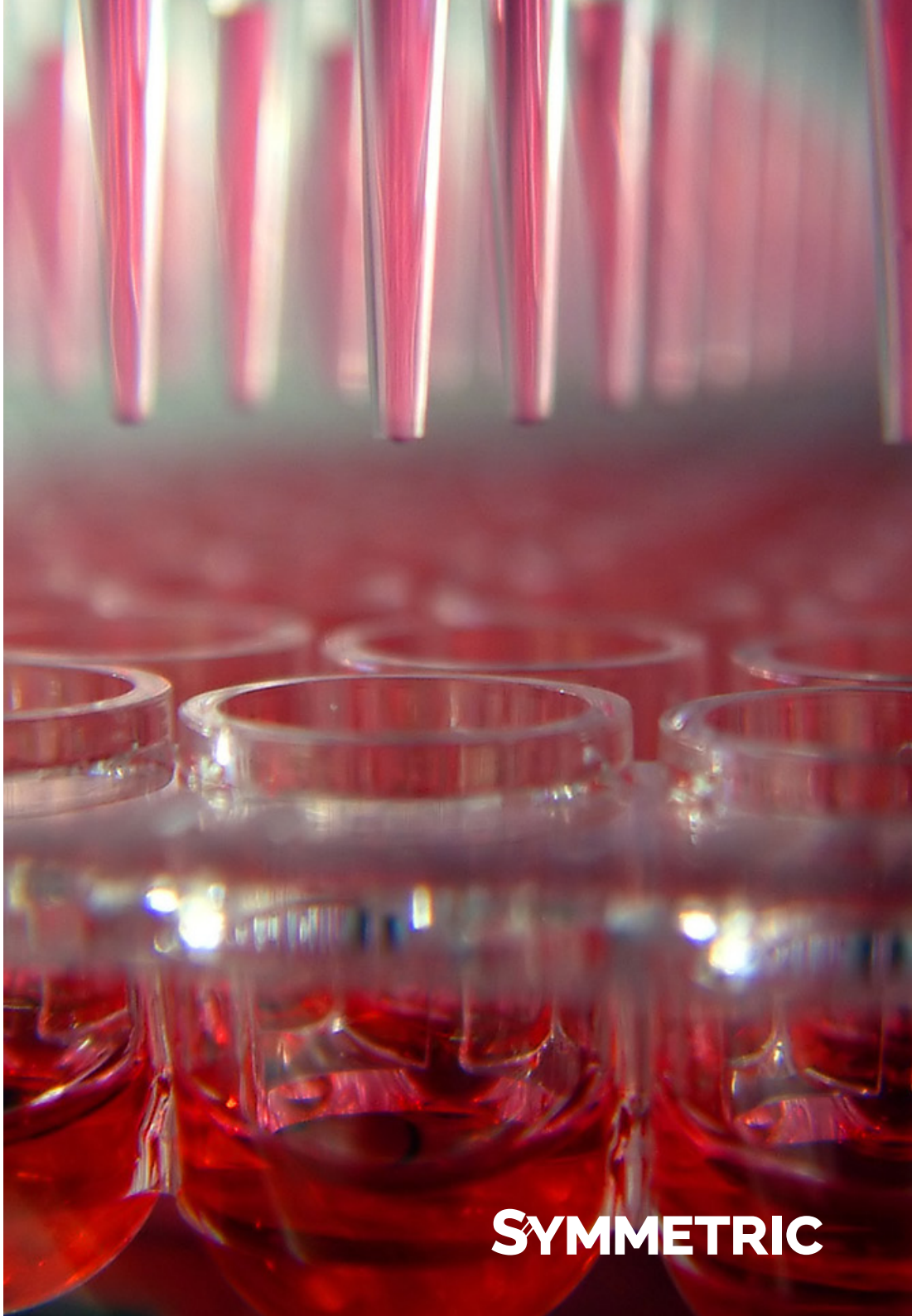
Sneak Peek

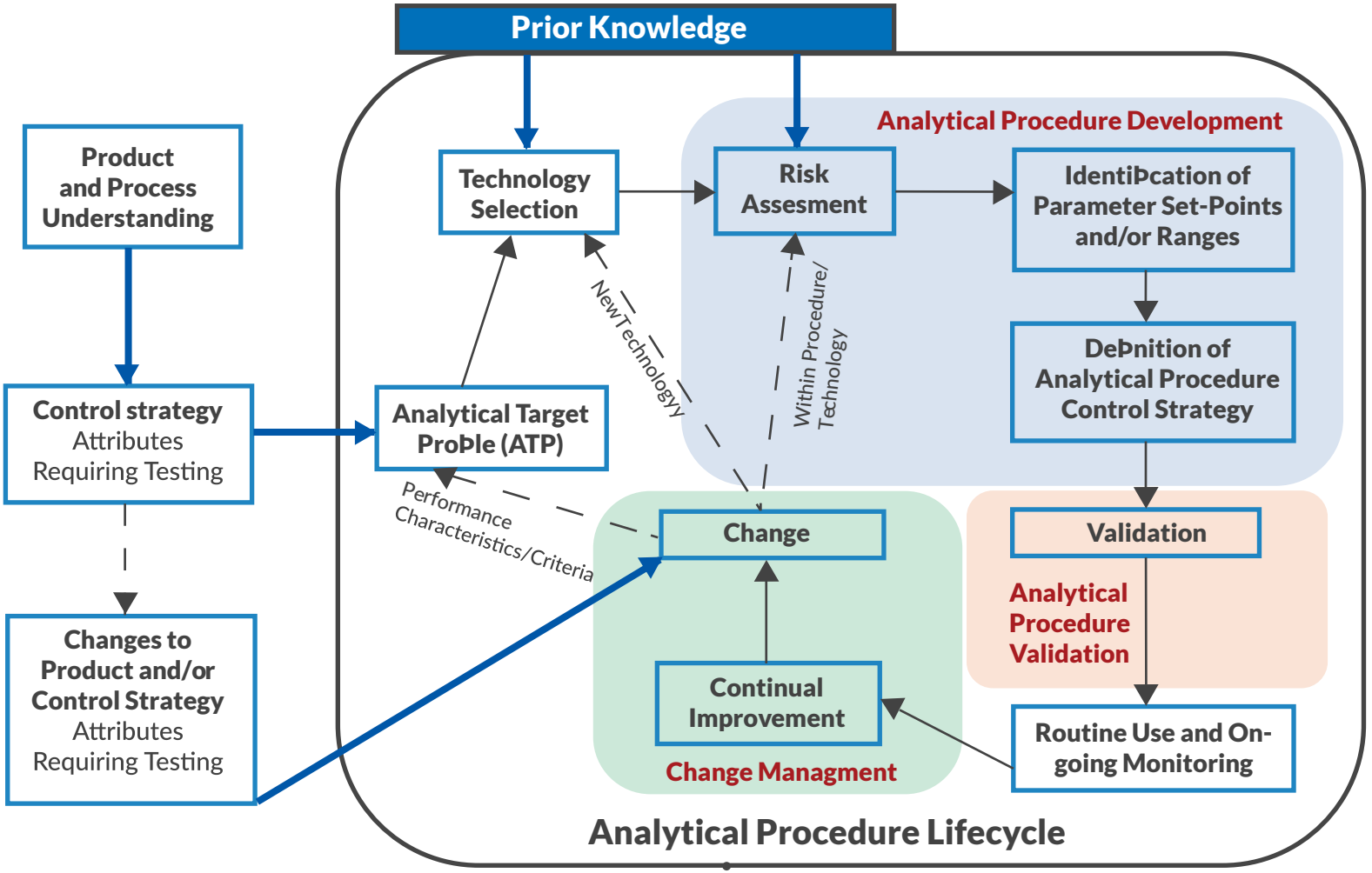
Development and Validation of Bioassays

Steven Walfish
Statistical Outsourcing Services, USA,
Independent Consultant

ONLINE
TRAINING



ICH Q14



Source: Steven Walfish

Randomization

- **A higher risk plate layout:**
  - Reference (R) and test samples (A & B) grouped together on the plate
  - A plate effect is likely to impact both series so can only be used for very well characterized assays

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	

- **Strip plot design:**
  - Randomize samples to rows to decrease potential plate effects
  - Reverse dilutions in the bottom half of the plate

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	
	R10	R9	R8	R7	R6	R5	R4	R3	R2	R1	
	A10	A9	A8	A7	A6	A5	A4	A3	A2	A1	
	B10	B9	B8	B7	B6	B5	B4	B3	B2	B1	

Source: Steven Walfish

DOE Example

Four factor study

- Analyst (1,2)
- Plate (1,2)
- Incubation Temp (38,40)
- Incubation Time (60,70)

Want to determine what factors are important to minimize bias

Response is difference from theoretical value of 100%

Source: Steven Walfish

Examples of Acceptance Criteria

Parameter	Acceptance Criteria
Repeatability & Intermediate Precision	Intra-run/overall %GCV should be within the expected limit
Accuracy or Dilutional Linearity	▸ Relative bias at each potency level (% recovery) should be within the specified interval and a trend should not appear in relative bias across potency levels ▸ A linear relationship should be observed between Estimated vs. Expected Potency with acceptable slope and Correlation coefficient ▸ Intercept should not be significantly different from zero ▸ The plot of Residuals vs. Estimated Potency should show a random distribution about zero
▸ Specificity/Selectivity	▸ Formulation buffer should not show any response ▸ Report for process intermediates, related and unrelated molecules
▸ Range	▸ The range of the method is demonstrated when the precision, accuracy, and dilutional linearity of the method meet the given acceptance criteria at each potency level

Source: Steven Walfish

Examples of Acceptance Criteria

Replication

- Independent replicates of concentration response curves are more effective than repeat aliquots (pseudo replicates) at reducing bioassay variability)

- Allows an assessment of outlying concentration response curves
- The number of replicates should be determined based upon the contribution of replicate variability to overall bioassay variability

Source: Steven Walfish

Combining Multiple Relative Potency

- Method 2 – Independent Assay Results, Homogeneity Assumed)

$$W_i = \frac{4t^2}{L_i^2}$$

$$\bar{R} = \frac{\sum_{i=1}^{n} W_i R_i}{\sum_{i=1}^{n} W_i}$$

$$SE = \frac{1}{\sqrt{\sum_{i=1}^{n} W_i}}$$

$$\bar{R} \pm t_{n-1;\alpha/2} SE$$

Source: Steven Walfish

Registration

07

Sneak Peek

Development and Validation of Bioassays

Mliekarenská 9, 821 09
Bratislava, Slovak Republic
ID: 47 068 124
VAT no: SK2023741973
Office: +421 948 262 346

100% Secure payments

Your details are protected and safe with us. Taxes calculated at the checkout.