Bridging PK Reference Standards Across Global Bioanalytical Studies: Strategies and Lessons Learned

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19 Nov 2025, 18th EBF Open Symposium, Barcelona, Spain

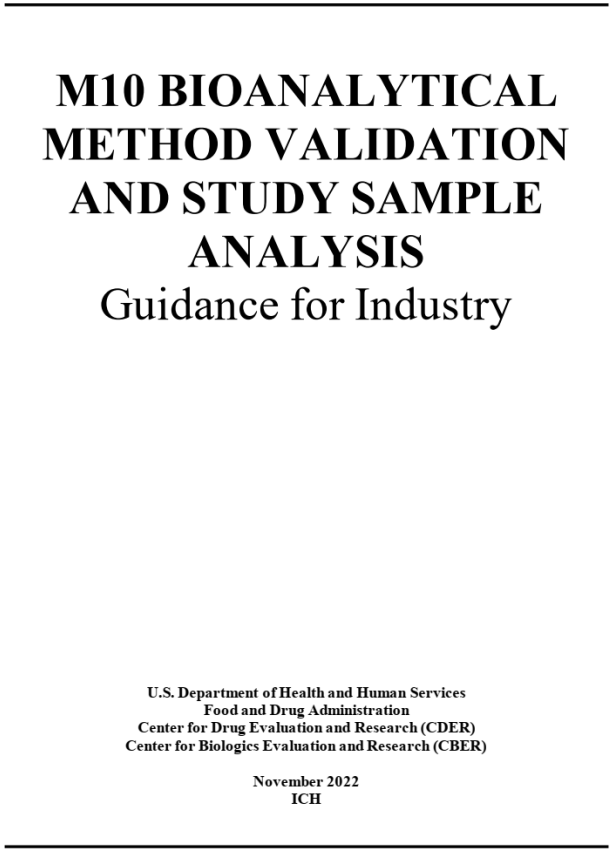
BioAgilytix

What is required to ensure comparability of different reference item lots in PK assays?

A potential issue occurring in large global studies.

Pharmacokinetics (PK) - ICH M10 Guideline

- Standard PK validation: six A&P runs on at least three days by two operators
- If the reference standard batch used for bioanalysis is changed, bioanalytical evaluation should be carried out with **QCs from the original material and the new material** prior to use to ensure that the performance characteristics of the method are within the acceptance criteria.



M10 BIOANALYTICAL METHOD VALIDATION AND STUDY SAMPLE ANALYSIS

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

November 2022
ICH

Established current standard at BioAgilytix

Comparison of old and new lot on one plate in one assay

- Calibration curve and two QC sets including ULOQ and LLOQ per reference item lot
- Cross evaluation of the QCs using old and new calibration curve

<table>
<tr><th>MSD</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td>STD1</td><td>STD9</td><td>ULOQ</td><td>ULOQ</td><td colspan="5" rowspan="7">NA</td><td>STD1</td><td>STD9</td><td rowspan="3">NA</td></tr>
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<tr><td>D</td><td>STD4</td><td rowspan="5">NA</td><td>LQC</td><td>LQC</td><td>STD4</td><td>ULOQ</td><td>ULOQ</td></tr>
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<tr><td>F</td><td>STD6</td><td colspan="2" rowspan="3">NA</td><td>STD6</td><td>MQC</td><td>MQC</td></tr>
<tr><td>G</td><td>STD7</td><td>STD7</td><td>LQC</td><td>LQC</td></tr>
<tr><td>H</td><td>STD8</td><td>STD8</td><td>LLOQ</td><td>LLOQ</td></tr>
</table>

Is this sufficient to ensure reference item lot to lot comparability in PK assays?

What can be adopted from Biosimilar quantification?

Scope: Assessment of using the same LBA to quantify both a biosimilar and its reference therapeutic.

Marini et al:

- Step 1: at least **three independent** runs made by **multiple operators over multiple days**. At least **three independent calibration curve preparations** each of the biosimilar and reference analyzed on the same plate
- Step 2: **Three independently prepared sets of QCs** from the reference and the biosimilar, respectively, and one independently prepared set of the calibration standards should be analyzed on the same plate in one run.
- Standard acceptance criteria plus **inter-batch mean bias (%RE) of ≤ 10% between the biosimilar and reference**

The AAPS Journal, Vol. 16, No. 6, November 2014 (© 2014)
DOI: 10.1208/s12248-014-9669-5

White Paper

Systematic Verification of Bioanalytical Similarity Between a Biosimilar and a Reference Biotherapeutic: Committee Recommendations for the Development and Validation of a Single Ligand-Binding Assay to Support Pharmacokinetic Assessments

Joseph C. Marini,[1,11] Michael Anderson,[2] Xiao-Yan Cai,[3] John Chappell,[4] Todd Coffey,[5] Dominique Gouty,[6] Aparna Kasinath,[7] Vera Koppenburg,[8] Philip Oldfield,[9] Shannon Rebarchak,[1] and Ronald R. Bowsher[10]

The AAPS Journal (2020) 22: 15
DOI: 10.1208/s12248-019-0397-8

Check for updates

Regulatory Note

Current Perspectives on Ligand-Binding Assay Practices in the Quantification of Circulating Therapeutic Proteins for Biosimilar Biological Product Development

T. M. Thway,[1,2] Y. M. Wang,[1] B. P. Booth,[1] K. Maxfield,[1] S. M. Huang,[1] and I. Zineh[1]

Intermediate approach to assess reproducibility in bridging

- Step 1: each **three independent calibration curve preparations** from both lots tested in one run to show comparability of lots
  - Cross evaluation of mean %RE of calibration curves
- Step 2: each **three independently prepared sets of QCs** from both lots evaluated using calibration curve from new reference item lot in one run
  - Target: bias ≤ 20% per QC level

MSD	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD9	STD1	STD9	STD1	STD9	STD1	STD9	STD1	STD9	STD1	STD9
B	STD2	STD10	STD2	STD10	STD2	STD10	STD2	STD10	STD2	STD10	STD2	STD10
C	STD3	Blank	STD3	Blank	STD3	Blank	STD3	Blank	STD3	Blank	STD3	Blank
D	STD4		STD4		STD4		STD4		STD4		STD4	
E	STD5		STD5		STD5		STD5		STD5		STD5	
F	STD6	NA	STD6	NA	STD6	NA	STD6	NA	STD6	NA	STD6	NA
G	STD7		STD7		STD7		STD7		STD7		STD7	
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MSD	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD9				ULOQ	ULOQ				NA	
B	STD2	STD10	NA			HQC	HQC				ULOQ	ULOQ
C	STD3	Blank				MQC	MQC				HQC	HQC
D	STD4	ULOQ	ULOQ	NA		LQC	LQC	NA			MQC	MQC
E	STD5	HQC	HQC			LLOQ	LLOQ				LQC	LQC
F	STD6	MQC	MQC			NA					LLOQ	LLOQ
G	STD7	LQC	LQC								NA	
H	STD8	LLOQ	LLOQ									

Case study: Bridging of new reference item lots

MSD	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD9	STD1	STD9	STD1	STD9	STD1	STD9	STD1	STD9	STD1	STD9
B	STD2	STD10	STD2	STD10	STD2	STD10	STD2	STD10	STD2	STD10	STD2	STD10
C	STD3	Blank	STD3	Blank	STD3	Blank	STD3	Blank	STD3	Blank	STD3	Blank
D	STD4		STD4		STD4		STD4		STD4		STD4	
E	STD5		STD5		STD5		STD5		STD5		STD5	
F	STD6	NA	STD6	NA	STD6	NA	STD6	NA	STD6	NA	STD6	NA
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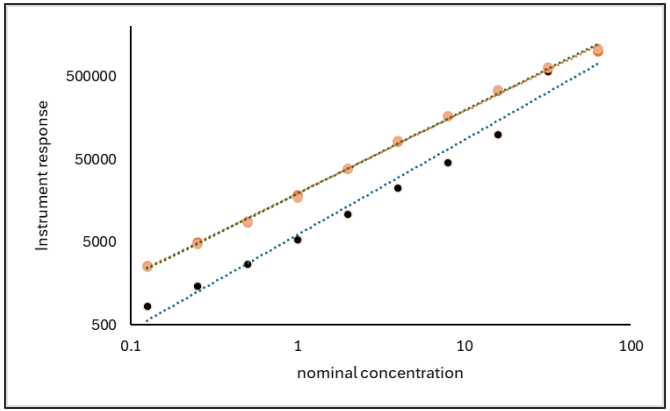
H	STD8		STD8		STD8		STD8		STD8		STD8	

Step 1: comparison of calibration curves

- 1/3 curves of validated reference item was incorrectly prepared

	instrument response		
[µg/mL]	curve 1	curve 2	curve 3
64	1001849	1007202	1079720
32	570673	630563	644122
16	99742	335303	338042
8	45899	164553	165748
4	22561	82049	84442
2	10739	38265	38771
1	5351	18299	17371
0.5	2682	8777	8952
0.25	1476	4987	4766
0.125	837	2566	2521



→ Plate Acceptance criteria must be clearly definded and distinguished from target acceptance critiera.

Case study: Bridging of new reference item lots

Step 2: QC set evaluation

Target Plate Acceptance Criteria:

- intra-batch RE and %CV for a QC level should be ≤ 20% (≤ 25% for LLOQ and ULOQ)
- Total error (TE) should not exceed 30 % (≤ 40 % at the LLOQ and ULOQ)
- The BCC for at least 67% of the QCs must be equal to or within ±20 % of the nominal concentrations (±25 % for ULLQ and LLOQ). At least two QCs at each level must pass.

Target Acceptance Criteria:

- The %RE of the BCC between each QC level of the two reference item lots should not exceed 20% difference

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Conclusions and Discussion

- Lessons learned: Acceptance criteria must be clearly defined
- Balance between robust data set and effort: more than one preparation ensures reliability
- Proposed set up for reference item bridging:
  - Two A&P runs: A calibration curve and two full QC sets for old and new lot independently prepared and tested on two days by two operators

MSD	1	2	3	4	5	6	7	8	9	10	11	12
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B	STD2	STD10	HQC	HQC						STD2	STD10	
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D	STD4	NA	LQC	LQC		HQC	HQC			STD4	ULOQ	ULOQ
E	STD5		LLOQ	LLOQ		MQC	MQC			STD5	HQC	HQC
F	STD6		NA			LQC	LQC			STD6	MQC	MQC
G	STD7					LLOQ	LLOQ			STD7	LQC	LQC
H	STD8					NA				STD8	LLOQ	LLOQ

Thank You

Ellen Gattkowski, PhD

Scientist III

Acknowledgement

- BioAgilytix colleagues for their support
- Our sponsors for their trust
- EBF