observed points from the line)
- Root MSE (assay variability) is typical for the potency assay (~0.15 log)
- The slope is statistically significant (P=0.01)

Studies supporting product licensure
Example of shelf life determination for a measles vaccine (cont.)

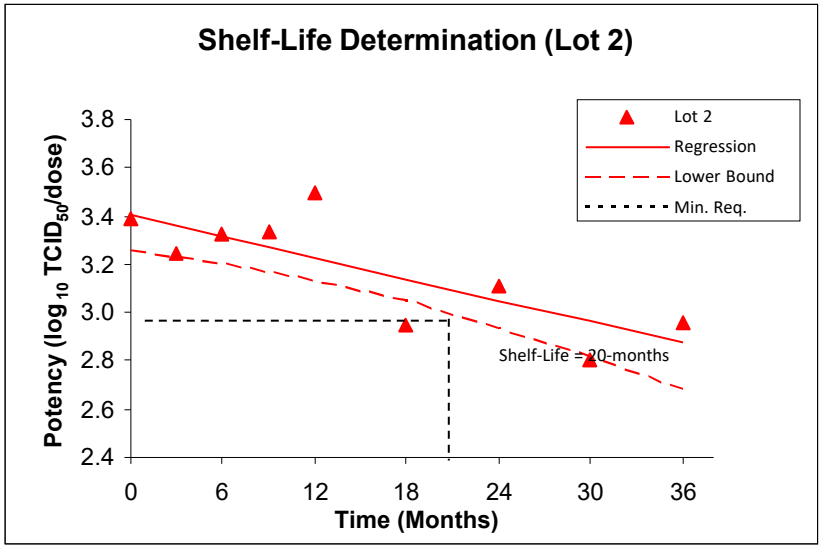
Evaluation of Lot 2 alone (cont.)

- Shelf-life determination

$$\text{Lower 95\% CB} = \hat{y} - t_{0.05,df} \cdot s \cdot \sqrt{\frac{1}{n} + \frac{(t - t_{avg})^2}{SXX}}$$

$$\text{where } \hat{y} = \hat{a} + \hat{b} \cdot t$$

Month	CB	
18	3.04	
19	3.03	
20	**3.01**	≥3.00
21	2.99	<3.00
22	2.97	
23	2.95	
24	2.93	



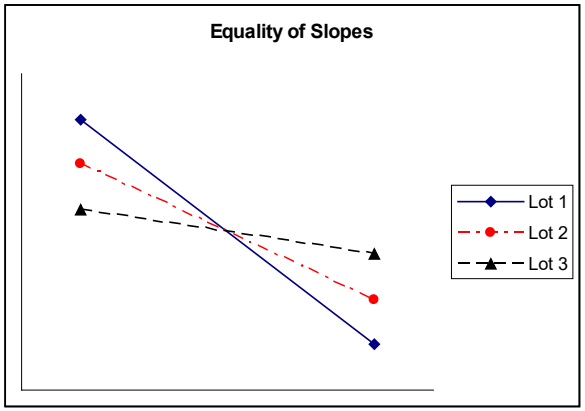
Studies supporting product licensure
Example of shelf life determination for a measles vaccine (cont.)

Evaluation using all lots

- Establishing poolability
  - Equality of slopes is established using a formal test of parallelism –

    parallel if P-value sufficiently high (P=0.4682 > 0.25)

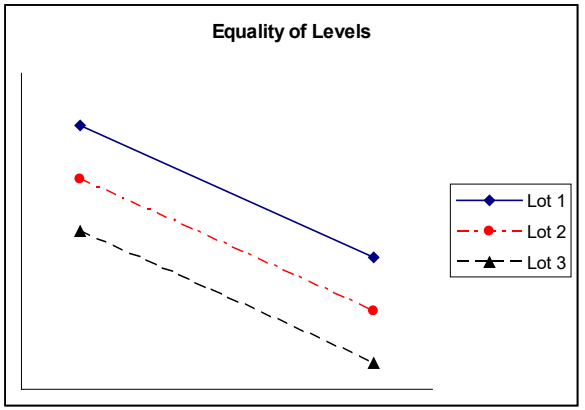
Source	DF	Type I SS	Mean Square	F Value	Pr > F
lot	2	1.29609630	0.64804815	59.83	<.0001
month	1	0.52348819	0.52348819	48.33	<.0001
month*lot	2	0.01704605	0.00852303	0.79	0.4682



  - Equality of levels is tested after fitting a common slope –

    equal levels if P-value is sufficiently high (P<0.0001 is not >0.25)

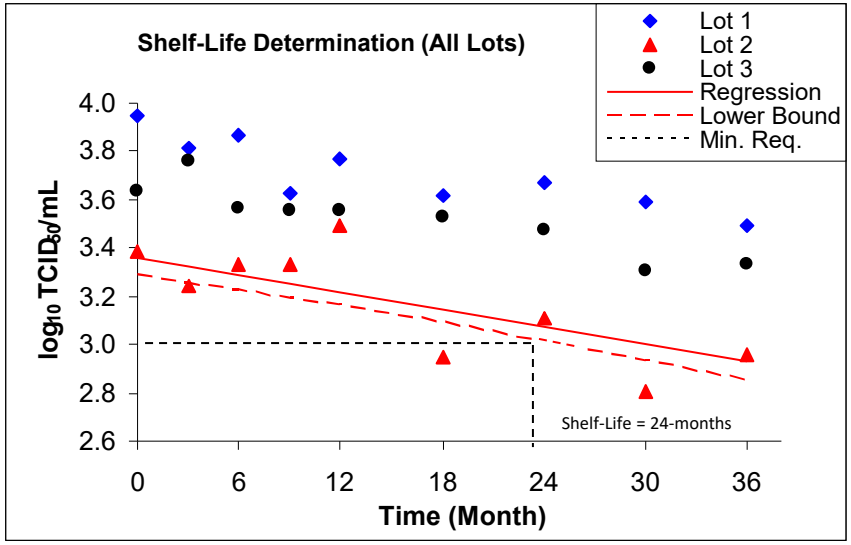
Source	DF	Type I SS	Mean Square	F Value	Pr > F
lot	2	1.29609630	0.64804815	60.96	<.0001
month	1	0.52348819	0.52348819	49.24	<.0001



Studies supporting product licensure
Example of shelf life determination for a measles vaccine (cont.)

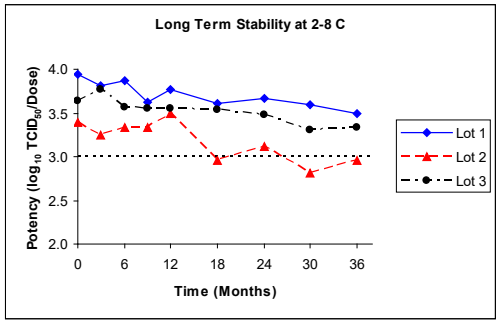
- Evaluation using all lots (cont.)
  - Shelf-life determination
    - Utilizing a model with common slope but different levels
    - Use “worst case” lot (lot 2) to determine shelf-life

Month	LB	
21	3.05	
22	3.04	
23	3.02	
24	**3.01**	≥3.00
25	3.00	<3.00
26	2.99	
27	2.97	



Studies supporting product licensure

Example of shelf life determination for a measles vaccine (cont.)

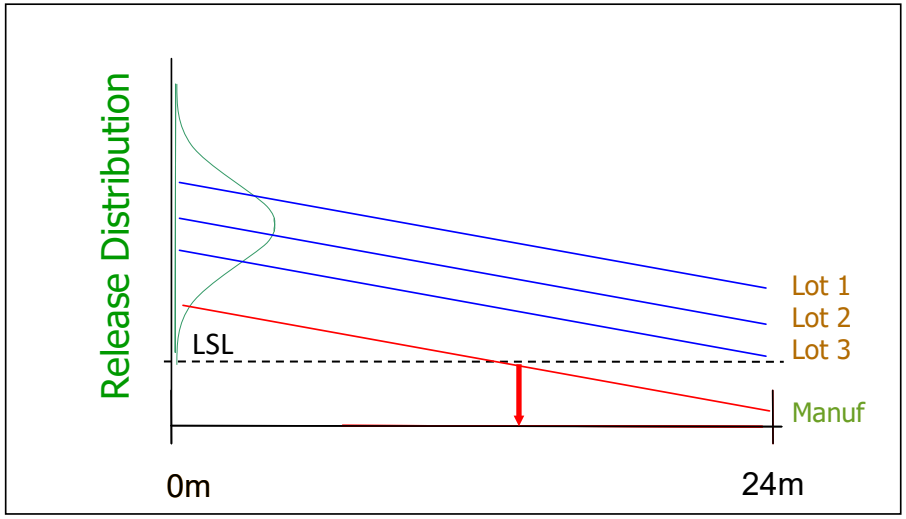


Comparison of approaches

Approach	Estimate	Comments
Individual Measurements	12-months	Does not effectively use all the data to show potency trend; post 12-month potencies are within specification.
Individual Lot	20-months	Effectively uses data for the worst case lot to estimate shelf-life; does not use the full power of the long term stability study. Recommended if the loss rates are not comparable among lots.
Combined Lots	24-months	Effectively uses all the data from the long term stability study. Recommended if the loss rates are comparable among lots.

Determination of release potency

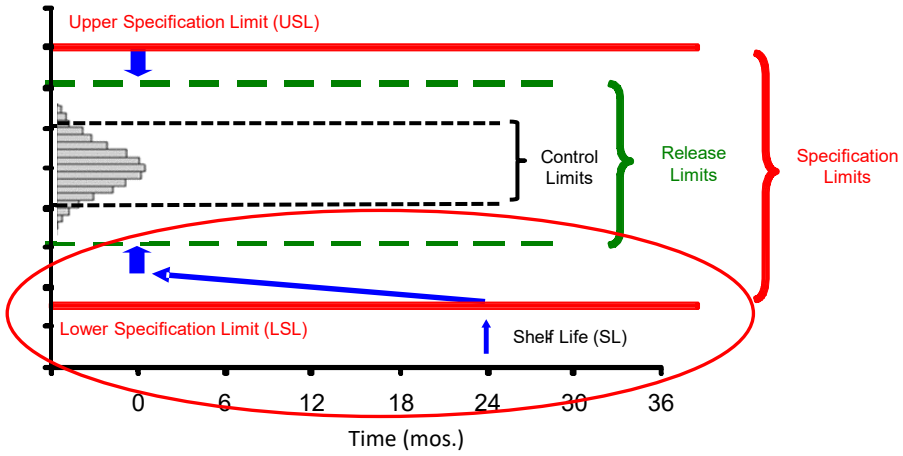
- ICH stability evaluation does not ensure quality of manufactured lots throughout shelf life



- ICH 1E: Determine shelf life from pooled (n = 3) or worst-case lot
- However, stability lots do not represent the total distribution of release potencies of future manufactured lots
- Manufactured lots which are released below the distribution of stability lots are predicted to fall below the lower specification limit (LSL) prior to expiry

Determination of release potency (cont.)

- A vision for the vaccine analytical control strategy



$$Lower\ Release = LSL + b \cdot SL + z_\alpha \sqrt{(s_b \cdot SL)^2 + s_{Assay}^2}$$

Schofield, *Licensure*, 2009

$b = slope/month$

$z_\alpha = statistical\ factor\ (95\%\ confidence)$

$s_b = variability\ of\ slope$

$s_{Assay} = assay\ variability$

- Scientifically/clinically justified upper and/or lower specification limits
- Release limits calculated to ensure quality at release and throughout shelf-life
- Control limits formulated to help manage manufacturing consistency

Accelerated Stability

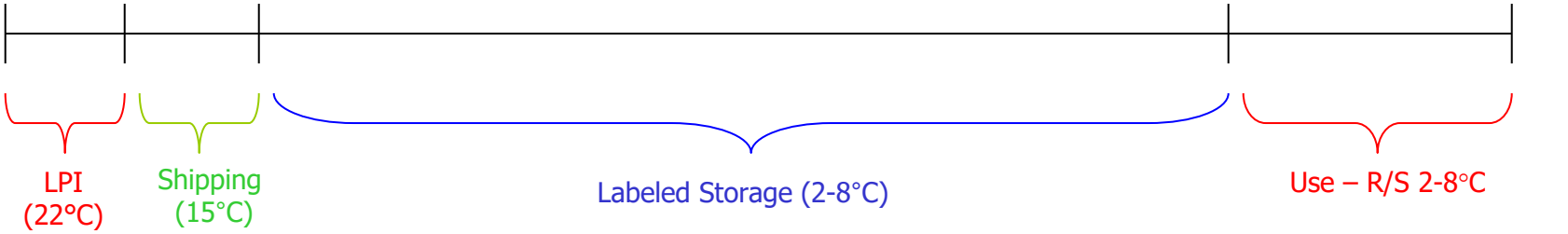
- Uses of accelerated stability
  - Development
    - Formulation development
    - Method validation
    - ***Release modeling***
    - Mechanism of degradation
    - Handling and use protocols
  - Post licensure
    - Stability monitoring
    - Stability comparability protocols
    - Managing excursions

Use of accelerated stability in the release model

- Guidelines
  - WHO *Guidelines on Stability Evaluation of Vaccines* (2006)
  - WHO *Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions* (ECTC, 2015)

$$Release = LSL + \sum t_i b_i + t_{df} \sqrt{\sum t_i^2 s_{b_i}^2 + s_{RelAssay}^2}$$

Note: ECTC includes 40°C for 3-days for WHO certification



Use of accelerated stability in the release model (cont.)

$$\text{Release} = \text{Expiry} + \sum t_i b_i + t_{df}\sqrt{\sum t_i^2 s_{b_i}^2 + s_{\text{Assay}}^2}$$

Condition	Temperature	Loss Rate	Std.Err.	Time at Condition	Loss	Error
Labeling, Packaging, & Inspection	20-25° C	0.0025/hr.	0.0005	24-hrs.	0.06	0.012
Self-Life	2-8° C	0.012/mo.	0.0017	24-mos.	0.29	0.041
Reconstitute & Store	2-8° C	0.0031/hr.	0.0005	8-hrs.	0.025	0.004
Release Assay Variability			0.08			0.08
					Total Loss = 0.3728 Clinical Minimum = 3.0	Total Error = 0.0907 Minimum Release = 3.5

$$\sum t_i \cdot b_i = 24 \cdot 0.0025 + 24 \cdot 0.012 + 8 \cdot 0.0031$$
$$= 0.06 + 0.29 + 0.025 = 0.3728$$

$$\sqrt{\sum t_i^2 \cdot s_{b_i}^2 + s_{\text{Assay}}^2} = \sqrt{24^2 \cdot 0.0005^2 + 24^2 \cdot 0.0017^2 + 8^2 \cdot 0.0005^2 + 0.08^2}$$
$$= \sqrt{0.012^2 + 0.041^2 + 0.004^2 + 0.08^2} = 0.0907$$

$$\text{Release} = 3.0 + 0.3728 + 1.645 \cdot 0.0907 = 3.5$$

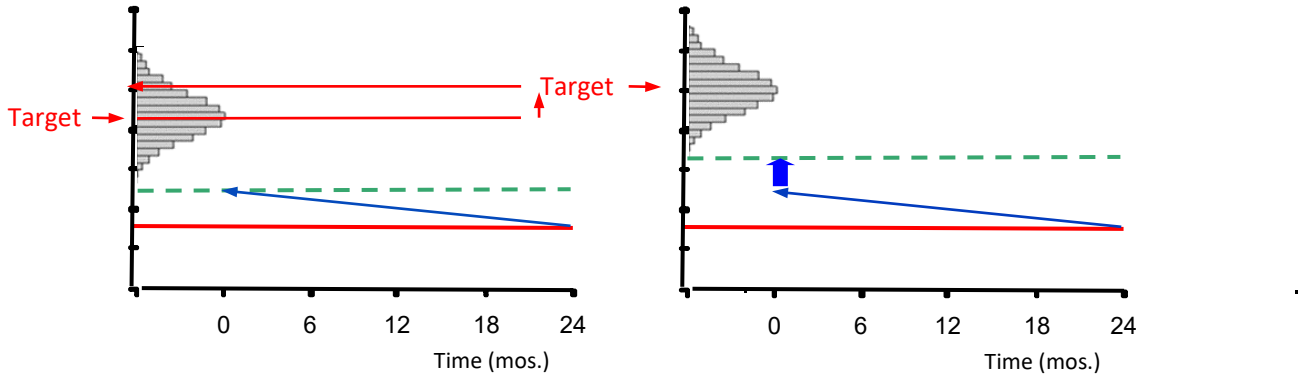
Managing the release limit through design

$$\text{Release} = 3.0 + 0.3728 + 1.645 \cdot 0.0907 = 3.5$$

- The "uncertainty" in the release limit calculation contributes:

  $1.645 \cdot 0.0907 = 0.15$ log (~30%) to the total loss + uncertainty

- Results in an increase in the manufacturing target



  - Forces production towards the upper specification limit (i.e., greater risk of OOS)
  - Decreases capacity, and thus availability to the customer

Managing the release limit through design
Reduce potency assay variability through strategic replication

$$Release = LSL + \sum t_i b_i + t_{df} \sqrt{\sum t_i^2 s_{b_i}^2 + s_{RelAssay}^2}$$

$$s_{RelAssay}^2 = \frac{s_{Between}^2}{r} + \frac{s_{Within}^2}{nr}$$

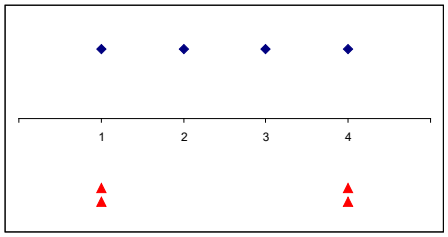
	Runs (r)			
n	1	6	9	12
1	44%	16%	13%	11%
2	41%	15%	12%	10%
3	39%	15%	12%	10%

- Variance components ($s_{Between}^2$ and s_{Within}^2) are from assay validation
  - Combinations of number of runs (r) and number of reps within runs (n) can be explored to reduce release assay variability
- The "Reportable Value" is the average
- The stability testing format needn't be the same as release testing
  - The goal of stability testing is different than the goal of release testing

Managing the release limit through design
Reduce slope variability through strategic stability study design

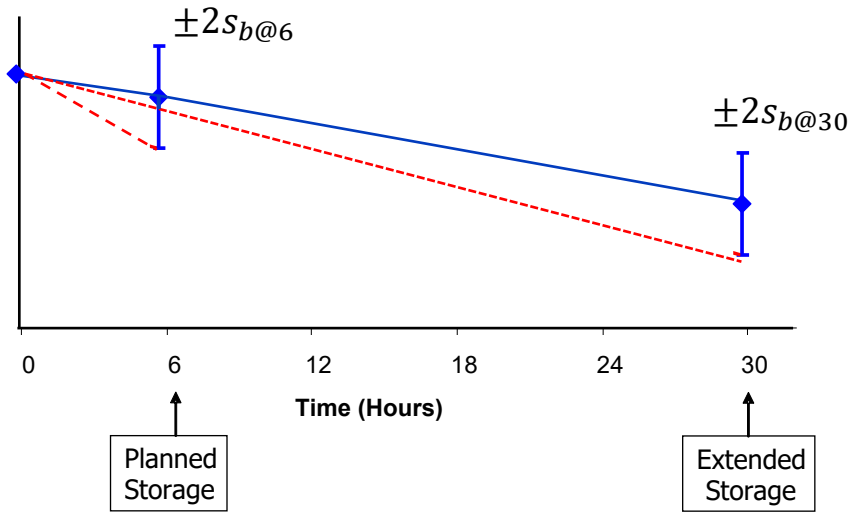
$$Release = LSL + \sum t_i b_i + t_{df}\sqrt{\sum t_i^2 s_{b_i}^2 + s_{RelAssay}^2}$$

Design 1	Design 2
1	1
2	1
3	4
4	4
s_b=0.45	s_b=0.33
Reduction=25%	



- Stability variability is managed through strategic selection of time points
  - Test at beginning and end of study
    - Significant reduction in variability (25%)
    - Note: assumes linear kinetics
  - Test at extended intervals

$$s'_{b@6} = s_{b@30}/\sqrt{5}$$



Managing the release limit through design
Other opportunities to reduce variability

- Calibration to a standard
- Reference to an unincubated sample (-70^{o} C) from the same lot
- Staged testing
  - Appropriate when the slope is the parameter of interest, and there is significant run-to-run variability
  - Withdraw samples at stability time points, store at -70° C, test together with unincubated samples
    - Requires sophisticated statistical design and analysis is required
      - Analysis of covariance

Some current barriers

- Lack of separate release and end-of-shelf-life limits
- Setting shelf life based on individual values
  - Last time point within specification
  - Modeling individual values using a prediction interval versus a confidence interval
- Incorrect kinetics modeling
  - Zero versus first order
- Sequential stability studies
  - A study holding a DS through shelf life, then formulated and held through DP shelf life
  - End-to-end modeling versus end-to-end studies

Summary

- Drug substance (bulk) evaluation addresses whether the intermediate with be suitable for filling into drug product (DP) throughout its shelf life
- Cumulative age may be addressed post licensure, or using accelerated stability to simulate suitability of aged DS
- Accelerated stability studies are used throughout development and post licensure to address development questions and to forecast impact on product which has been exposed to elevated temperatures
- Statistical methods make use of all the data to obtain a reliable estimate of product shelf-life
- A release model can be used to help assure a commercial lot will maintain adequate potency after handling, shipment, long-term storage, and use

References

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Thank you

Questions?

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