IMMUNOGENICITY STUDIES: WHAT TO KNOW FOR AN ANALYTICAL INSPECTION

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All data in this presentation are modified, and were crafted specifically as example scenarios

LEARNING OBJECTIVES

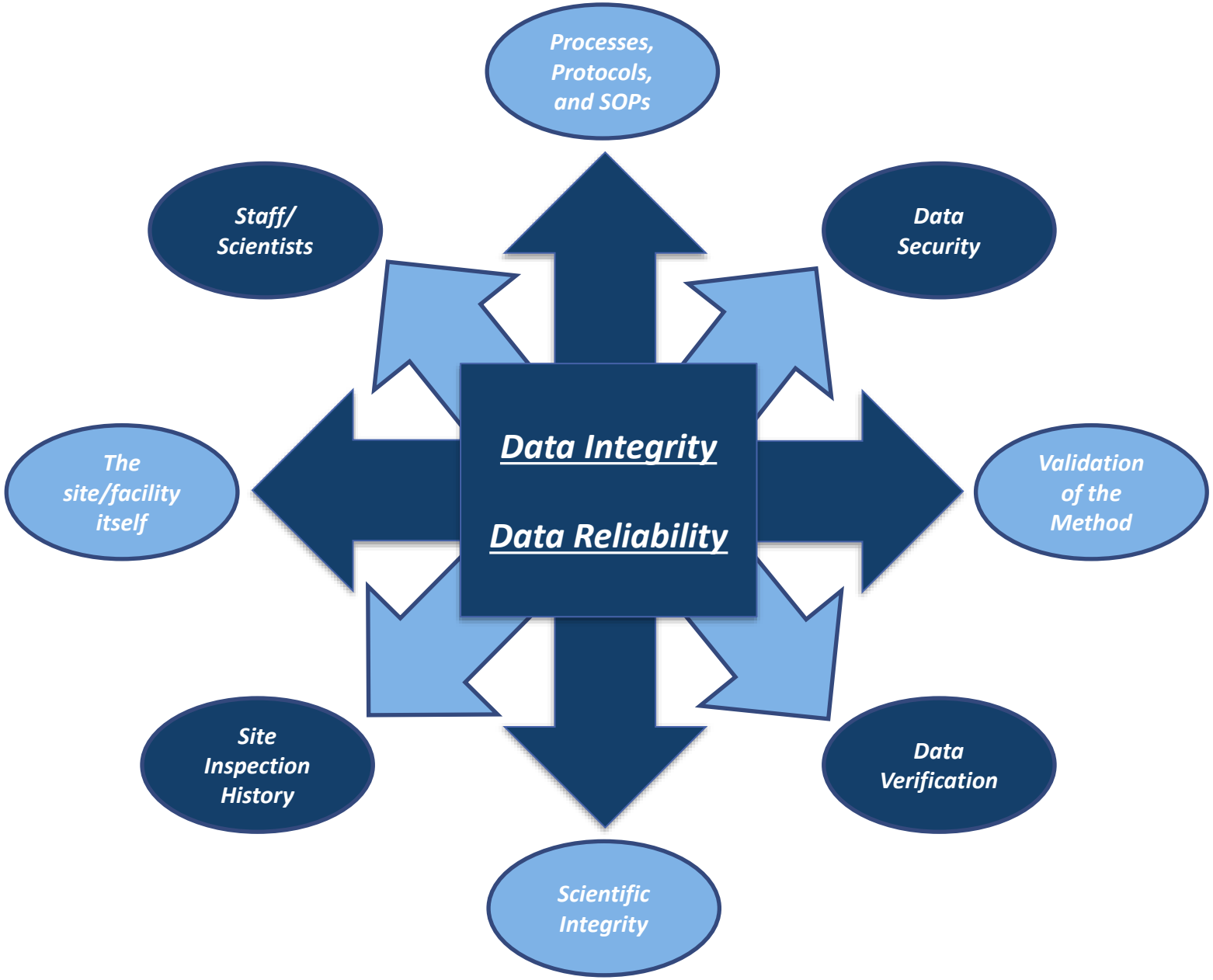
- *Are Analytical Inspections Different for Large Molecules/Biosimilars?*
- *Are There Any Applicable Guidances?*
- *What to Know About Method Validation Parameters for Biosimilar Studies?*
  - *PK Assays*
  - *Immunogenicity Assays*
- *What to Know About Sample Analysis for Biosimilar Studies?*
  - *Immunogenicity Assays*

Are Analytical Inspections Different for Large Molecules/ Biosimilars?

Yes and No……

(I know….. Not super helpful, right?!?!?!)

Analytical Inspections



- *Will review method validation and study records – paper and electronic*
- *Sample management/handling documentation and practices*
- *SOPs: up-to-date; relevant for large molecules; are they followed*
- *Audit Trails: are they active; are they reviewed regularly?*
- *Documentation: Contemporaneous, thorough, detailed*

Are Analytical Inspections Different for Large Molecules/ Biosimilars?

But, there ARE differences……

- ***Some of the inspectional elements will be different:***
  - ***Different method validation parameters***
  - ***Multiple method validations/methods within the same study***
  - ***Different equipment/instrumentation and software***
- ***Will likely involve a larger number of personnel***
  - ***Multiple method validations and studies could mean multiple teams of people***
- ***Will likely involve more data/more records***
  - ***Again, multiple method validations and studies result in a lot of data***
  - ***Don't underestimate how time-consuming a biosimilar inspection can be***

Are There Any Applicable Guidances?

Glad you asked that, because yes, there are!

- *For PK Assays: Bioanalytical Method Validation Guidance for Industry (May 2018)*

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry

- *For Immunogenicity Assays: Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection (February 2019)*

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/immunogenicity-testing-therapeutic-protein-products-developing-and-validating-assays-anti-drug

Method Validation Parameters: PK Assays

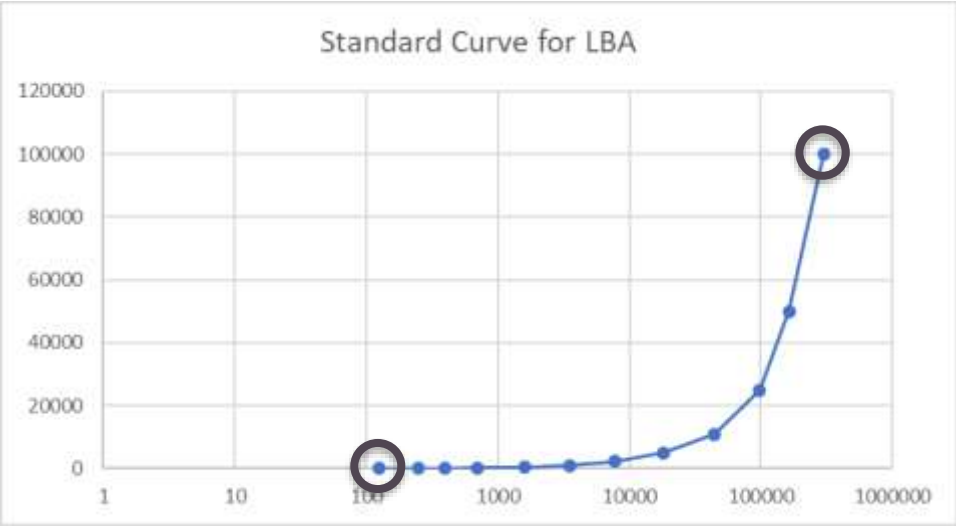
- Calibration Curves
- Duplicate Samples
- %CV
- 4- or 5-parameter logistical curve fit
- Anchor Points

Nominal concentration (ug/mL)	Assay Response Replicate 1	Replicate 2	Average	%CV
25	124	126	125	1.60
50	248	252	250	1.60
100	350	450	400	25.0
200	690	710	700	2.86
450	1500	1700	1600	12.5
1000	3300	3700	3500	11.4
2200	7400	8000	7700	7.79
5000	18000	18600	18300	3.28
11000	43200	45200	44200	4.52
25000	97000	97800	97400	0.82
50000	160000	166000	163000	3.68
100000	290000	310000	300000	6.67

%CV ≤20% (25% for LLOQ and ULOQ)

100 ug/mL standard has %CV = 25%; unacceptable; remove from the curve

Too many replicates with unacceptable %CV could indicate a potential problem with the assay



Ligand binding assay signals are typically not linear and will plateau; thus, use a 4- or 5-parameter logistical curve fit

Anchor points can be used to improve the curve fit; not considered a new LLOQ or ULOQ; no acceptance criteria applied

Method Validation Parameters: PK Assays

Precision and Accuracy

6 P&A Runs

Total Error

No Acceptance Criteria for QCs

QC	Nominal Conentration (ug/mL)		Average	%Accuracy	%CV
LLOQ	50	49	50	100	2
		50			
		51			
LQC	150	155	158.3	106	1.82
		160			
		160			
MQC	2000	1990	2000	100	0.5
		2000			
		2010			
HQC	37500	39500	40000	107	1.25
		40000			
		40500			
ULOQ	50000	50000	51000	102	1.96
		51000			
		52000			

A P&A run should not fail unless:

1) ***The calibration curve is unacceptable***
2) ***There is a <u>CONTEMPORANEOUSLY DOCUMENTED</u> technical/instrument error***

*** "Failed" QCs DO NOT result in a failed P&A run***

QC	Nominal Conentration (ug/mL)	P&A Run #		Average	%Accuracy	%CV	Total Error
LLOQ	50	1	49	50	100	2	2
			50				
			51				
		2	***70***	***65***	***130***	***13.3***	***43.3***
			70				
			55				
		3	45	47.7	95.3	5.3	10
			48				
			50				
		4	48	52.3	105	7.2	12.2
			54				
			55				
		5	51	51.3	103	8.8	11.8
			47				
			56				
		6	48	52.3	105	8.6	13.6
			52				
			57				
		<u>Avg</u>	***53.1***				
		<u>%Accuracy</u>	***106***				
		<u>%CV</u>	***13.2***				
		<u>Total Error</u>	***19.20%***				

Total Error: ±30% (±40% for the LLOQ)

- ***All QC results from P&A runs should be included in the assessment of inter-run P&A***
- ***Identified outliers should be included in inter-run P&A***
- ***If linked to a contemporaneously documented error, results can be presented with and without the outlier***

Method Validation Parameters: PK Assays

MRD

- *MRD = Minimal Required Dilution – dilution applied to all samples in an assay, including calibration standards, QCs, and samples*
- *An MRD is used to minimize non-specific assay signal/interference that could confound results*
- *Sample dilution yielding the highest signal-to-noise ratio*
- *Sample dilution yielding a signal closest to assay diluent*
- *Typically involves assessing multiple control concentrations in serial dilutions of matrix; comparison to the same concentration in assay diluent*
- *Ensure an adequate signal-to-noise ratio across the range of the assay*
- *MRD should not exceed 1:100*

Method Validation Parameters: PK Assays

Comparability

- During sample analysis, the biosimilar and the reference drug(s) will be assessed; one assay for all drugs
- Use biosimilar for standard curve
- At minimum:
  - Prepare QCs using the biosimilar and the reference drug(s)
  - Assess precision and accuracy against the biosimilar standard curve
  - Same acceptance criteria; all QCs for all drugs should meet criteria
- Key validation parameters (e.g., selectivity, stability) should also be considered in determining comparability for a biosimilar assay

QC	Nominal Conentration (ug/mL)	Biosimilar A	Average	%Accuracy	%CV	US-licensed reference	Average	%Accuracy	%CV
LLOQ	50	49 50 51	50	100	2	48 52 57	52.3	105	8.62

Method Validation Parameters: Immunogenicity Assays

Key Differences

- Assays are semi-quantitative: no calibration curve/standards
  - No Accuracy measurements/criteria
  - Precision is king in immunogenicity assays
- Cut Points: the level of assay response that defines a sample as being positive or negative
  - Influenced by matrix, assay background, assay variability, etc.
- Establishing an low positive control (LPC) concentration based on assay sensitivity
- Drug Tolerance: immunogenicity samples will likely contain the study drug, which can interfere with an assay; drug tolerance establishes the allowable drug concentration to ensure reliable results

Method Validation Parameters: Immunogenicity Assays

Precision

- ADA and NAb assays yield results of “positive” or “negative”
- While assays are not quantitative, and thus, do not have accuracy criteria, the concept of precision and assay variability is critical
  - Intra- and Inter-assay precision are critical components of method validation
- Precision assessments should include:
  - Runs on different days, using different analysts, and different instruments; inclusion of all possible variability
  - Six independent runs; six replicates of each positive and negative control
  - %CV ≤20%
- Precision should be assessed for all assay tiers:
  - Screening
  - Confirmatory; precision of unspiked samples; spiked samples; percentage inhibition
  - Titer; precision of titer dilutions

Method Validation Parameters: Immunogenicity Assays

Precision

Screening Assay

	NC-RLU	LPC-RLU	HPC-RLU
	70	90	3000
	74	100	2850
	78	97	3100
	69	103	3050
	71	92	2940
	68	91	3210
Average	71.7	95.5	3025
SD	3.72	5.32	126
%CV	***5.20***	***5.57***	***4.15***

Confirmatory Assay

	NC-RLU	NC-RLU Drug-Spiked	NC % Inhibition	LPC-RLU	LPC-RLU Drug-Spiked	LPC % Inhibition	HPC-RLU	HPC-RLU Drug-Spiked	HPC % Inhibition
	70	71	-1.43	90	66	26.7	3000	100	96.7
	74	69	6.76	100	70	30	2850	150	94.7
	78	75	3.85	97	71	26.8	3100	160	94.8
	69	72	-4.35	103	78	24.3	3050	145	95.2
	71	66	7.04	92	68	26.1	2940	120	95.9
	68	73	-7.35	91	70	23.1	3210	110	96.6
Average	71.7	71.0	0.8	95.5	70.5	26.2	3025	131	95.7
SD	3.72	3.16	6.03	5.32	4.09	2.39	126	24	1
%CV	***5.20***	***4.45***		***5.57***	***5.80***	***9.13***	***4.15***	***18.47***	***0.89***

Titer Assay

	MRD	*1:2*	*1:4*	*1:8*	*1:16*	*1:32*	*1:64*
1	3000	1500	750	300	155	80	75
2	2900	1600	700	290	158	75	70
3	3100	1700	800	280	155	79	71
4	3200	1450	725	310	147	80	69
5	2800	1650	810	305	160	81	70
Average	3000	1580	757	297	155	79	71
SD	158	104	47.4	12.0	4.95	2.35	2.35
%CV	***5.27***	***6.56***	***6.26***	***4.05***	***3.19***	***2.97***	***3.30***

Method Validation Parameters: Immunogenicity Assays

Cut Points

- Determination of assay cut points is a critical, fundamental part of method validation
- In the absence of a quantitative assay, the cut point is the value used to determine whether a sample is ADA-positive or ADA-negative
- Appropriate statistical methodology is crucial

- Cut points are determined for each tier of an ADA assay scheme
- Screening Cut Point:
  - determined using at least 50 individual treatment-naïve matrix lots
  - designed to yield a 5% false-positive rate
  - Can be fixed or floating; a floating cut point accounts for expected plate-to-plate variability

- Confirmatory Cut Point:
  - In a competitive inhibition assay format, is expressed as percentage inhibition (drug-inhibition of treatment-naïve matrix lots)
  - Designed to yield a 1% false positive rate (higher specificity)
  - Typically fixed

- Titer Cut Point:
  - Can be the same as the screening cut point
  - Often an alternate titer cut point is used to due to variability

Method Validation Parameters: Immunogenicity Assays

Cut Points

- Once cut points are established, criteria for positive and negative controls should be established
  - These are in addition to precision criteria discussed in the previous slides
- Examples:
  - Negative controls (NC): 3 of 4 replicates must yield an assay signal < screening cut point (SCP)
  - LPC replicates must have an assay signal > screening cut point
  - Confirmatory LPC replicates must yield assay signal inhibition > confirmatory cut point (CCP)

SCP = 79	NC-RLU
	78
	69
	71
	68
Average	71.5
SD	4.51
%CV	***6.31***

SCP = 79	NC-RLU
	78
	80
	81
	68
Average	76.8
SD	5.97
%CV	***7.77***

SCP = 79	LPC-RLU
	97
	103
	92
	91
Average	95.75
SD	5.50
%CV	***5.74***

SCP = 79	LPC-RLU
	97
	78
	75
	91
Average	85.3
SD	10.5
%CV	***12.3***

CCP = 22.2%	LPC-RLU	LPC-RLU Drug-Spiked	LPC % Inhibition
	90	66	26.7
	100	70	30
	97	71	26.8
	103	78	24.3
	92	68	26.1
	91	70	23.1
Average	95.5	70.5	26.2
SD	5.32	4.09	2.39
%CV	***5.57***	***5.80***	***9.13***

CCP = 22.2%	LPC-RLU	LPC-RLU Drug-Spiked	LPC % Inhibition
	90	87	3.3
	100	70	30
	97	71	26.8
	103	99	3.9
	92	68	26.1
	91	70	23.1
Average	95.5	77.5	18.9
SD	5.32	12.63	12.02
%CV	***5.57***	***16.30***	***63.73***

Method Validation Parameters: Immunogenicity Assays

Sensitivity and the LPC

- In a semi-quantitative assay, determining positive control concentrations is not as straightforward as in a PK assay

- Importance of the LPC:
  - Monitors performance of the assay at a signal range close to the cut point
  - Precision
  - The LPC should consistently yield positive results; ensure reliability of unknown sample results

- The LPC concentration is typically determined from sensitivity assessments
  - Sensitivity: the concentration at which the assay signal crosses the cut point
  - Determined using serial dilutions of the PC
  - The LPC is subsequently calculated from the sensitivity; based on a 1% failure rate (i.e., the LPC should be responsible for run failure approximately 1% of the time) – in real-world language, the LPC should yield negative results every now and again!

Method Validation Parameters: Immunogenicity Assays

Sensitivity and the LPC

	PC Concentration ng/mL							
Run	640	320	160	80	40	20	10	Sensitivity Concentration (ng/mL)
1	3000	1500	750	300	155	90	75	15
2	2900	1600	700	290	158	80	70	12
3	3100	1700	800	280	155	91	71	15.5
4	3200	1450	725	310	147	93	69	14
5	2800	1650	810	305	160	95	70	16
								14.5

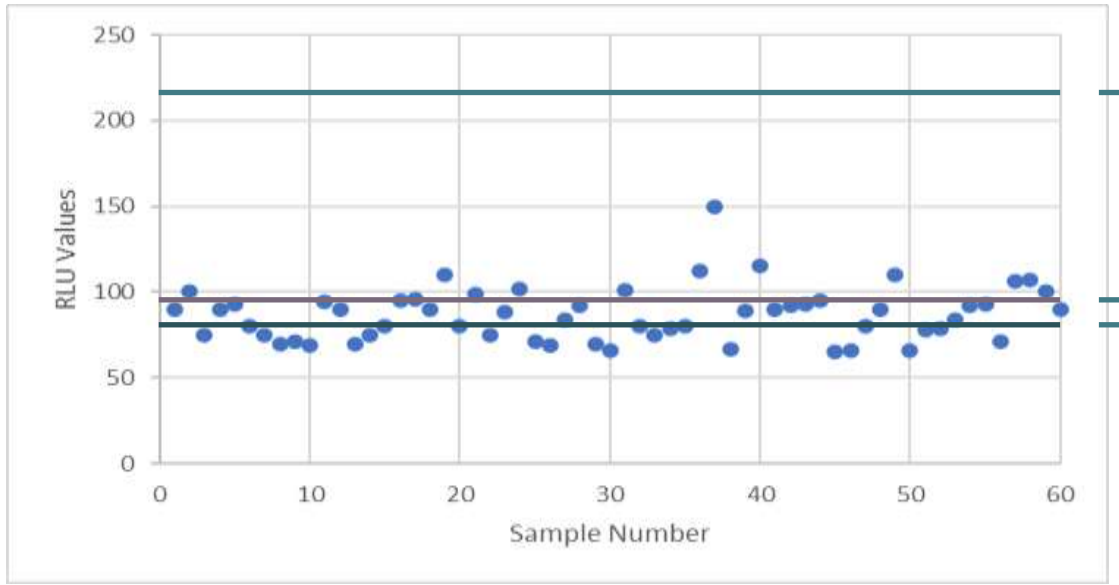
A concentration of 20 ng/mL was chosen as the LPC

1) *Does the LPC yield a 1% run failure rate?*
2) *Is the LPC signal relevant to study samples?*

SCP = 79	LPC-RLU
	97
	103
	92
	91
Average	95.75
SD	5.50
%CV	***5.74***

SCP = 79	LPC-RLU
	97
	78
	75
	91
Average	85.3
SD	10.5
%CV	***12.3***

The LPC does indeed occasionally cause a run to fail



Method Validation Parameters: Immunogenicity Assays

Drug Tolerance

- *Immunogenicity samples may also contain the drug product; particularly true in long-term, multiple-dose studies*
- *Due to the nature of ADA and NAb assays, the drug product can potentially interfere with the assay, resulting in false positive or false negative results*
- *Sub-optimal drug tolerance may require additional assay optimization steps (e.g., acid dissociation)*
- *Determined by assessing PC samples without the drug product and in the presence of increasing drug concentrations*
  - *The highest concentration allowing detection of a positive control sample is the validated drug tolerance*

SCP = 79	LPC (20 ng/mL)	Biosimilar Drug Concentration (ug/mL)	RLU Value	US-Reference Drug Concentration (ug/mL)	
	20 ng/mL	0	108	0	110
		25	111	25	112
		50	93	50	96
		100	***84***	***100***	***89***
		250	75	250	78
		500	70	500	75
		1000	71	1000	75

The validated drug tolerance is 100 ug/mL – both for the biosimilar and the US-reference drug (comparability)

These data indicate that assay results of samples with >100 ug/mL drug may not be reliable; false negatives

Challenge Question #1

- *Is the following statement True or False?*
- *Precision and Accuracy are critical parts of immunogenicity assay method validations*

Challenge Question #2

- *Biosimilar bioanalytical inspections*
  - *a) Involve many of the same assessments as small molecule bioanalytical inspections*
  - *b) Involve different assessments compared to small molecule bioanalytical inspections*
  - *c) May involve multiple methods, method validations, and studies*
  - *d) Will assess data integrity and data reliability*
  - *e) All of the above*