It's time to rethink your CRO.™

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How to Implement a Validated Method

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CPSA 2010

"A Practical Guide to Bioanalysis"

- Familiarize yourself with the method:
  - Review the method
  - Review the method validation report
  - Examine the stability claims
  - Build the “assets” required to run the method
  - Establish competency

- Reference standards
  - Identity
  - Purity
  - Storage condition
  - Expiration
- Sampling
  - Compare in-life protocol to method’s requirements
  - Matrix, species, anticoagulant
  - Preservatives, inhibitors, blocking agents
  - Timing, temperature
  - Containers

- Stability
  - Review the validation stability data:
    - Most windows will be for less than few weeks
    - Was validation data generated against a freshly prepared and extracted calibration curve?
  - Will you need to extend the stability data to protect the period from collection until analysis?
  - Is ongoing sample storage consistent with the stability data?

- Sample Analysis Plan
  - The “go to” agreement on how to run the study
  - Declares appropriate choices for: reference standards, regulatory level, calibration range, location of QC samples, acceptance criteria, etc.
  - Plan consolidates proprietary SOP content, but plan can also trump SOP.
  - Convenient for sponsor customization; prevents misunderstandings

- Sample prep preliminaries:
  - Make and compare stocks
  - Screen internal standard
  - Screen matrix
  - Calibration standards, QC samples
  - Reagents, mobile phases, system suitability sample
- Establish instrumental performance
  - Calibration still current?
  - Requalifications up to date?
  - Meeting system suitability

- Establishing Prestudy Competency
  - Manual methods
    - Analyst qualification
      - Prepare a core validation run
      - Linearity, precision, accuracy, specificity, carryover
  - Robotic methods
    - Method variation lessened by executing version-controlled files
    - Consistency from month to month
  - Audit trails- continuous ON

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Prestudy Qualification Run, 96 well plate

Sys ✓	Blank	Blank + ISTD	Blank + ISTD	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6	Cal 7	Cal 8
Carry over	Carry over	QC Low	QC Mid	QC High	QC High	QC Low	QC Mid	QC Low	QC High	QC Mid	QC Low
QC Mid	QC Mid	QC High	QC Low	QC Mid	QC High	QC High	QC Low	Cal 8	Cal 7	Cal 6	Cal 5
Cal 4	Cal 3	Cal 2	Cal 1	Sys ✓							

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Acceptance of Qualification Run

- Inactivate any samples with known error or instrumental malfunction
- Integrate consistently with single parameter set
- Calculate:
  - Drop any Cal > 15% bias from intended; > 75% remain?
  - Blank, Blank + ISTD, Carryover < (20% *Cal 1)?
- QC performance:
  - ≤ 15% CV
  - ≤ ±15% mean bias from intended

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Method Sample Analysis Run

Sys ✓	Blank	Blank + ISTD	Blank + ISTD	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6	Cal 7	Cal 8
Carry over	Carry over			QC High							
QC Low								QC Mid			
			QC High								
						QC Mid					
										QC Low	
		Cal 8	Cal 7	Cal 6	Cal 5	Cal 4	Cal 3	Cal 2	Cal 1	Sys ✓	

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Acceptance of Sample Analysis Run

- Inactivate any samples with known error or instrumental malfunction
- Integrate consistently with single parameter set
- Calculate:
  - Drop any Cal > 15% bias from intended; > 75% remain?
  - Blank, Blank + ISTD, Carryover < (20% *Cal 1)?
- QC performance:
  - At least 4/6 within ±15% of nominal
  - No two samples can fail at the same concentration

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Look at Early Study Sample Results

- Are the sample concentrations well distributed throughout the range?
  - Pause after 2-3 runs are completed
    - High and middle QC's should bracket range of C_{max}
    - Low QC anchors the low end at 3 x LLOQ
    - Range should cover 4-5 half-lives on the elimination phase
  - Special scrutiny for BE studies!
- Fixes
  - Relocate the curve and QC's (single validation run)
  - Add more QC's and revise acceptance rules

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Looking Deeper into the Data

- Consistency of internal standard response:
  - Cals/QC's *vs.* incurred samples
  - Between subgroups of incurred samples
- Divergence between the front and back sets of calibration standards?
- Drift in internal standard response
- Drift in system check sample response

- Pre-establish drop criteria in SOP (poor chrom, instrument malfunction, attributable error, etc.)
- Sample tracking integrity during sample storage and analysis
- Watch carryover-
  - Investigate affected pairs
  - Is CO factor responsible for more than 5%?
- Fractured, convoluted runs- just fail them!

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Audit Focal Points

- Avoid reprocessing; integrate, then accept consequences.
- Remediate any lapse in stability coverages
- Document any deviations or investigations and discuss their impact on study
- Carefully justify any repeat analysis requests
- Retain and report all runs performed