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Combining DOE with an empirical approach to improve vaccine formulation development

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Better Health, Brighter Future

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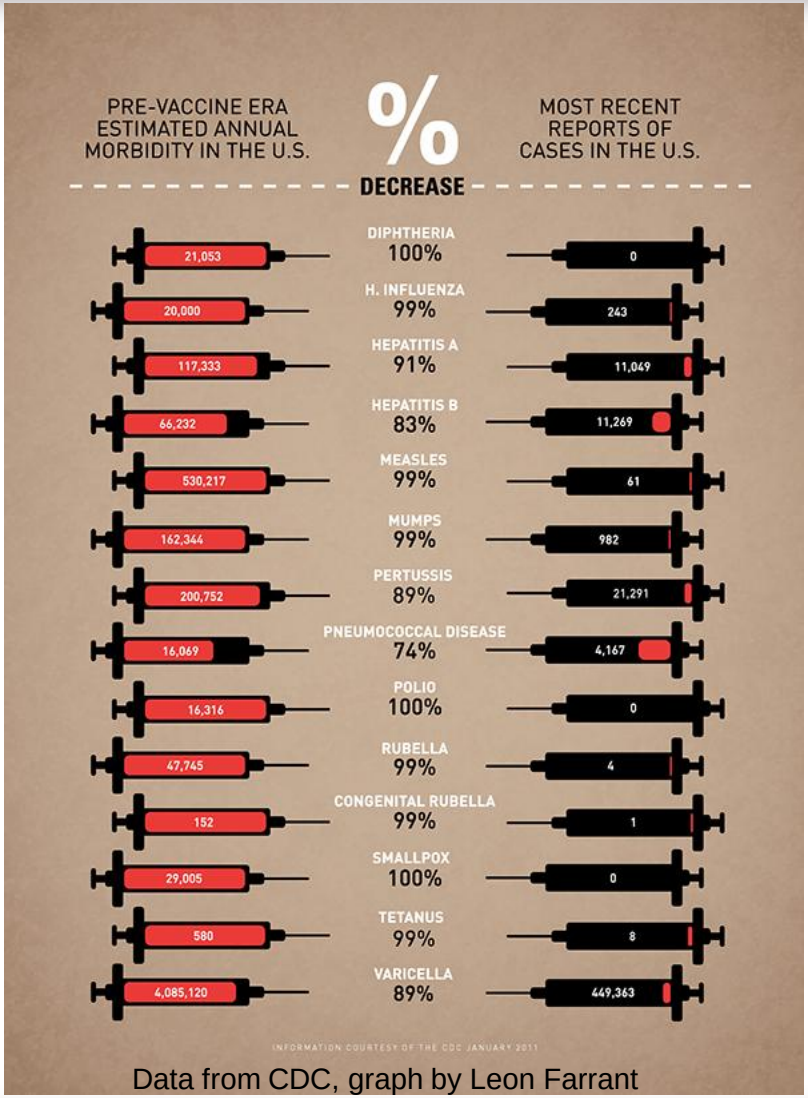
Jill A. Livengood, Ph.D.

Vaccine Business Unit

Vaccines

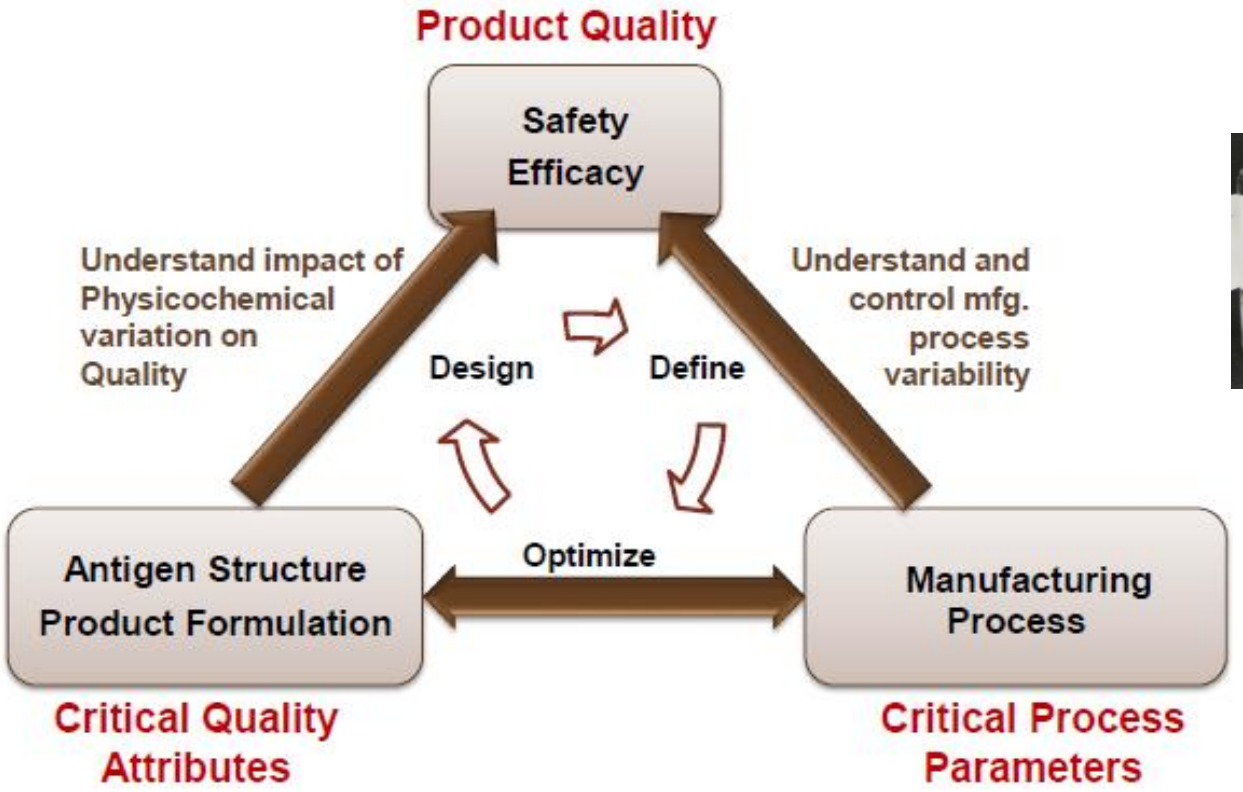
1. One of the 10 greatest Public Health Achievements of the 20th Century (MMWR 1999)
2. Antigen or attenuated live virus to trigger immune response for disease protection
3. Biological Products
   - Stability is critical
4. Pediatric and Adult
   - Complex immunological environment
   - Healthy subjects

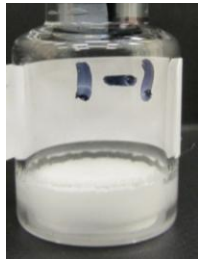


Data from CDC, graph by Leon Farrant

Quality by Design

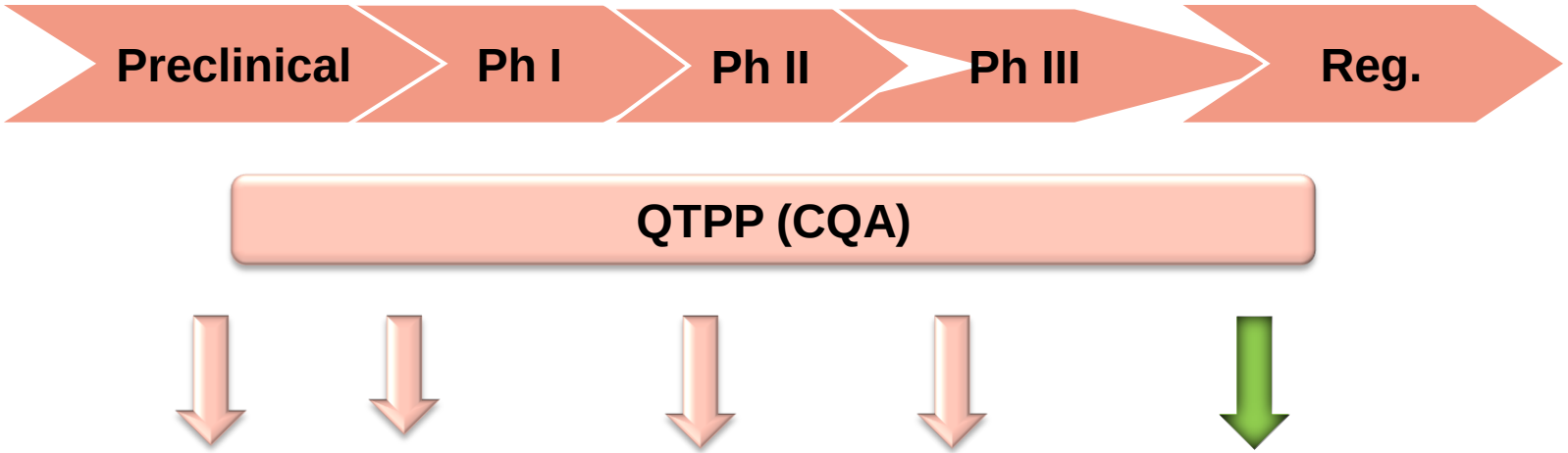
Begin vaccine formulation with the end in mind





QbD: “a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management in order to ensure the quality of the product” - ICH Q8

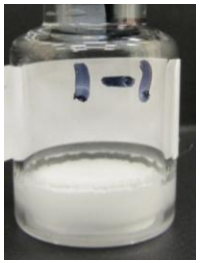
Building a Quality Target Product Profile QTPP



Begin (*formulation development*) with the end in mind

QTPP for Live Attenuated Vaccines *(example)*

1. Potency (live biological product)
   - Initial retention of titer: $\leq$ 0.5 $\log_{10}$ loss of titer
   - Shelf life ~ 2 years at 4° C
     - lyophilized
   - Stability at 4º, 25º and 40ºC storage: $\leq$ 0.5 $\log_{10}$ loss of titer
   - Freeze-thaw liquid stability: $\leq$ 0.5 log loss of titer
   - Stable in liquid state during processing



2. Physical Attributes
   - Re-constitution time: $\leq$ 2 minutes to a clear, colorless solution
   - pH: physiologic (7.2 to 7.4)
   - isotonicity: 280 to 390 mOsm/L
   - Cake appearance: white, dense, uniform cake, no evidence of collapse
3. Animal-derived protein-free formulation

Better Health, Brighter Future

Part I: Formulation Development – Empirical Approach

Vaccine Business Unit

Excipient Identification and Screening

Empirical approach

1. Product Biology
2. Literature search
3. Freedom to Operate
4. Analytics
   - Stability indicating assay
   - High throughput
5. Buffer Screen: liquid

Excipients	
Proteins/Polymers	Human serum albumin (0.05-2.0%)
	Gelatin (0.05-3.0%)
	Dextran(1-10%)
Sugars	Sucrose (2-12%)
	D-Trehalose dihydrate (1-10%)
	Lactose (1-10%)
Polyols	Sorbitol (2-7%)
	Mannitol (1-10%)
Surfactants	Pluronic® block copolymers (0.0001-1.0%)
	Polysorbate 80 (0.05%)
Amino acids	Monosodium l-glutamate or Potassium glutamate (10-100mM)
	Arginine (5-100mM)
	Alanine (1-100mM)
	Methionine (2.5mM)
Osmolytes	Urea (0.2 to 0.5%)
Salts	Magnesium chloride, Sodium chloride (50 to 150mM)
Chelating agents	EDTA (1-10mM)

Buffer Screen / Liquid Stability / Lyo feasibility

Empirical approach

Buffer Choice (Liquid)	Liquid Buffer Screen	Sugar plus excipient **pre/post** lyo	Sugar plus excipient: Stability at **25°C for 2 weeks**
Histidine	Histidine, Trehalose	Trehalose/HSA	Sucrose/HSA
HEPES	HEPES, Trehalose	Trehalose/Lactose	Sucrose/Lactose
Tris	Tris, Trehalose	Trehalose/Sucrose	Sucrose/MSG
PBS	PBS, Trehalose	Trehalose/Sorbitol	Sucrose/Alanine
Citrate	PBS, Trehalose	Trehalose/Mannitol	Sucrose/Methionine
NaCl	PBS, Trehalose	Trehalose/MSG	Sucrose/Urea
		Trehalose/Alanine	Sucrose/EDTA
		Trehalose/Methionine	Trehalose/Lactose
		Trehalose/PF127	Trehalose/HSA
		Trehalose/Polysorbate 80	Trehalose/Mannitol
		Trehalose/Urea	Trehalose/MSG
		Trehalose/MgCl2	Trehalose/Alanine
		Trehalose/EDTA	Trehalose/Urea
		Sucrose/HSA	Trehalose/Methionine
		Sucrose/Lactose	Trehalose/EDTA
		Sucrose/Trehalose	Trehalose/Polysorbate 80
		Sucrose/Sorbitol	
		Sucrose/Mannitol	
		Sucrose/MSG	
		Sucrose/Alanine	
		Sucrose/Methionine	
		Sucrose/PF127	
		Sucrose/Polysorbate 80	
		Sucrose/Urea	
		Sucrose/MgCl2	
		Sucrose/EDTA	

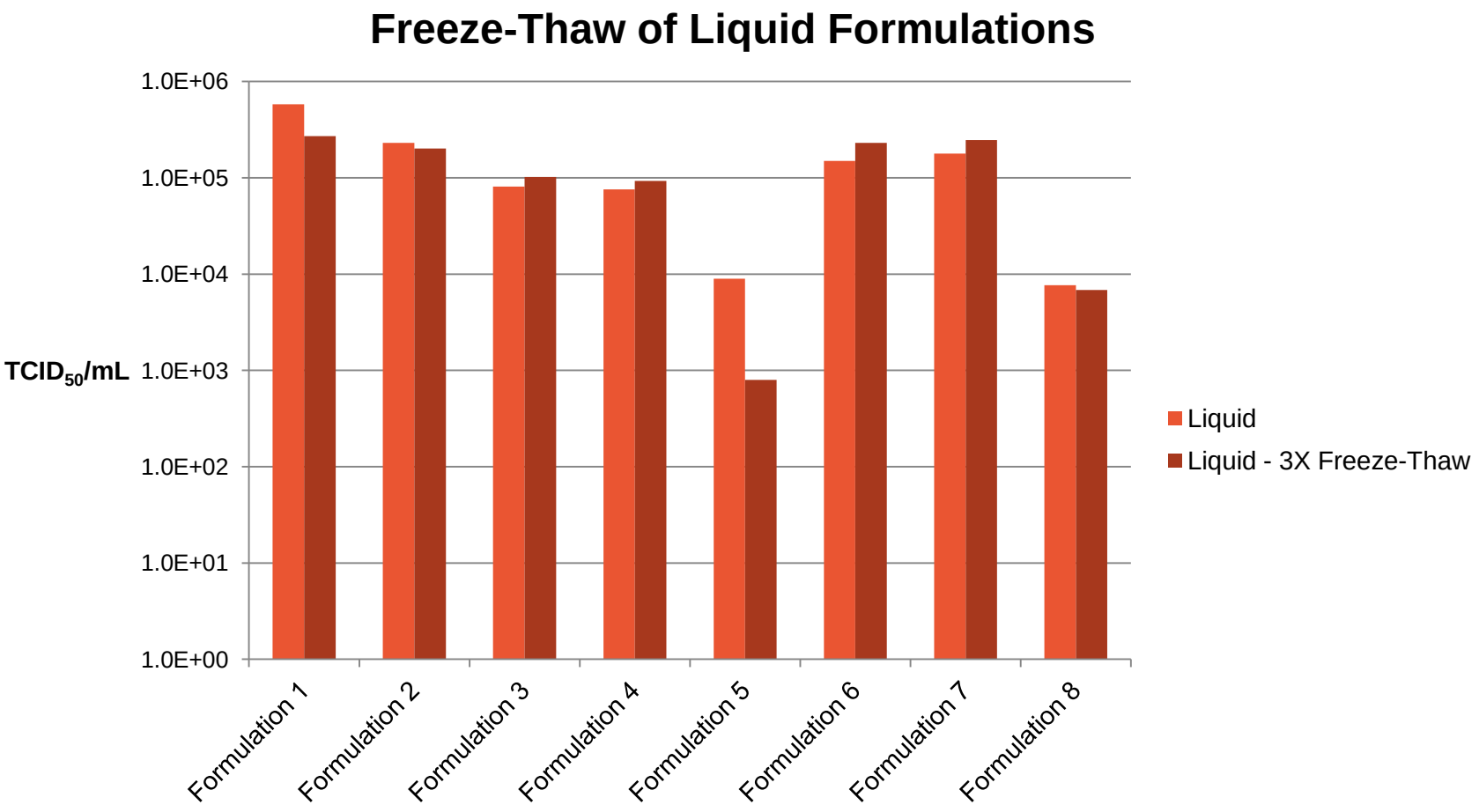
Feasibility of Lyophilized Formulations

Empirical approach

Vaccine drug substance buffer exchanged, lyophilized and subjected to accelerated and real time stability

1. disaccharide/ protein complex/organic buffer/inorganic salt
2. disaccharide/ protein complex/monosaccharide
3. disaccharide/amino acid
4. disaccharide/ amino acid
5. disaccharide/ amino acid/inorganic salt
6. disaccharide/ monosaccharide/amino acid
7. disaccharide /protein complex/ inorganic buffer/ inorganic salts
8. monosaccharide/ protein complex

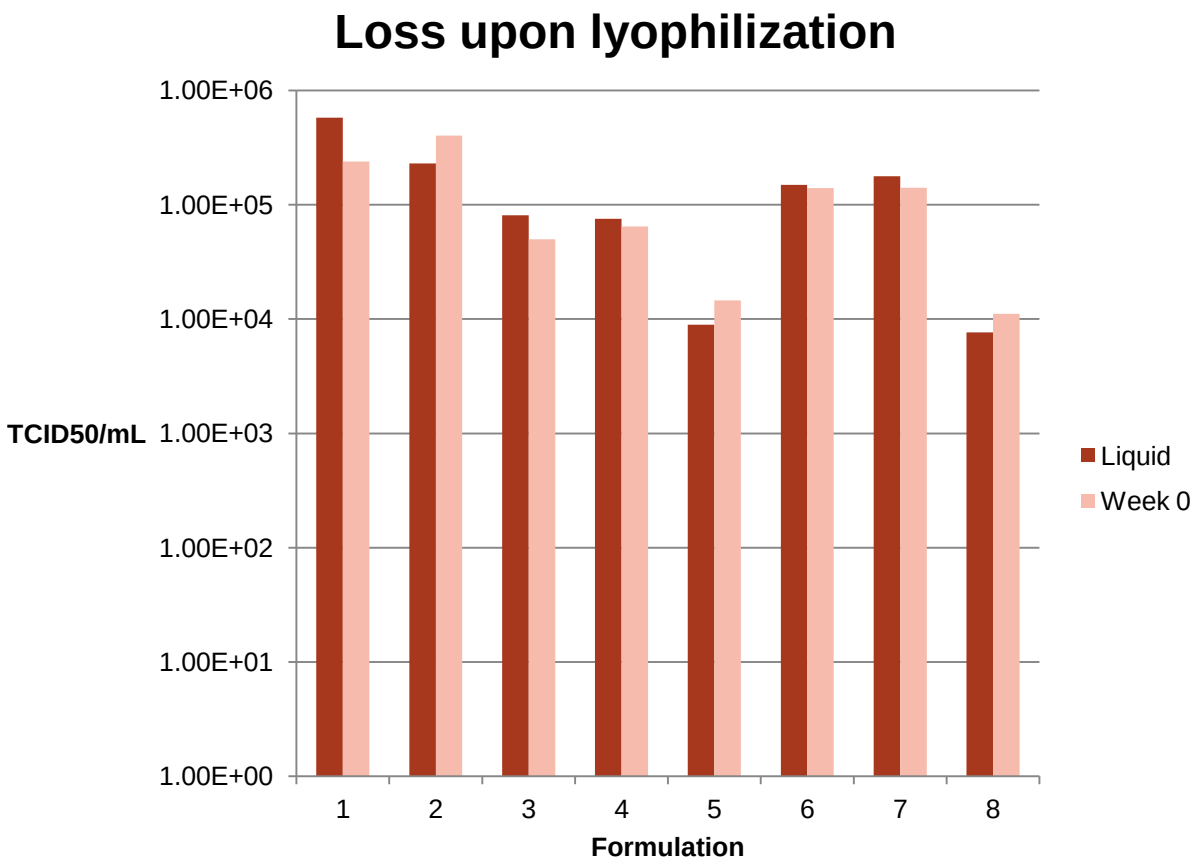
Effect of Freeze-Thaw
Potency CQA



Liquid and Post-Lyophilization Titers
Potency CQA

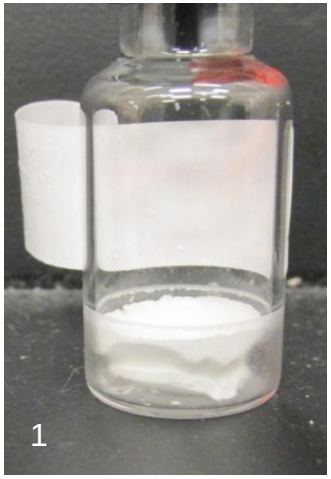
Target Titer = 2E5 $TCID_{50}$/mL

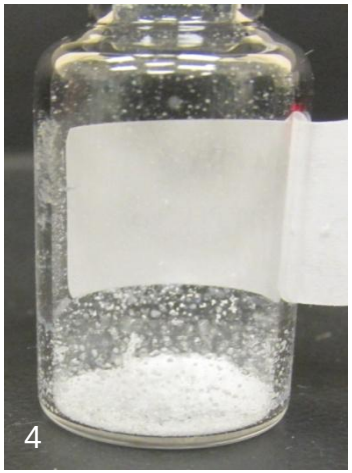
RX	Liquid	Lyophilized
1	5.79E+05	2.40E+05
2	2.30E+05	4.04E+05
3	8.09E+04	5.00E+04
4	7.57E+04	6.48E+04
5	8.92E+03	1.46E+04
6	1.49E+05	1.40E+05
7	1.78E+05	1.41E+05
8	7.64E+03	1.11E+04

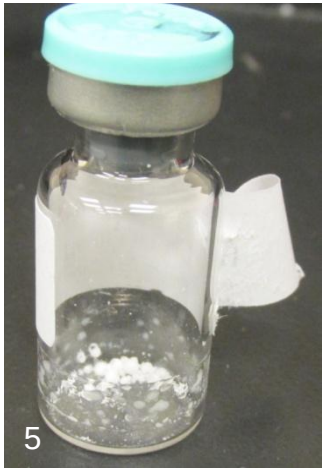


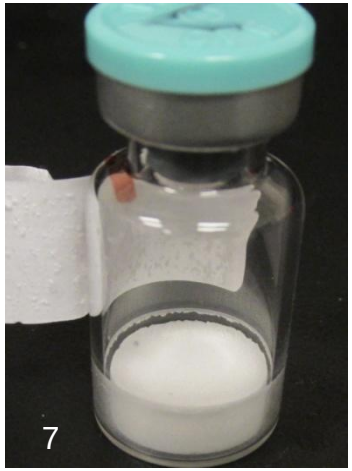
Cake appearance after Lyophilization

Physical Attributes CQA







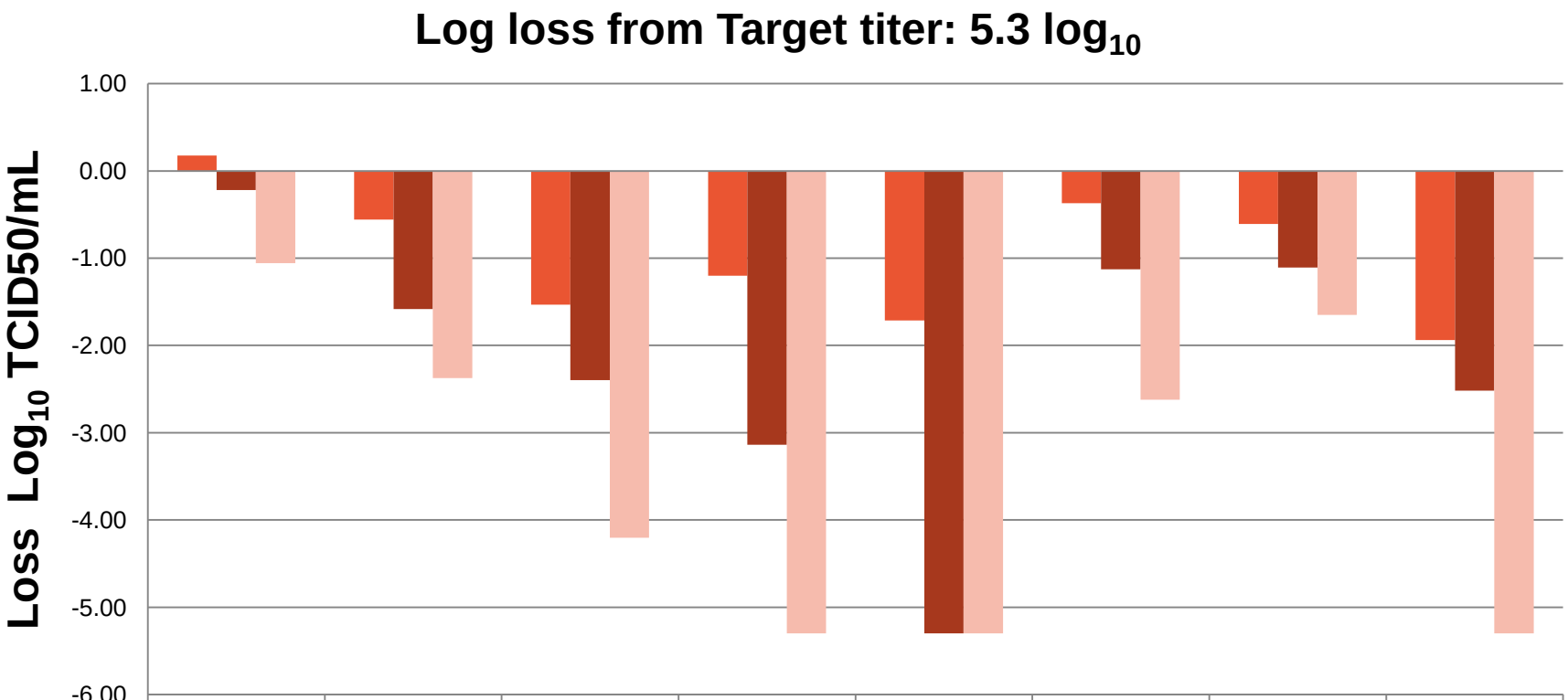


Physical Product Characteristics

post lyophilization T=0

Formulation	Average Reconstitution Time (sec)	Average pH	Cake Appearance Score	Description	Predicted mOsm/L	Residual Moisture %
I	72	7.15	3	Shrinking on sides and bottom	352	1.79
II	17	6.87	5	Whole	283	0.77
III	15	7.55	3	Cracked	292	1.04
IV	14	7.50	1	Powder	311	2.21
V	24	7.15	1	Small Spheres	386	3.21
VI	23	7.52	4	Melting on bottom	310	0.47
VII	29	7.01	5	Whole	273	1.15
VIII	29	7.33	4	Shrinking on sides, beige	287	0.71

Stability
Potency CQA

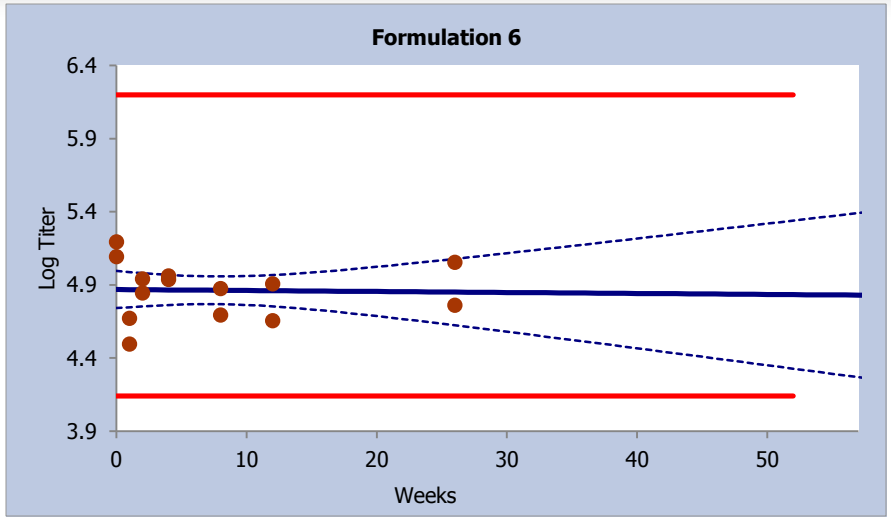


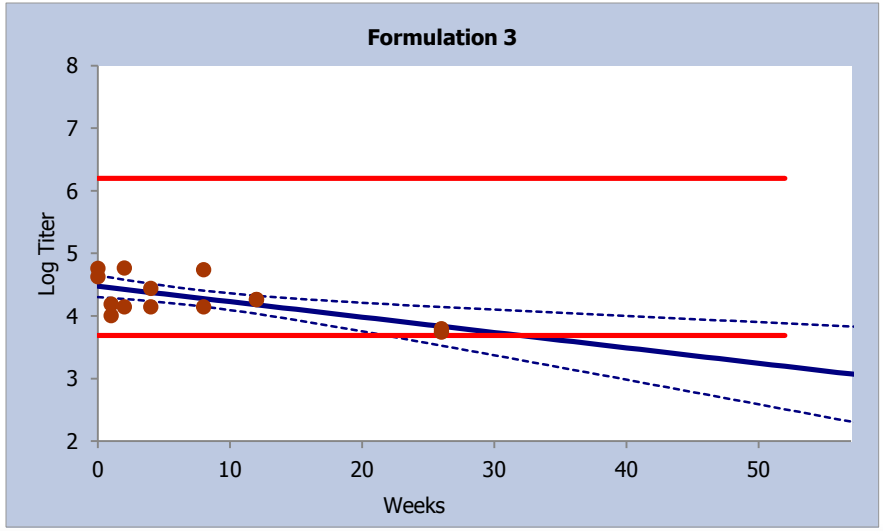
	Rx1	Rx2	Rx3	Rx4	Rx5	Rx6	Rx7	Rx8
4C, 26W	0.18	-0.56	-1.53	-1.20	-1.71	-0.37	-0.61	-1.94
25C, 12W	-0.22	-1.58	-2.40	-3.14	-5.30	-1.13	-1.11	-2.52
40C, 4W	-1.06	-2.37	-4.20	-5.30	-5.30	-2.62	-1.65	-5.30

Estimated Shelf Life – 4°C

Potency TPP

	Study 1 Log_{10} Titer		
Formulation	Week 0	Expiry	ESL (Weeks)
Formulation I	5.37	4.37	>52
Formulation II	5.50	4.50	18
Formulation III	4.69	3.69	21
Formulation IV	4.80	3.80	22
Formulation V	4.16	3.16	36
Formulation VI	5.14	4.14	>52
Formulation VII	5.14	4.14	46
Formulation VIII	4.05	3.05	26





Select Best Formulation based on QTPP

Study 1: empirical approach

Rx	Reconstitution time	LIQ F/T	Cake structure	4°C stability	No animal derived products
1	X	X	X	X	
2	X	X	X		
3	X	X	X		
4	X	X			
5	X				
6	X	X	X	x	x
7	X	X	X		
8	x	x	x		

Part II: Formulation Development - DOE

Preclinical → Ph I → Ph II → Ph III → Registr.

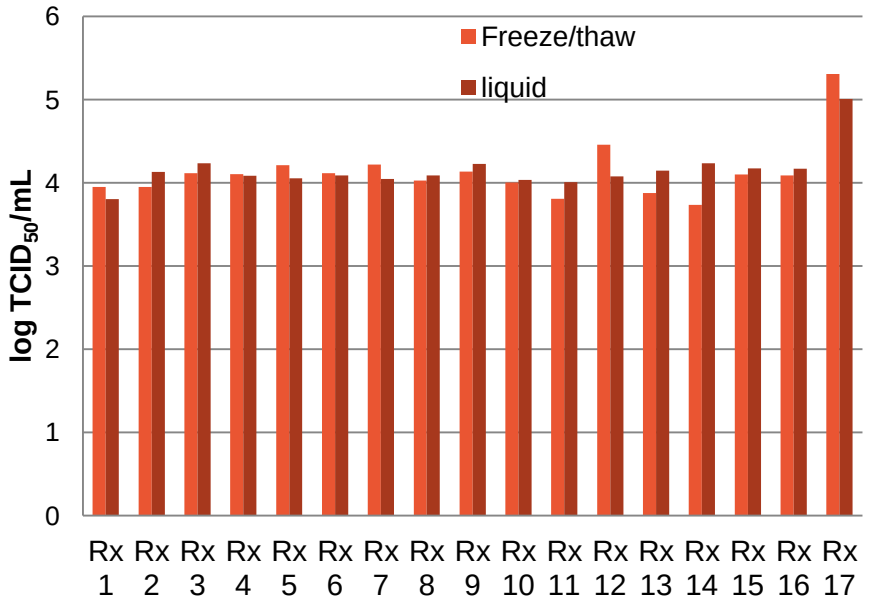
QTPP (CQA)

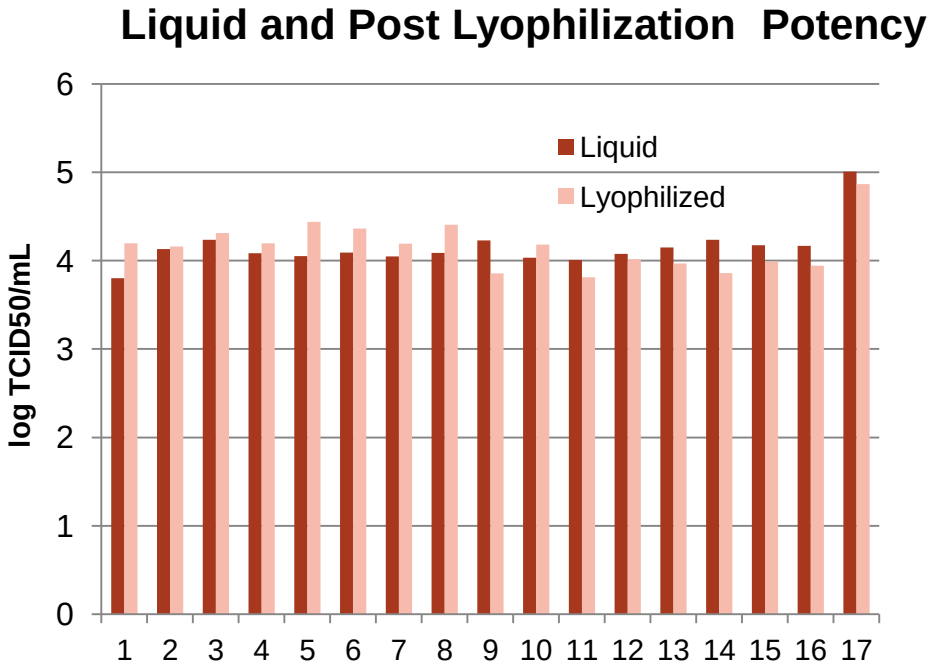
Design of Experiments (DOE)

- Study 1 identified formulation 6 as a potential formulation
- DOE designed based on:
  - Buffer: fixed, pH 7.2
  - Excipient 1: 10 to 40 mg/mL
  - Excipient 2: 5 to 20 mg/mL
  - Excipient 3: 20 to 50 mg/mL
- Definitive screen designed 16 formulations within the defined ranges
  - Formulations determined using JMP's DOE functionality
- New formulations manufactured and lyophilized
- Samples placed on stability at 4, 25 and 40°C
  - 4°C: 0, 0.5, 1, 2, 3, 6, 12, 18 and 24 months
  - 25°C: 0, 0.5, 1, 2, 3 and 6 months
  - 40°C: 0, 2, 4 and 6 weeks

Effect of Freeze-Thaw and Lyophilization

Potency CQA

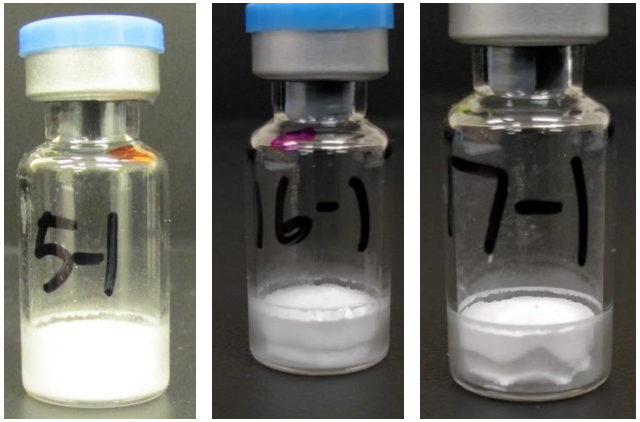




Cake Appearance

Physical Characteristics CQA

- Week 0-Lyo
  - Whole cakes with some shrinkage
- 4ºC and 25ºC stability
  - No change through week 12
- 40ºC stability
  - Week 2: Collapse of 11 of 17 formulations
  - Week 4: 12 of 17 formulations
  - Week 6: 13 of 17 formulations



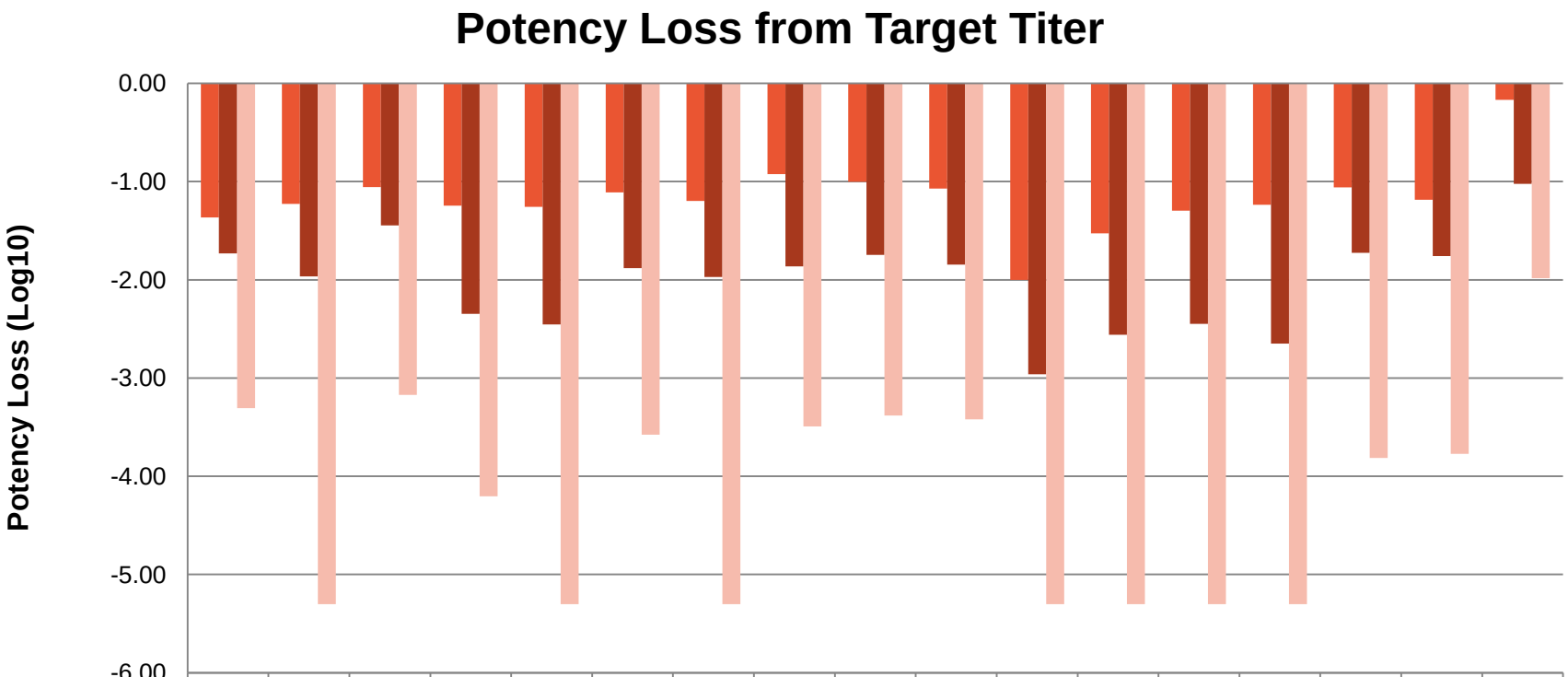
Week 0-Lyo

5 (W4) 9 (W6) 17 (W6)

40ºC

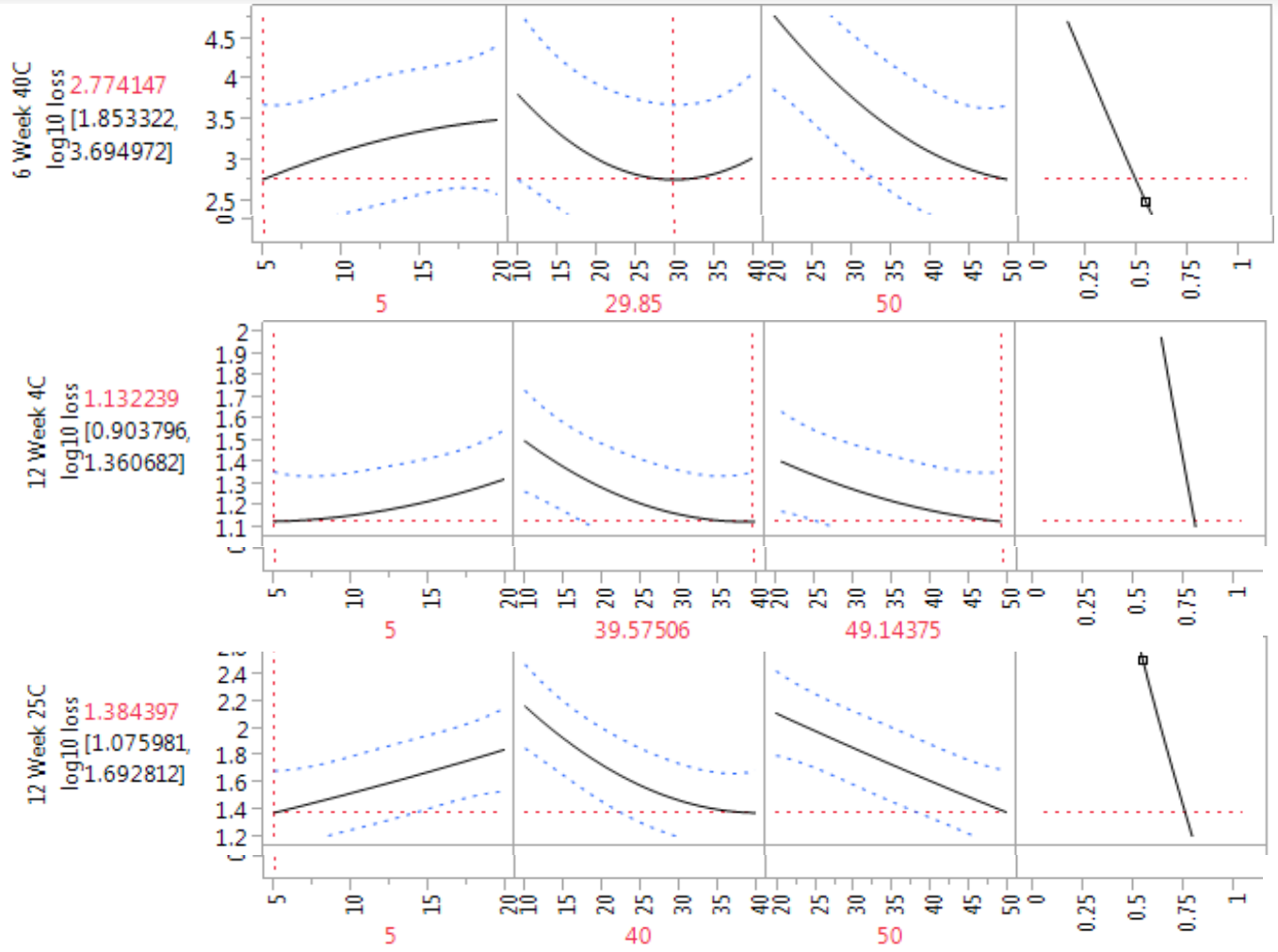
Formulation RX	Residual Moisture %
1	0.71
2	0.5
3	0.46
4	1.01
5	0.45
6	0.74
7	1.06
8	0.72
9	0.98
10	1.08
11	0.87
12	0.98
13	1.46
14	0.6
15	1.21
16	0.77
17	1.39

Stability
Potency CQA

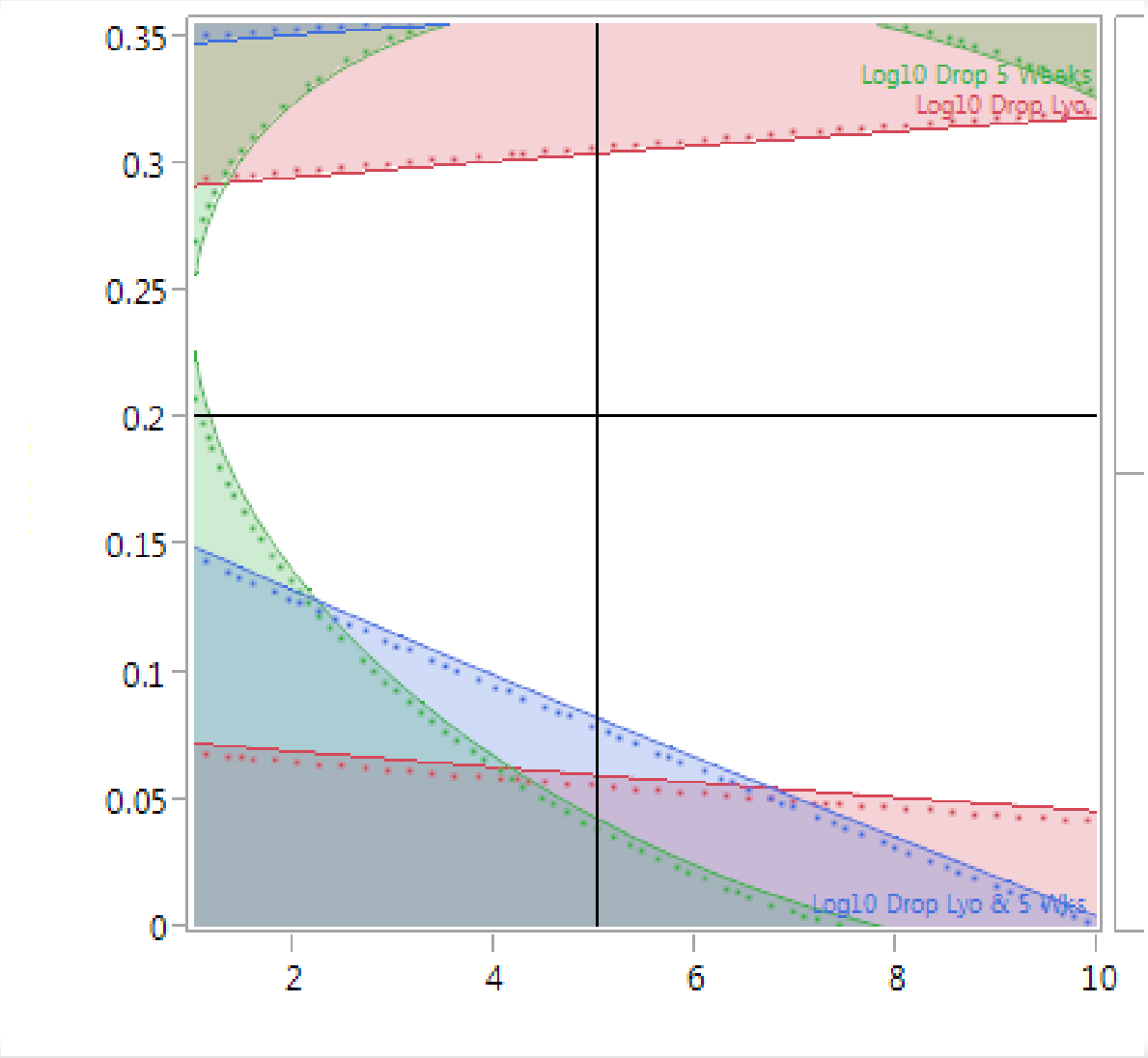


	Rx1	Rx2	Rx3	Rx4	Rx5	Rx6	Rx7	Rx8	Rx9	Rx10	Rx11	Rx12	Rx13	Rx14	Rx15	Rx16	Rx17
4C, 26 W	-1.36	-1.23	-1.06	-1.24	-1.26	-1.11	-1.20	-0.92	-1.00	-1.07	-2.00	-1.53	-1.30	-1.24	-1.06	-1.19	-0.17
25C, 26 W	-1.73	-1.97	-1.45	-2.35	-2.46	-1.88	-1.97	-1.86	-1.75	-1.84	-2.96	-2.56	-2.45	-2.65	-1.72	-1.76	-1.02
40C, 6W	-3.31	-5.30	-3.17	-4.20	-5.30	-3.58	-5.30	-3.49	-3.38	-3.42	-5.30	-5.30	-5.30	-5.30	-3.81	-3.77	-1.98

Statistical analysis of DOE: Most desirable formulations predicted



Design space with lead excipients



Study 2 down-selection

Rx	Reconstitution time/moisture	LIQ F/T	Cake structure	Stability	animal derived products
1	x	x		x	x
2	x	x			x
3	x	x		x	x
4	x	x		x	x
5	x	x	x		x
6	x	x		x	x
7	x	x			x
8	x	x		x	x
9	x	x		x	x
10	x	x		x	x
11	x	x	x		x
12	x	x	x		x
13	x	x	x		x
14	x	x			x
15	x	x		x	x
16	x	x	x	x	x
17	x	x	x	x	

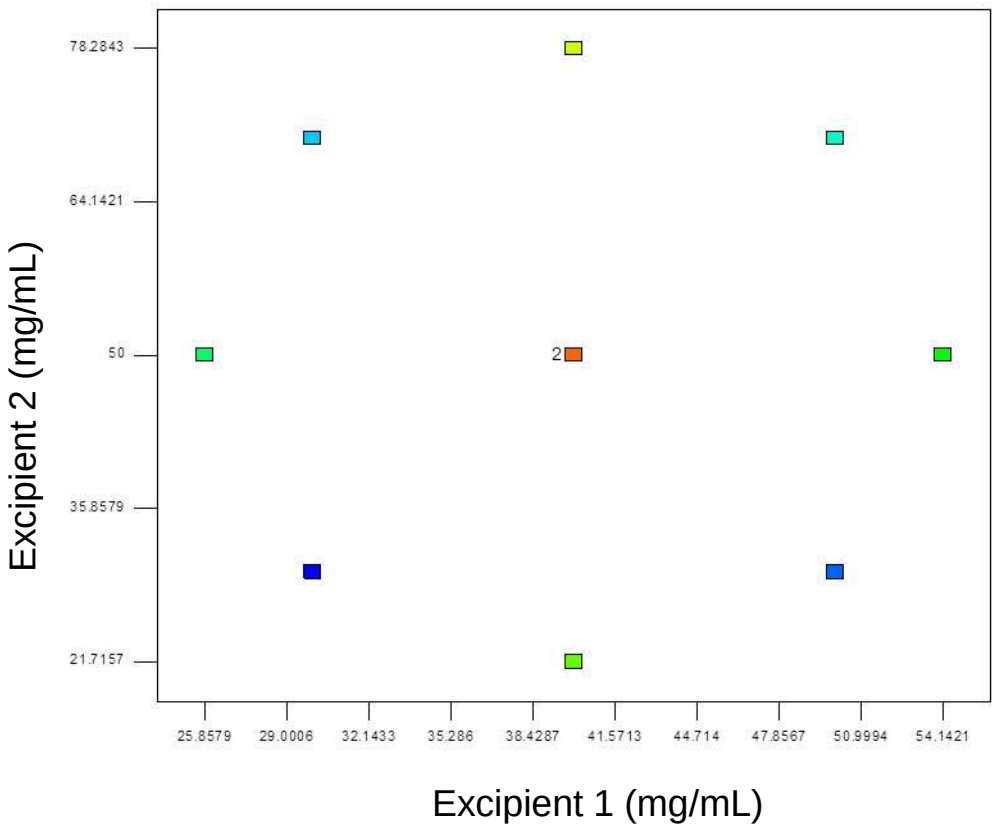
Part III: DOE Response Curve / Central Composite Design

Preclinical → Ph I → Ph II → Ph III → Registr.

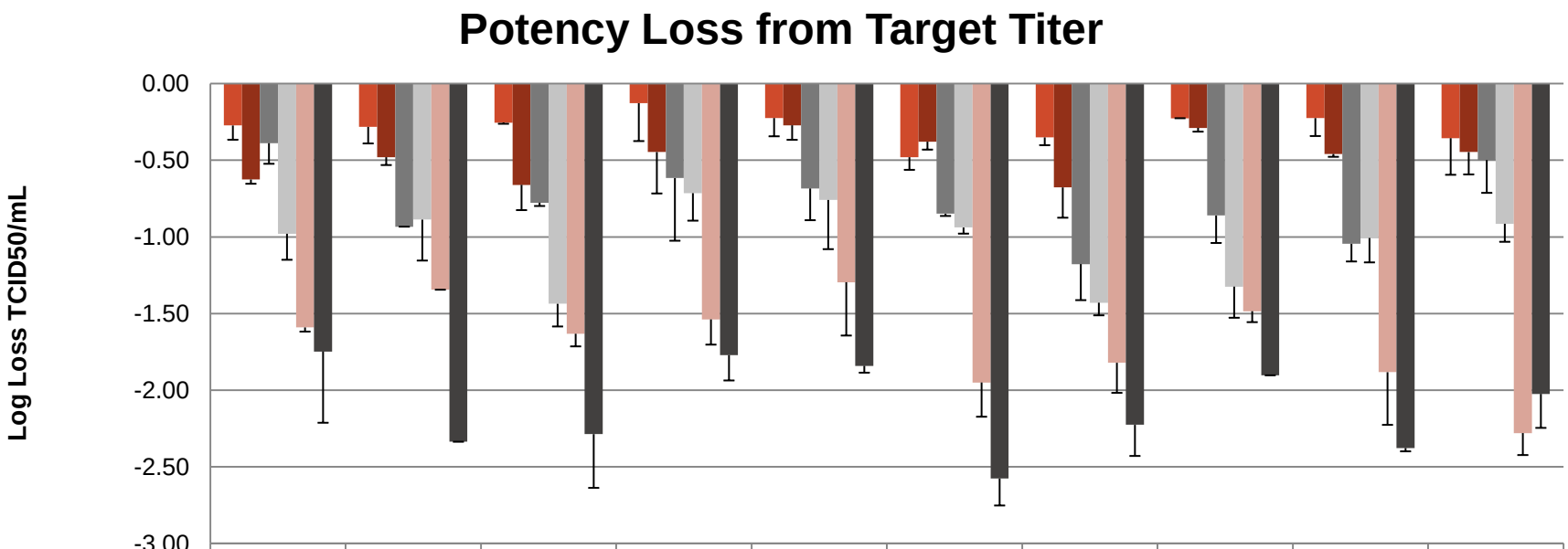
QTPP (CQA&ATP)

Study 3: DOE
central composite design with 2 center points

Rx	Excipient 1	Excipient 2
	mg/mL	mg/mL
1	50.00	70.00
2	40.00	50.00
3	40.00	50.00
4	25.86	50.00
5	30.00	70.00
6	54.14	50.00
7	50.00	30.00
8	40.00	78.28
9	30.00	30.00
10	40.00	21.72



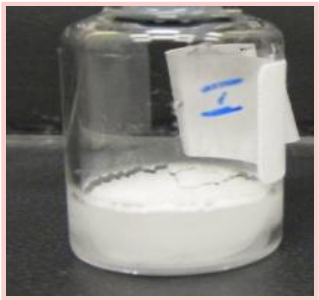
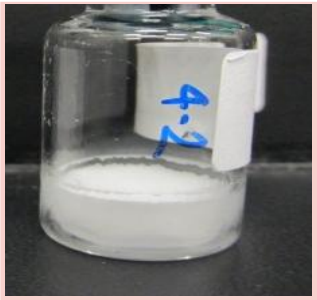
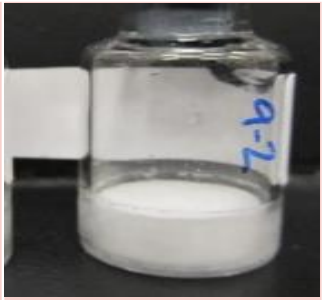
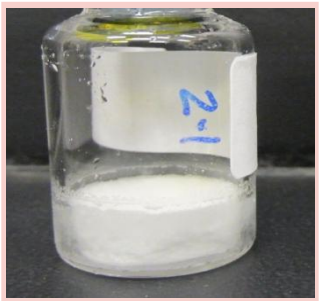
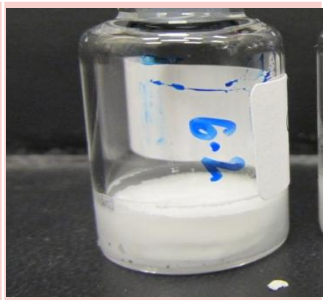
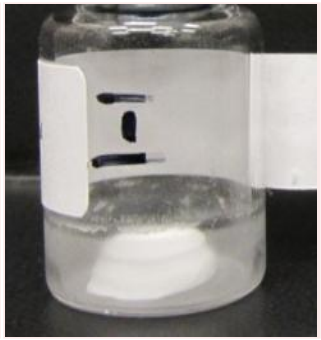
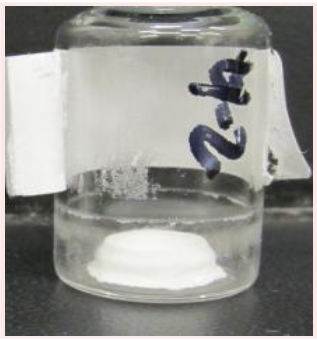
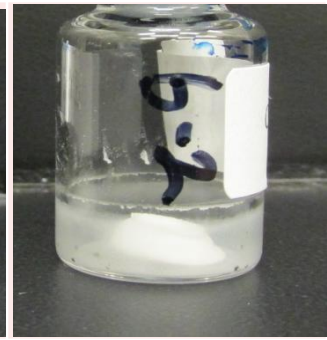
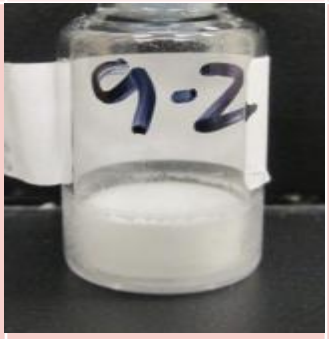
Stability Study 3
Potency CQA



	1	2	3	4	5	6	7	8	9	10
Liquid	-0.27	-0.28	-0.26	-0.13	-0.22	-0.48	-0.35	-0.23	-0.22	-0.36
Week 0	-0.63	-0.48	-0.66	-0.45	-0.27	-0.38	-0.68	-0.29	-0.46	-0.45
25C Week 2	-0.39	-0.93	-0.78	-0.62	-0.68	-0.85	-1.18	-0.86	-1.05	-0.50
25C Week 4	-0.98	-0.89	-1.44	-0.71	-0.76	-0.94	-1.43	-1.32	-1.01	-0.91
40C Week 2	-1.59	-1.34	-1.63	-1.54	-1.30	-1.95	-1.82	-1.48	-1.88	-2.28
40C Week 4	-1.75	-2.34	-2.29	-1.77	-1.84	-2.58	-2.23	-1.90	-2.38	-2.02

Cake Appearance Study 3

Physical Characteristics CQA

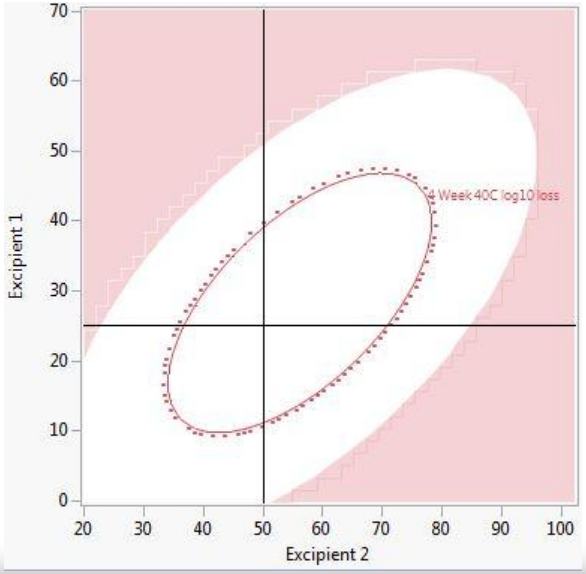
RX	1*	4*	9**	2***	6***
Week 0					
40°C Week 2					
25°C Week 4					

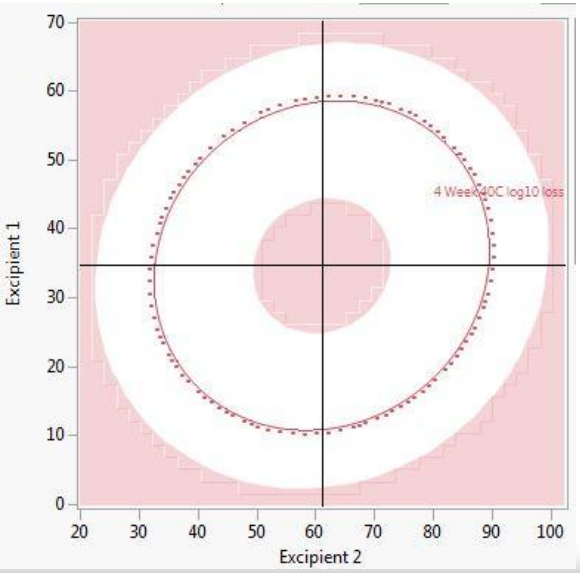
*= highest stability ** = best cake structure *** = lowest stability

Down selection – based on TPP

1. Potency
   - Stability
2. Physical Characteristics
   - Cake structure
   - Osmolality

3. *Choose 3 formulations to move to next stage*





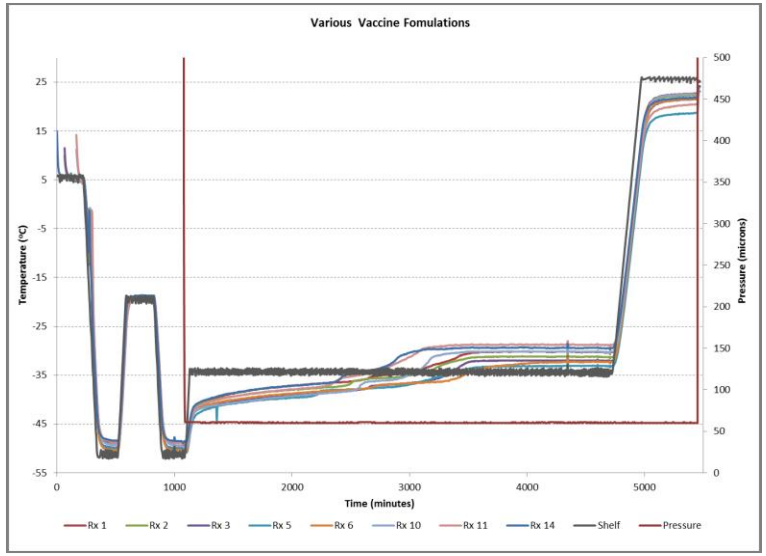
Part IV: Lyophilization Cycle Design Process Engineering

Preclinical → Ph I → Ph II → Ph III → Registr.

QTPP (CQA)

Process Engineering of Lyophilization Cycle

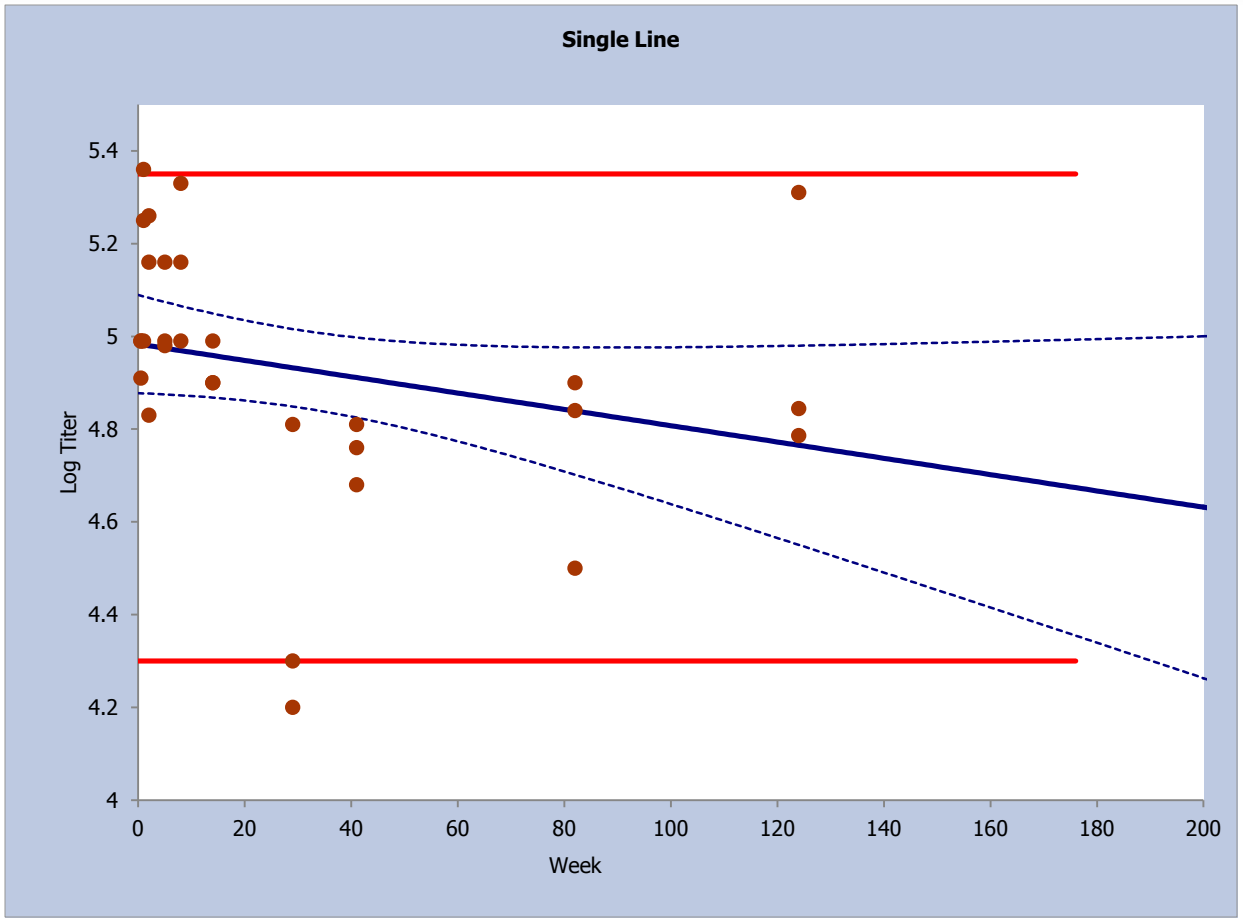
- Thermal Properties Characterization of Candidate Formulations
  - Freeze Drying Microscopy, Differential Scanning Calorimetry & Electrical Resistance
- Cycle design and engineering
  - Determine Lyophilization Critical Process Parameters (CPPs)
    - Shelf temp
    - Chamber pressure
    - Cycle duration
  - Refine parameters
    - Thermal treatment?
    - Produce range of residual moistures
  - Confirm target CPP and CQA
    - At large scale
- Stability Studies
    - On range of residual moistures



Preclinical → **Ph I** → **Ph II** → **Ph III** → **Registr.**

QTPP (CQA)

TPP:
Estimated Shelf Life – 4°C

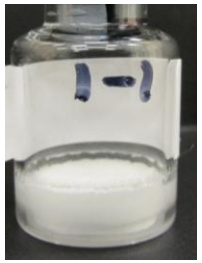


Estimated Shelf Life (90% Confidence): >176 Weeks

Begin (formulation development) with the end in mind

QTPPs for Live Attenuated Vaccines *(example)*

1. Potency (live biological product)
   - Initial retention of titer: $\leq$ 0.5 $\log_{10}$ loss of titer
   - Shelf life ~ 2 years at 4° C
     - lyophilized
   - Stability at 4º, 25º and 40ºC storage: $\leq$ 0.5 $\log_{10}$ loss of titer
   - Freeze-thaw liquid stability: $\leq$ 0.5 log loss of titer
   - Stable in liquid state during processing



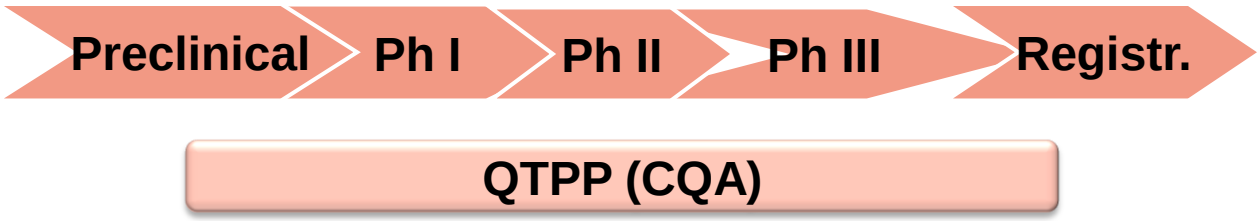
2. Physical Attributes
   - Re-constitution time: $\leq$ 2 minutes to a clear, colorless solution
   - pH: physiologic (7.2 to 7.4)
   - isotonicity: 280 to 390 mOsm/L
   - Cake appearance: white, dense, uniform cake, no evidence of collapse
3. Animal-derived protein-free formulation

Take home message

Combining empirical approach with DOE enables:

- Shorter development time
- Better understanding of design space
- Understanding of excipient interactions
- Evaluate more factors in analysis
- Use statistical approach to justify excipient choices and ranges
- Apply to TPP

- Compare and contrast purely empirical with combined empirical /DOE



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Takeda

Vaccine Business Unit