PRA Health Sciences

Different Interpretations on New FDA Guidance 2019 for ADA Validations - *a CRO's Perspective*

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Introduction

- **Based on the new FDA ADA guidance[1], the pharmaceutical industry and CROs adapted their ADA validation approach.**

- **From a CRO's perspective it became apparent that several pharmaceutical companies interpret some assay design elements differently.**

1. U.S. Department of Health and Human Services, Food and Drug Administration, et al., Guidance for Industry – Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Product (2019).

Different interpretations on new FDA guidance 2019 for ADA validations
- *a CRO's perspective*

Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

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)

January 2019
Pharmaceutical Quality/CMC

Content of the presentation

- **Cut point determination**
  - Cut point determination in healthy volunteer matrix
  - Evaluation of the cut point in the study population
  - Use of commercial matrix
- **Use of QCs**
  - Low positive control QC and sensitivity
  - Intermediate QC
- **Precision of the assay**
- **Documentation of method development and assay life cycle**

Cut point determination

- Healthy volunteer matrix -

"The cut point should be determined statistically with an appropriate number of treatment-naïve samples, generally around 50, from the subject population."

Cut point determination

- Healthy volunteer matrix -

Differences observed between sponsors

1. In PRA's SOP it is described as follows: "Preferably, the SCP is determined with around 50 treatment-naïve samples."

2. 48 matrix units is more feasible for practical reason (96 well plate, 48 replicates on the plate).

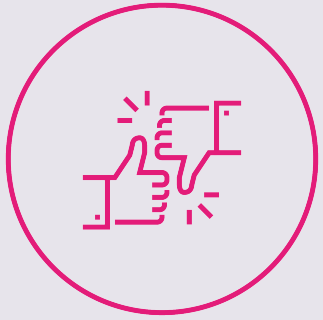
3. Sponsor X: 100 matrices in 3 runs on at least 2 different days. All data is pooled with a total of 300 values.

4. Sponsor Y: In total 200 units tested by 3 analysts. 75% of the matrices consisted of diseased matrix that are relevant for the trial.

5. Sponsor Z: At least 50 accepted results for cut point calculation per run (in 3 runs). For practical reasons 60 units are used, so 10 may be rejected on outlier or CV.

Cut point determination

- Evaluation of the cut point in the study population -

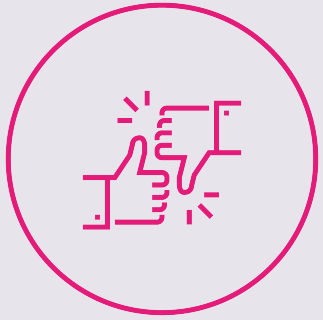


"Because samples from different target populations and disease states may have components that can cause the background signal from the assay to vary, different cut-points may be needed for discrete populations"

"Therefore, it is necessary to confirm that the cut point determined during assay validation is suitable for the population being studied. A sufficient number of samples from the target population should be used, justification for the number used should be provided."

Cut point determination

- Evaluation of the cut point in the study population-



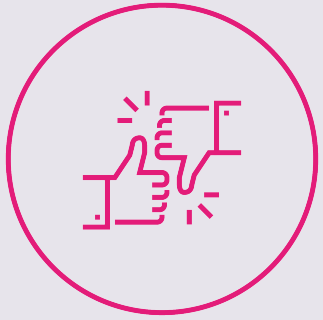
Differences observed between sponsors

1. <u>PRA approach:</u>
   a) Test matrix variability in the validation with 10 diseased units
   b) Perform false positive assessment on pre-dose samples of the clinical trial.
   c) If necessary, a study-specific cut point can be calculated.

2. <u>Sponsor Y:</u>
   a) 50 specimens taken prior to the 1st dose should be analyzed in the initial screening assay.
   b) In case the false positive rate is less than 5% or higher than 15% a new CPF should be determined based on results from the new population.
   c) If this new CPF does not differ by more than 20% from the initial CPF, the initial CPF will be used. Otherwise a new CPF must be established according to this pre-study validation SOP.

Cut point determination

- Evaluation of the cut point in the study population-



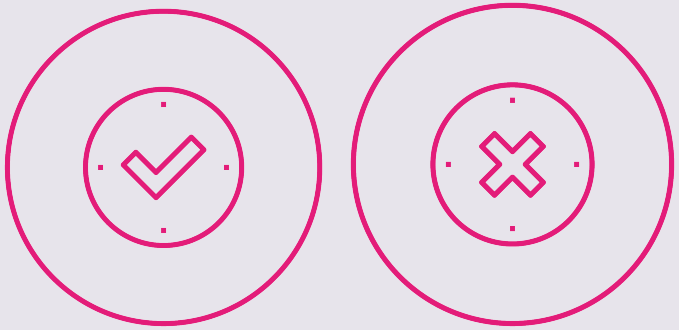
Differences observed between sponsor

3. Sponsor X

In case the validated cut points are determined on healthy matrix and the target population is a disease population, establish disease population-specific cut points using individual drug-naïve matrix samples of this particular disease population.

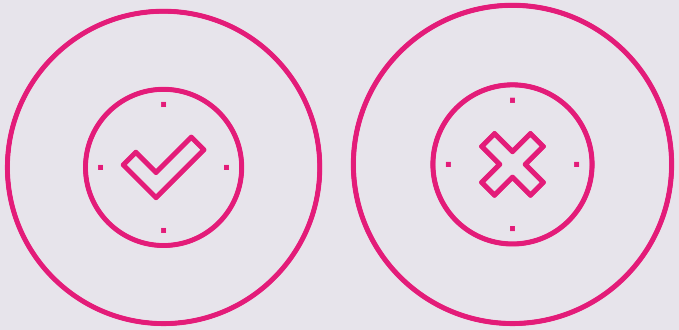
This can be determined using commercially available matrix samples or pre-dose study samples.

Use of commercial matrix



"If treatment-naïve samples from the appropriate subject population are not available for the pre-study validation exercise, alternative samples may be used.
Frequently, these are samples from commercial sources.
When alternative samples are used to determine the cut-point in validation exercise, the cut-point should be confirmed once samples from the appropriate population are available;

Use of commercial matrix



Questions that arise:

- Commercial sources: how representative are the samples?
  - Bags versus tubes
  - Plasma / serum preparation conditions?
  - What do we really know from the collection procedures or matrix donors?
  - Rare matrices: what can realistically be expected?

- Diseased populations, what is relevant?

Use of QCs – Low-positive QC and sensitivity

For the low-positive QC sample, we recommend that a concentration be selected that, upon statistical analysis, would lead to the rejection of an assay run 1% of the time.

Use of QCs – Low-positive QC and sensitivity

PRA SOP

1. Sample spiked with PC antibody at a high response limit
2. A 2-fold dilution (until negative score), with at least 6 dilution steps.
3. 4 runs analyzed
4. Each run contains 3 independently prepared dilution curves resulting in a total of 12 dilution curves

Differences observed with other sponsors

- 3 runs with 3 curves = 9 curves in total
- 3 runs with 4 curves = 12 curves in total
- 4 runs with 3 curves = 12 curves in total
- 6 runs with 4 curves = 24 curves in total

Use of QCs – Low-positive QC and sensitivity

Questions arise:

1. How to determine if the Low-positive QC has the correct value? In theory, judgment can only be made after 100 runs

- Sponsor Y: On every plate 2x low-QC, based on 0.5% failure rate, no QC can fail.

- Sponsor X: assessment of sLPC/cLPC performance and positional differences
by analysis of 40/20 sLPC/cLPC samples of which 1 is allowed to score below the SCP/CCP.
If not, elevate the PC concentration (e.g. 20%)

2. What should be the procedure if the Low-QC starts failing during sample analysis due to, for example, the use of new labelled materials?

Use of QCs – The intermediate QC in validation and bioanalysis

"The intermediate value is useful for assessing precision during assay validation. […] Intermediate-value QC samples for detection of ADA are generally not needed for monitoring system suitability during routine assay performance".

Use of QCs – The intermediate QC in validation and bioanalysis

- In PRA SOP the Mid-QC is used during:
  - Cut point runs: for precision only
  - All other validation parameters: for run acceptance and precision
  - Not used during sample analysis

Precision of the assay

"To provide reliable estimates, the sponsor should evaluate both intra-assay (repeatability) and inter-assay (intermediate precision) variability of assay responses"

Precision of the assay

PRA SOP

- Inter-assay: QC data from all validation runs will be used for the assessment of inter-assay precision (with a minimum of 6 runs)
- Intra-assay (separate experiment) -> 6 independent preparations of the same samples (QCs), on a single plate prepared by a single Lab Analyst.

Sponsor X

- Inter- and intra-assay precision determined in 6 runs in total (may be combined with other validation parameters)

Sponsor Y

- Inter-assay determined over all performed runs after final SCP, CCP and sensitivity
- Intra assay: One run with final QC levels

Documentation of assay development and life-cycle management

"The sponsor should examine robustness during development phase and determine whether aspects of assay robustness should be validated. For example, the efficiency and stability of labeled reagents and incubation times and temperature should be established."

"Lastly, a discussion should be provided regarding life-cycle management of approved immunogenicity assays, including an assay requalification schedule and assay transfer to contract testing laboratories for post marketing surveillance."

Documentation of assay development and life-cycle management

Questions and solutions

- Method development optimization steps such as MRD, temperature conditions and incubation times
  - Conducted by CRO → Method development report
  - Conducted by Sponsor → Transfer of method details to CRO

- Efficiency and stability of labeled reagents
  - CoA's for all labeled materials performed by a specialized vendor

- Assay Life Cycle Management report

Documentation of assay development and life-cycle management

Method development report

-1: Preparation of Method Development

	Description
Scope	**Theoretical Approach**
Purpose of the Project	Goal is to develop a PK method for the detection of study drug X
Availability of assay method	Method has been transferred, but likely needs optimization
Required reagents and materials	**Critical material**:

Type	Name (PRActic)	batch	PRA ID
Drug			
Capture			
Detection 1*			
Detection 2*			

Predicted Cmax of bio-analysis samples	400 ug/mL
Matrix	Human Serum
Patient/subject population	
Limitations and constraints	NA
Specific sponsor requests	
Assay principle	

Testing transferred assay

	Description
MD-1	Etc.

Assay optimization with normal mouse serum (NMS)

	Description
MD-5	Etc.

Pre-validation

	Description
	Test Precision/accuracy, dilution, selectivity and target interference. Etc.

Documentation of assay development and life-cycle management

1. BIOANALYTICAL METHOD LIFE CYCLE INFORMATION

Anti-XX-antibodies	Method validation #	Clinical study #1	Clinical study #2	Clinical study #3
Analyte	Anti-XX antibodies			
Validation type	Full validation	In-study	In-study	In-study
Method ID for liquid drug	PRA-NL-LML-0000, version 01	PRA-NL-LML-0000, versions 01, 02, 03, 04 and 05	PRA-NL-LML-0000, versions 02 and 03	PRA-NL-LML-0000, version 05
Method ID for lyophilized drug	NA	NA	NA	PRA-NL-LML-0001, versions 01, 02 and 03
Study period	18-Mar-2016 – 06-Apr-2016	23-Sep-2016 – 02-Apr-2019	02-Mar-2017 – 16-Aug-2018	06-Aug-2019 – 21-Apr-2020
Bioanalytical site	PRA Health Sciences - Early Development Services, Bioanalytical Laboratory, Amerikaweg 18, 9407 TK, Assen, The Netherlands			
Matrix	Serum			
Platform	Electrochemiluminescence Immunoassay (ECLIA)			
Format	A validated bridging format using XX-Biotin as capture and XX-Sulfotag as detection material			
Stock reference (liquid drug)	Compound X, batch number XY, expired 06-Nov-2016	Compound X, batch number Y, expired 31-Dec-2019	Compound X, batch number Y, expired 31-Dec-2018	Compound X, batch number XY, expired 31-Dec-2018
Stock reference (lyophilized drug)	NA	NA	NA	Compound XY, batch number Z, expiry date 10-Sep-2020
Calibration range	NA			
Matrix study population	Normal and diseased (patients with XY)	Patients with XZ	Patients with XYZ	Patients XX
Link to reports	Appendix 1.1 and Appendix 1.2	Appendix 2.1	Appendix 2.2	Appendix 2.3
Synopsis of amendment history	NA	NA	NA	Qualification of new stock reference

Documentation of assay development and life-cycle management

1.1 Clinical study #2

Method performance in study XX			
*Bioanalytical Report (**Error! Reference source not found.**)*	Determination of anti XX antibodies in human serum samples from clinical trial XX by ECLIA		
Source and lot of critical reagents	• Capturing: XX-DP-Biotin, batches XX_BIO_13Feb17 and XX_BIO_07Nov2017 • Detection: XX-DP-Sulfotag, batches XX_Sulfo_13Feb17 and XX_Sulfo_07Nov20; • Negative control: pooled blank serum (drug-naïve) with PRA IDs XX and XX • Positive control: rabbit polyclonal anti XX pool, batch XX		
Assay passing rate (Table 2)	4 screening runs passed acceptance criteria out of a total of 5 runs; 3 confirmation runs passed acceptance criteria out of a total of 3 runs; 2 titration runs passed acceptance criteria out of a total of 2 runs; 1 qualification run passed acceptance criteria.		
QC criteria for screening (Table 3)	Neg-QC	Total CV ≤20.0%	1 screening run was rejected according to QC criteria
	Low-QC	Response ≥ SCP	
	High-QC	Response > SCP	
QC criteria for confirmation (Table 4)	Neg-QC	Signal reduction < CCP	All confirmation runs were accepted according to QC criteria
	Low-QC	Signal reduction ≥ CCP	
	High-QC	Signal reduction > CCP	
QC criteria for titration (Table 5)	Neg-QC	Total CV ≤ 20.0%	All titration runs were accepted according to QC criteria
	Low-QC	Response ≥ SCP	
	High-QC	Response > SCP	
Study sample analysis / stability (Section 12)	In total 112 samples from 36 subjects were screened and a total of 11 samples from 8 subjects scored positive in the screening assay. In the confirmation assay 1 sample was confirmed positive for anti-XX antibodies. This positively confirmed sample was titrated and had measurable titer of 2.		

Acknowledgement

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