Cross Validation and Matrix Effect – two Critical Factors in Ensuring Successful Regulated Bioanalysis

Luke Bi

Covance Shanghai

Feb 25, 2015

COVANCE
SOLUTIONS MADE REAL™

Outline

- Introduction to Covance Shanghai
- Cross Validation and Matrix Effect in Regulated Bioanalysis
- Case Studies of Cross validation & Matrix Effect
  - Cross validation
  - Matrix effect evaluation
- Conclusions

LOGO GOES HERE

COVANCE
SOLUTIONS MADE REAL™

Need for Regulated Bioanalysis in China

Global Pharma entering into China

- Drug registration trial includes a Phase 1 PK and a Phase 3 study
- Global Phase 2 and 3 trials include China sites

Bioanalysis performed within China meets global quality requirements

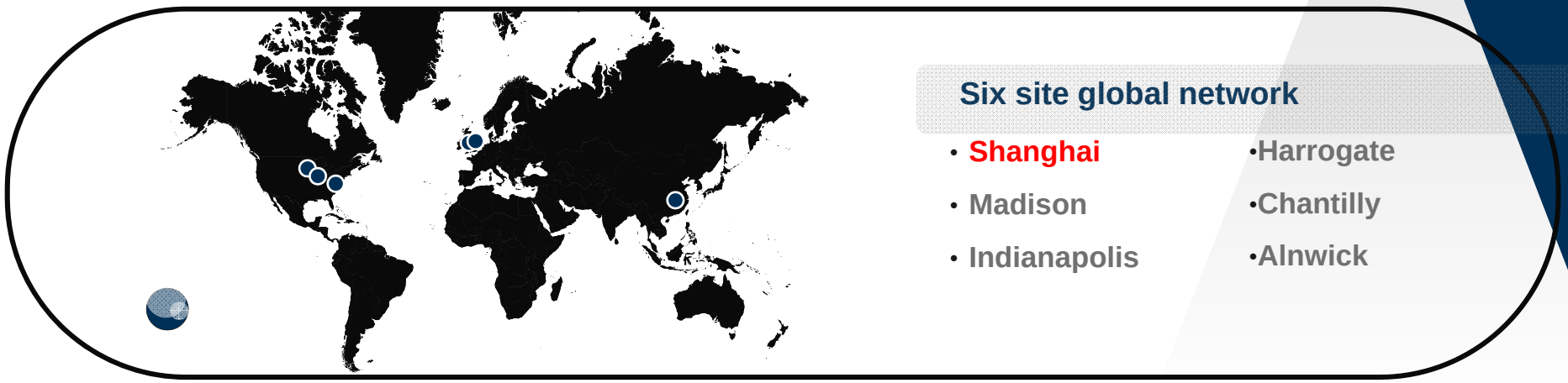
China Pharma going to Global

- Chinese pharmaceutical and biotech companies are entering into global market. i.e. dual IND filing strategy in China and US/EU

Bioanalysis meets CFDA, US FDA and EMA GLP regulations

COVANCE
SOLUTIONS MADE REAL™

Global Consistency & High Quality



Six site global network

- **Shanghai**
- Madison
- Indianapolis
- Harrogate
- Chantilly
- Alnwick

Global operating procedures

Common technology platforms

Global Scientific/Technical Network

COVANCE
SOLUTIONS MADE REAL™

Compliance

- Passed multiple international regulatory inspections
  - Belgium OECD GLP inspections in June 2011 and June 2013
  - UK MHRA GLP inspection in May 2013
- First international CRO obtaining CFDA GLP certificate
  - Understanding CFDA requirements
    - SOP, documentation, Instrument calibration, QA inspection, protocol and report
  - Inspected by CFDA for dual-filing studies regularly
  - Drug products approved by CFDA with bioanalytical support from Covance Shanghai
- Full compliance in GCP regulations for clinical bioanalysis
- Passed 80+ sponsor technical/QA audits

COVANCE
SOLUTIONS MADE REAL™

Bioanalytical Team

Integrated project management approach

- Scientists follow through method development, validation and sample analysis
  - Continuation of the knowledge & experience
  - Project ownership and accountability

Staff career development

- Culture of caring and understanding
- Building PK/TK capability, understanding the study design
- Training on client communication and project management
- Expanding LC/MS/MS applications in ADC and peptide

We have built a great team-experienced, versatile and nimble

COVANCE
SOLUTIONS MADE REAL™

Key Accomplishments

50+ clients: large pharmaceutical and biotech companies and Japanese companies

Supported bioanalysis for 70+ new drug development at different stages

Clinical:Preclinical =70:30

Method development and validation:

- 230+ methods developed or transferred
- Establishing 60 new methods per year

Analytical operation:

- 65,000 samples analyzed per year
- Batch Success: 96% (completed runs)
- 200+ ISR analysis, 99% success rate
- 60+ cross validation studies, 100% success

COVANCE
SOLUTIONS MADE REAL™

Covance global SOP on cross validation

- Blinded and randomized study design
- Cross validation study samples
  - Incurred pooled samples from previous study (N=6)
    - 2 samples between MQC and HQC
    - 2 samples between LQC and MQC
    - 1 Sample at BQL
  - QC samples or spiked samples (N=6)
    - Same as above
  - Total 60 samples completely randomized

Acceptance Criteria:

- Incurred pooled study samples:
  - 2/3 results within ±20%
- QC or spiked samples:
  - Mean QC of each lab should be within 15% of each other

Case 1: Cross Validation for Result Verification

Issue:

Sponsor noticed significant higher plasma exposure in APAC and conducted a cross validation study between sponsor's lab and Covance Shanghai.

Solution:

- Cross validation included 3 QC samples (n=3) and 4 pooled study samples.
  - Both labs were blinded to cross validation samples during analysis
  - Acceptance criteria: 2/3 of samples within ±20%. The mean of QC of each lab should be within 15% of nominal value.

Results:

- Covance Shanghai results met the acceptance criteria
- Cross validation study validated the APAC study results

COVANCE
SOLUTIONS MADE REAL™

Case 1: Cross Validation Results and Comparison (n=6)

Sample Name	Sample Name	Covance Shanghai Results (ng/mL)	Sponsor Lab Results (ng/mL)	% Diff
QC300	Mean	294	296	0.6
	CV%	0.6	2.3	
QC2000	Mean	2000	1970	-1.5
	CV%	1.9	2.5	
QC40000	Mean	41033	38207	-7.1
	CV%	2.0	1.9	
Sample Low	Mean	6657	6421	-3.6
	CV%	1.8	2.2	
Sample Mid	Mean	21083	20097	-4.8
	CV%	2.0	1.4	
Sample High	Mean	40100	37664	-6.3
	CV%	1.2	1.7	
Sample >HLQ	Mean	61700	58722	-4.9
	CV%	0.9	1.3	

Case 2: Cross Validation for Vendor Selection

Issue:

- Sponsor organized a feasibility test in CRO selection to support China registration trial.
  - The feasibility test contained 60 samples at 3 levels
- Covance Shanghai was selected based on results

Assay Date	Concentration (ng/mL)		
23-Feb-2012	Sample A	Sample B	Sample C
Mean	0.197	1.38	13.5
CV%	3.5%	2.$	1.4%
N	20	20	20

Case 2: Cross Validation for Vendor Selection

Covance conducted a cross validation with sponsor lab

- There were 60 aliquots from 6 incurred samples (N=10)
- The samples were randomized and analyzed in blind.

Results

- 80% of samples were within ±20%.
- Covance results had < 5.7% variation.

Random Code		Sponsor Lab Results (ng/mL)	Covance Shanghai Results (ng/mL)
A	Average	0.243	0.205
	CV (%)	6.2	5.7
B	Average	0.878	0.756
	CV (%)	3.7	3.8
C	Average	6.44	5.98
	CV (%)	2.5	3.1
D	Average	4.76	4.93
	CV (%)	12.1	2.1
E	Average	0.161	0.175
	CV (%)	14.5	5.1
F	Average	0.644	0.762
	CV (%)	30.1	2.1

Case 3: Failure in Cross Validation and Investigation

We recently encountered a failure in a cross validation study

- Cross validation QCs of one metabolite failed and had consistent >10% bias. Cross validation passed for the other two analytes in the assay.
- We found Covance stock was ~10% lower in the comparison of stock solutions between the 2 labs
  - During investigation, we found that the reference material (same lot) had ~4% difference in potency
  - The reference material is hygroscopic and may have reduced potency over time due to absorbing moisture

Case 3: Failure in Cross Validation and Investigation

Stock solution

Covance prepared stock solutions on two occasions over 2 months. We performed stock comparison to ensure accuracy of the preparation

Follow up:

Sponsor will send reference material to both labs at the same time.

Cross validation test for this metabolite will be repeated

Case 4: Matrix Effect in Cross Validation

- One cross validation study had 30 incurred study samples from previous studies.
  - 15 samples were within the established curve range
  - 15 samples were out of range and required 10 fold dilution
- Results:
  - Overall 20/30 met the acceptance criteria
  - Samples without dilution:
    - 9 out of 15 failed. On average these 15 samples had consistent bias with mean bias of 23%.
  - Sample with 10 fold dilution:
    - 1 out of 15 failed. Samples had random bias without a clear trend with mean bias is 2.1%.

Matrix Effect Evaluation

- Covance Shanghai often evaluates method performance in Chinese matrix to support China registration
- Matrix effect at LQC & HQC in six lots of Chinese matrix
- Selectivity at LQC in six lots of Chinese matrix
- We have conducted cross validation using QCs prepared in Chinese matrix
- In General, we have not observed method performance issue in Chinese or Caucasian matrix

Matrix Effect Identification and Evaluation

- Consistent high IS response was observed in patient samples in a Phase 2 statin study
- Matrix effect was identified through sample dilution
- Using stable labeled ISTD could minimize matrix effect

Sample ID	Original IS response (Run 2)	Mean IS Response of CALs & QCs (Run 2)	% of Mean IS (Run 2)	Mean IS Response of CALs & QCs (Run 4)	IS Response 2X Dilution (Run 4)	% of Mean IS (Run 4)	IS Response 10X Dilution (Run 4)	% of Mean IS (Run 4)
Sample 1	24662.7	11291.0	218	11030.3	12593.4	114	10725.9	97.2
Sample 2	22642.7		201		11054.2	100	13155.1	119.3
Sample 3	21808.3		193		13363.3	121	14873.8	134.8
Sample 4	22651.9		201		10604.4	96	12370.2	112.1

Lovastatin Acid Results (ng/mL)

Sample ID	Original Results	2X Dilution	% Diff	10X Dilution	% Diff
Sample 1	25.6	24.0	-6.3	24.1	-5.9
Sample 2	29.3	26.6	-9.2	27.8	-5.1
Sample 3	21.6	19.8	-8.3	19.3	-10.6
Sample 4	19.2	21.9	14.1	17.5	-8.9

Matrix Effect Identification and Evaluation

Matrix Effect Identification:

- Most assays use stable labeled ISTD and CALs/QCs are prepared from Caucasian matrix
- IS response of batch CALs/QCs and study samples are monitored closely
- Covance SOP defines sample reassay criteria based on ISTD response and data reporting decision tree.

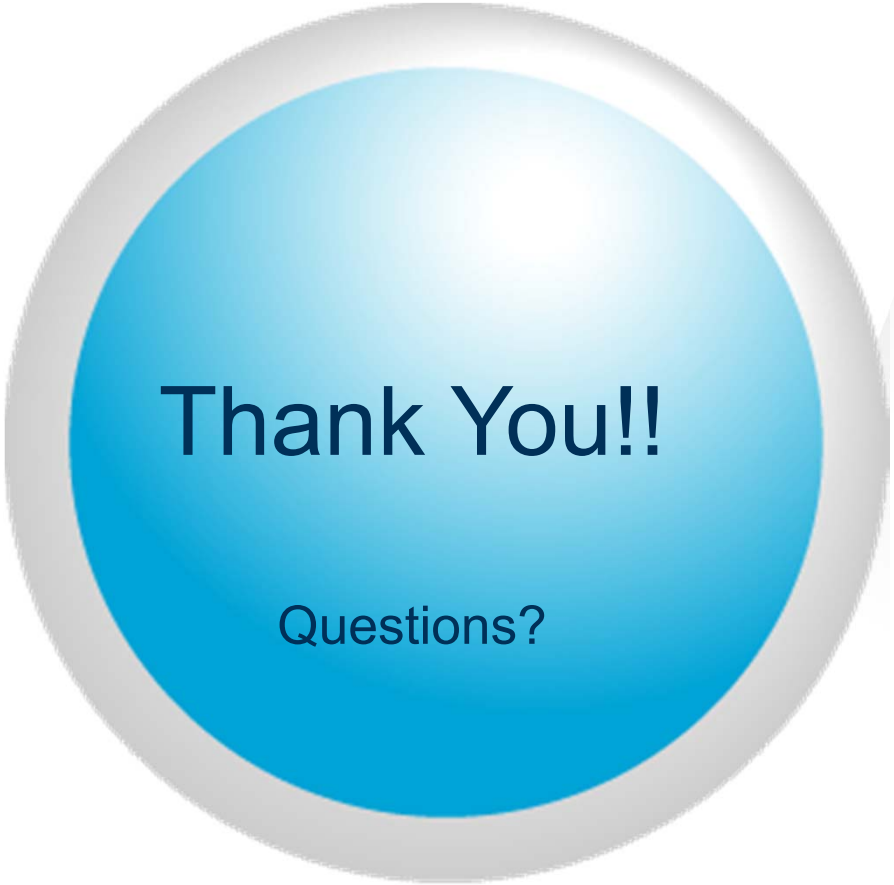
> Internal standard peak area less than 10.0%, (50.0% for samples which have a concentration below the LLOQ), or greater than 150.0% of the mean internal standard peak area for the analytical run acceptable calibration standards and QC samples (stable isotope labelled IS). See the decision tree in Appendix 1 for repeat selection

Summary

- Cross validation are very effective to ensure successful method transfer and consistency between labs
- Cross validation samples should be blinded and randomized contains spiked QC and/or incurred samples
- Covance Shanghai operates on a global quality system and has successfully conducted cross validation studies and matrix effect evaluation in Chinese matrix
- Covance Shanghai understands CFDA requirements and has supported China registration trials and helps sponsor obtain drug approval in China
- Covance Shanghai meets current regulatory requirements of CFDA, US FDA & OECD GLP and GCP. Bioanalytical data has been accepted by CFDA, FDA and EMA.

Acknowledgment

Covance Shanghai Bioanalytical Team



COVANCE
SOLUTIONS MADE REAL™