

# Transfer of Methods Supporting Biologics and Vaccines

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## Outline

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- Terminology
- Methods and specifications for biologics and vaccines
- USP <1224> Transfer of Analytical Methods
- Method transfer approaches
- Comparison of approaches
- Acceptance criteria for method transfer
- Equivalence approach
- Total error approach

## Terminology



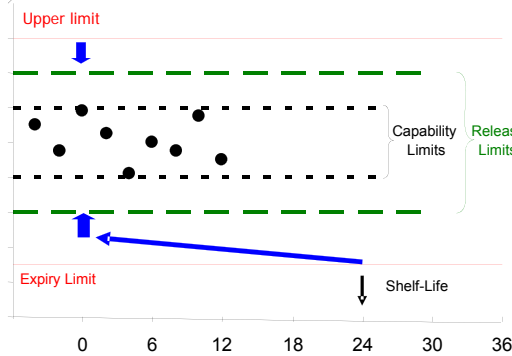
- **Method transfer** – technology transfer of an analytical method from a development laboratory to one or more manufacturing laboratories
- **Method verification** – “The verification process for *compendial test procedures* is the assessment of whether the procedure can be used for its intended purpose, under the actual conditions of use for a specified drug substance and/or drug product matrix.” – USP <1226>
- **Sending laboratory** – the lab from which the method is being transferred, also called the transferring lab
- **Receiving laboratory** – the lab(s) to which the method is being transferred
- **Reproducibility** – interlaboratory variability
- **Trueness** - “the closeness of agreement between a test result and the accepted reference value” – ISO [5,6]
  - Can be either the known value or measured value from the transferring lab

## Methods for Biologics and Vaccines



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|--|--|
| ■ Protein concentration <ul style="list-style-type: none"><li>– UV absorbance, amino acid analysis</li></ul>             | ■ Host cell residual assays <ul style="list-style-type: none"><li>– qPCR, ELISA, Western blots</li></ul> |
| ■ Purity <ul style="list-style-type: none"><li>– Electrophoresis, HPLC</li></ul>   | ■ pH, appearance, particulates, moisture   |
| ■ Aggregation & fragments <ul style="list-style-type: none"><li>– HP-SEC, biophysical methods</li></ul>                  | ■ CDC, ADCC, neutralization assays   |
| ■ Glycosylation profile <ul style="list-style-type: none"><li>– CE, HPLC, MS</li></ul>                                   | ■ Potency <ul style="list-style-type: none"><li>– Antigen binding and bioassays</li></ul>                |
| ■ Structural characterization <ul style="list-style-type: none"><li>– Peptide mapping, MS, biophysical methods</li></ul> |  |

## A Specification Model



- Starts with scientifically supportable maximum and minimum requirements, from release through end of shelf-life
- Release limits calculated from shelf life, stability, and release assay variability
- Process capability (control) limits within release limits

- Properties of specifications
  - Specifications  $\neq$  Capability Limits
  - Need good process capability to derive a meaningful basis for comparing laboratories

## USP <1224> Transfer of Analytical Procedures

- Appears in USP 35–NF 30 which will become official on May 1, 2012
- Types of transfers
  - Comparative testing
  - Covalidation (reproducibility), complete or partial validation
  - Transfer waiver
    - Product is comparable and lab already has experience
    - Compendial procedure (verification by <1226>)
    - Procedure is similar to a method already in use
    - Equipment and personnel are transferred
- Transfer protocol
  - A single lot and/or expired, aged, or spike samples
  - Appropriate analytical performance criteria
  - Experimental design and acceptance criteria
- Limitations
  - Does not encompass microbiological or biological procedures
  - Chapter does not provide statistical methods

## Method Transfer Approaches

- The statistical quality control approach
  - Minimal approach assuming adequate training
    - Sample size based on feasibility
  - Conventional statistical quality control charts (SPC)
    - “Trueness criterion” is met if the mean falls within its control limits
    - “Precision criterion” is met if the range falls within its control limit
- The descriptive approach
  - Several samples tested by the sender and receiver
    - Point estimates of the difference ( $S - R$ ) and the receiving lab variability ( $s_R$ ) are calculated
  - “Trueness criterion” is met if:  $S - R \in (-\Delta, \Delta)$
  - “Precision criterion” is met if:  $s_R < AC$

## Method Transfer Approaches (cont.)

- The statistical significance approach
  - Usual difference hypothesis:
  - Criteria based on t-test for means and F-test for variances – “nonsignificance”
    - “Trueness criterion” met if  $t < t_{crit}$
    - “Precision criterion” is met if  $F < F_{crit}$
- The statistical equivalence approach
  - Equivalence hypothesis:
  - Criteria based on 90% confidence intervals for means and standard deviations
    - “Trueness criterion” met if  $(LCL, UCL) \in (-\Delta, \Delta)$
    - “Precision criterion” is met if  $UCL < AC$

$$H_0 : \mu_S - \mu_R = 0$$

$$H_a : \mu_S - \mu_R \neq 0$$

$$H_0 : |\mu_S - \mu_R| \geq \Delta$$

$$H_a : |\mu_S - \mu_R| < \Delta$$



## Comparison of Approaches



- The approaches differ in the matter of whether, and degree to which they acknowledge decision risks

		True Transfer Situation	
		Successful	Not Successful
Transfer Decision	Successful	Correct Decision	Invalid Site Passed
	Not Successful	Valid Site Failed	Correct Decision

- “Valid Site Failed” – usually called producer’s risk
  - Consequence – checking study procedure, additional development work, additional training, etc.
  - Relatively small cost
- “Invalid Site Passed” – usually called customer’s risk
  - Consequence – failure to meet release and stability requirements
  - Much higher cost, extensive investigation, potential regulatory action

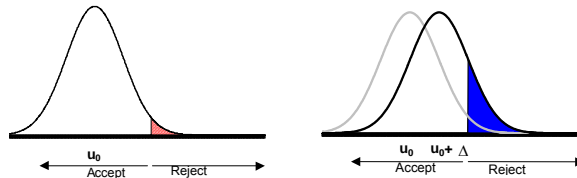
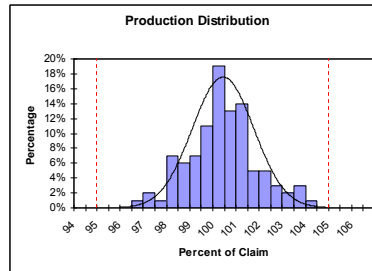
## Comparison of Approaches (cont.)



- The QC and descriptive approaches make no provision for “customer risk” (i.e., the more severe risk of passing an invalid site)
- The statistical significance approach is unable to conclude equivalence
  - The producer’s risk ( the less severe risk) is strictly controlled
  - Lack of significance may be due to excess variability or poor power (sample size)
- The statistical equivalence approach allows the manufacturer to conclude equivalence
  - The customer’s risk (the more severe risk) is strictly controlled

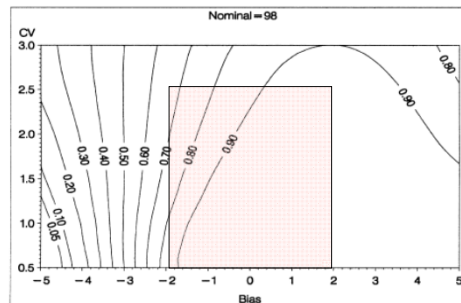
## Acceptance Criteria for Method Transfer

- Determine  $\Delta$  that yields the maximum tolerable failure rate
  - Use the production distribution, development data, and/or simulation
  - Requires satisfactory process capability
    - Recall the release model



## Acceptance Criteria for Method Transfer (cont.)

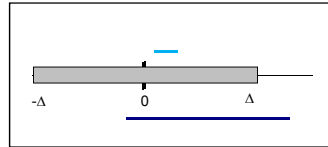
- Perform simulations to determine acceptance criteria on the difference and on receiving lab CV which results in a maximum tolerable failure rate



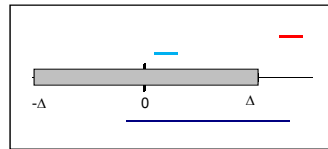
- The region of  $\pm 2\%$  difference and  $< 2.5\%$  CV yields an acceptable failure rate for release of future batches

## Implementation of Equivalence Approach

- Two possible conclusions
  - Declare equivalence if the 90% confidence interval (CI) falls within  $\pm\Delta$
  - Can not declare equivalence if the CI is not fully embraced by  $\pm\Delta$



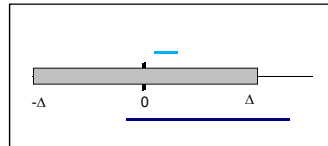
- Alternative of 3 possible conclusions/follow-up
  - Declare equivalence if the 90% confidence interval falls within  $\pm\Delta$
  - Can not declare equivalence if the CI falls completely outside of  $\pm\Delta$
  - Investigate and perform more runs if the CI is not fully embraced by  $\pm\Delta$



## Implementation of Equivalence Approach (cont.)

- Controlling risks through transfer study sample size

- The width of the CI, and thereby the risk that the CI will not fall within the equivalence margin, is controlled by sample size
  - Depends upon whether the acceptance criterion is on bias or on difference
- The estimate of intermediate precision is subject to uncertainty and has an associated confidence interval
  - For  $n = 3$  the upper one-sided 95% confidence bound on the estimated variability (8% CV) is ~60%
  - For  $n = 8$  the upper one-sided 95% confidence bound on the estimated variability (8% CV) is ~15%

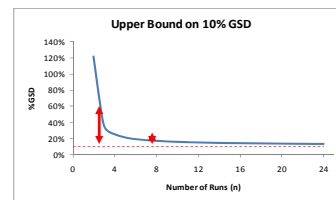


$$\text{Bias: } \bar{x}_R \pm t_{\alpha} \cdot s \cdot \sqrt{\frac{1}{n}}$$

$$n \geq \left\lceil \frac{(t_{\alpha} + t_{\beta}) \cdot s}{AC} \right\rceil$$

$$\text{Difference: } \bar{x}_S - \bar{x}_R \pm t_{\alpha} \cdot s \cdot \sqrt{\frac{2}{n}}$$

$$n \geq 2 \cdot \left\lceil \frac{(t_{\alpha} + t_{\beta}) \cdot s}{AC} \right\rceil$$



## Implementation of Equivalence Approach (cont.)

- Sample size can be managed using a combination of runs and replicates
  - Variance components between and within runs comprise the confidence interval on the receiving lab results
  - An optimal combination of number of runs (I) and number of reps within runs (J) can be determined to minimize risks of transfer failure

$$[\hat{\mu}_R - k\hat{\sigma}_{RI}, \hat{\mu}_R + k\hat{\sigma}_{RI}] \in [\mu(1 - \lambda), \mu(1 + \lambda)],$$

where

$$k = t_{f, \frac{1+\beta}{2}} \sqrt{1 + \frac{J\hat{R} + 1}{N(\hat{R} + 1)}}, \quad f = \frac{(\hat{R} + 1)^2}{\left(\frac{\hat{R} + 1}{I - 1}\right) + \left(\frac{1 - \frac{1}{J}}{N}\right)},$$

$\hat{\mu}_R$  = mean from receiving lab,

$\hat{\sigma}_{RI} = \sqrt{\hat{\sigma}_{RB}^2 + \hat{\sigma}_{RW}^2}$  = intermediate precision,

$\mu$  = known potency of the lot,

$\lambda$  = allowable margin,

$\hat{R}$  = ratio of between - run and within - run variances,

I = # runs,

J = # reps within run,

N = I · J

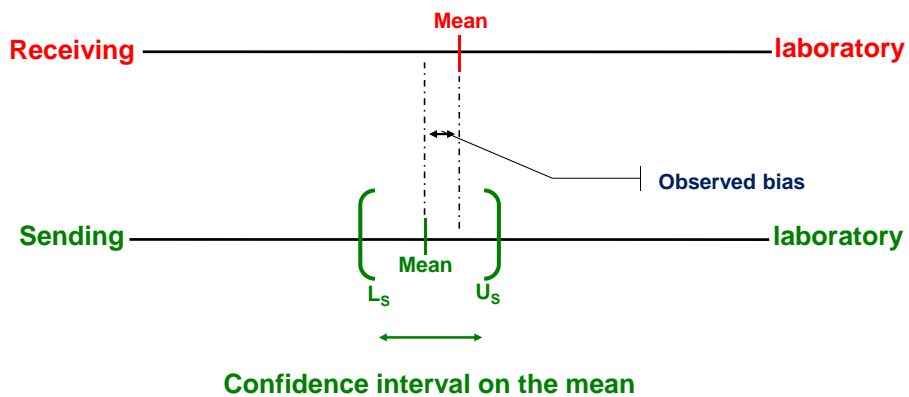
## Total error approach

- On method transfer, it is not possible to separate the systematic error (trueness) from the random error (precision) because the true value of the sample is usually unknown;
- Total Error = Systematic Error + Random Error
  - = Trueness + Precision
  - = Bias + Standard Deviation

## Tolerance Interval Method



Step 1: Calculate the  $100 \cdot (1 - \alpha)$  % confidence interval for the mean of the results from the sending laboratory.



## Tolerance Interval Method



Step 2: Calculate the  $\beta$ -expectation tolerance interval for the receiving laboratory. This interval will predict where individual future results will fall with  $100 \cdot \beta$ % probability

Step 3: Calculate Adjusted Acceptance Limits

The true value of the samples are unknown, but estimated by the sender. Due to this, we should adjust the acceptance limits for the uncertainty in the senders measurements

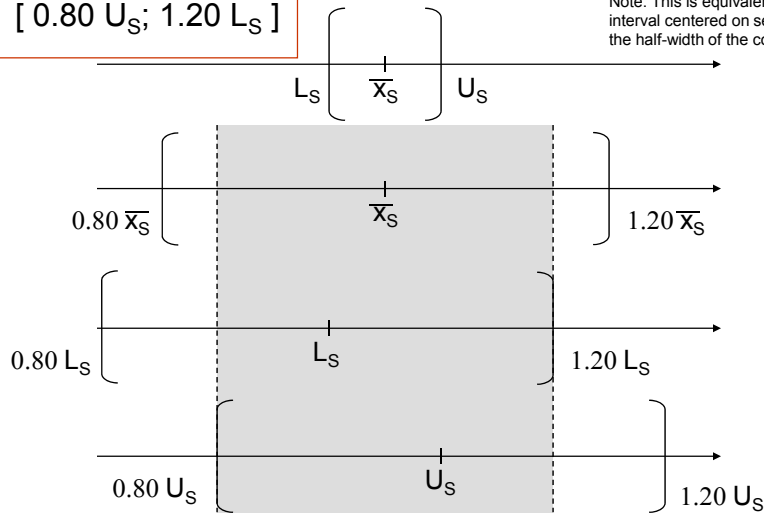
Example: Acceptance Limits =  $\pm 20\%$

## Tolerance Interval Method

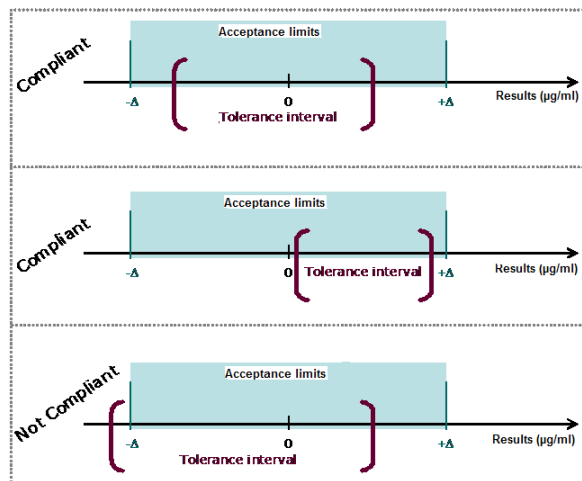


$[0.80 U_S; 1.20 L_S]$

Note: This is equivalent to truncating the interval centered on sending lab mean by the half-width of the confidence interval.



## Tolerance Interval Method



## Study Samples



- Multiple samples types to account for the variety of samples that are to be tested
  - Process intermediates
  - Final formulated product
- Multiple levels to account for potential sample range
  - A key assay parameter is the range
    - The greatest potential for method failure is at the assay extremes
    - The transfer should reaffirm the range in the receiving lab
  - Stability samples
  - Spike/dilution

## Summary



- Discrimination between specifications and control limits is required to be able to conduct a risk based method transfer study
- An assessment of product failure rate can be a basis for establishing a tolerable difference between the sending and receiving labs
- Only equivalence and total error approaches properly account for the more important consumer risk
- The combined impact of bias and variability should be assessed in method transfer
- The method transfer should be powered to minimize study risks, and their consequent costs

## References



- USP <1224> Transfer of Analytical Methods, 35–NF 30
  
- Kringle, R., et. al. (2001) “A Unified Approach for Design and Analysis of Transfer Studies for Analytical Methods,” Drug Information Journal, 35, pp. 1271-1288.
  
- Rozet, E., et. al. (2006) “The transfer of a LC-UV method for the determination of fenofibrate and fenofibric acid in Lidoses: use of total error as decision criterion,” Journal of Pharm. and Biomed. Anal., 42, pp.64-70.
  
- Dewé, W., et. al. (2007) “Using total error as decision criterion in analytical method transfer,” Chemometrics and Intelligent Laboratory Systems, 85, pp. 262-268.