Experiences and Challenges in Evaluation of ADA Assay Performance Across Multiple Laboratories

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EBF, Focus Workshop, Points to Consider on Cut Points

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Outline

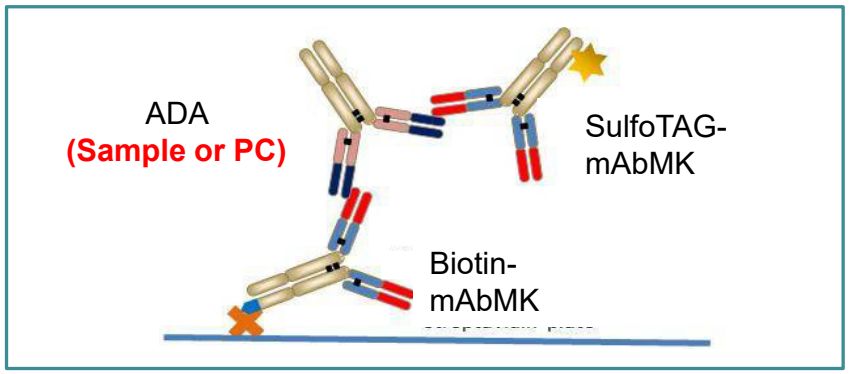
- Purpose of sharing experiences and challenges during ADA assay transfer and cross lab evaluation
- Case study:
  - Clinical ADA assay format and validation parameters
  - Experience during ADA assay transfer and validation at 5 CROs
    - Assay performance and challenges - CP, sensitivity/LPC, precision, DT and stability
  - ADA assay cross lab evaluation at 3 CROs
    - Study design and results
- Summary

Purpose of Sharing Experience and Challenges during ADA Assay Transfer and Cross Lab Evaluation

- Assay transfer is unavoidable to support global clinical studies
  - With the HGRAC restriction on sample export, setting up the assays in China is needed.
  - For in-licensed drug candidates, the assay may need to be transferred to the alternative vender(s)
  - Need multiple vendors to support larger studies
- ADA assay cross lab evaluation/comparison
  - To ensure the method validated at different laboratories provides comparable data to support global studies
  - There is no regulatory guidance on how to conduct ADA cross validation
- Sharing experiences and challenges would help to foster discussions on best practice.

Case Study - Clinical Anti-mAbMK Antibody (ADA) Assay

- Bridging format
- Labeled mAbMK drug for capturing and detection
- PC: a monoclonal antibody against mAbMK
- Matrix: human serum
- MRD: 100
- Tiered approach – screening, confirmatory and titer



Sample Acid Dissociation

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Sample incubation with Bio-mAbMK and TAG-mAbMK in neutralizing buffer

(For Confirmatory assay, prepare the capture/detector solution with mAbMK)

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Sample incubation on blocked Streptavidin MSD plate

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Read the plate on MSD analyzer

ADA Assay Validation Parameters and Considerations

- Assay cut points
  - At least 50 individual serum samples from healthy naïve subjects, analyzed by ≥2 analysts in 3 different days for total 6 individual runs with and without drug treatment.
  - Balanced design – Provided plate map template and statistical analysis by MSD for all CROs
- Sensitivity and LPC determination
  - At least 6 runs of PC dilution curves (minimum 6 dilutions/curve) over ≥3 days by ≥2 analysts
  - Balanced design – Provided plate map template and statistical analysis by MSD for all CROs
  - Define the LPC concentration by taking the higher value between screening and confirmatory LPCs
- Intra- and inter-assay precision (LPC, MPC and HPC)
  - At least 6 independent runs by ≥2 analysts over multiple days using multiple instrument, if available
- Drug tolerance (screening and confirmatory assay)
  - At least 2 individual runs in screening and confirmatory assay and calculate the mean.
  - Generally, target DT around trough concentration of therapeutic dose at 100 ng/mL PC

ADA Assay Validation Parameters and Considerations (cont.)

- Selectivity (screening and confirmatory assays)
  - At least 10 individual lots, freeze spiked LPC over 12 hrs; ≥ 80% of the blank samples should be below the screening CP and ≥80% of the samples spiked with PC at the LPC concentration should be positive.
  - At least 1 lot of hemolyzed and 1 lot of lipemic samples; if failed, additional test to ensure ≥ 80% pass
  - NMPA (China) requires the testing at HPC as well
- Stability (screening and confirmatory assays)
  - Short term stability (RT and 2-8°C) – at least 4 hours to cover the sample preparation
  - F/T – at least 5 cycles, if applicable
  - Describe any restrictions in the report and method based on the result of stability tests
- Prozone (Hook) Effect (screening and confirmatory)
- Robustness in screening and confirmatory assays
  - Incubation time ranges for capture and/or acid dissociation, etc.

ADA Assay Performance and Challenges at 5 Different CROs

CRO Labs	CPs*	LPC*	Precision	Issues Observed
1	SCP: 1.26 S/N; CCP: 19.51% inh; TCP: 1.58 S/N	24 ng/mL	No issue	No issue
2	SCP: 1.09 S/N CCP: 22.0% inh; TCP: 1.25 S/N	21 ng/mL	No issue	Low CP and did second round of mini-pool/ large pool selection; memo for justification
3	SCP: 1.07 S/N; CCP: 12.4% inh; TCP: 1.16 S/N	19 ng/mL	No issue	Used locally acquired individual serum samples for CP; BioIVT pool for the rest of validation and study support
4	SCP: 1.24 S/N; CCP: 20.4% inh; TCP: 1.47 S/N	80 ng/mL	After changing LPC, precision passed	LPC signal < SCP or CCP in multiple precision runs; 6 additional PC curves were added to estimate the assay sensitivity, and LPC was moved from 24 to 80 ng/mL.
5	SCP: 1.13 S/N; CCP: 12.6% inh; TCP: 1.18 S/N	21 ng/mL	Passed after control reading time within 2 min	Inter-assay precision for LPC (confirmatory), MPC and HPC (screening) were between 20% - 25.5%. The incubation time after adding read buffer needs to be controlled within 2 min.

* All statistical analysis on CP, sensitivity and LPC levels were conducted by MSD statisticians.

ADA Assay Performance and Challenges at 5 Different CROs (cont.)

CRO Labs	Drug Tolerance (target to 50 µg/mL)	Stability	Dilution /Hook Effect	Issue Observed
1	50 µg/mL at 100 ng/mL PC; 10 µg/mL at LPC	6 F/T cycles; 26h54min at RT; 27h at 2-8ºC	No hook effect up to 200 µg/mL PC	No issue
2	55 µg/mL at 100 ng/mf PC	6 FT; 11h at RT (19h LPC S/N ↑); 19 h at 2-8°C	5000-fold dilution; No hook effect up to 50 µg/mL PC	Stability: LPC signal increase at 19 h RT (reduced to 11h for stability)
3	100 µg/mL at 100 ng/mL PC; 47 µg/mL at LPC	9 F/T; 24 h at RT or 24 h at 2-8°C	No hook effect up to 30 µg/mL PC	Stability: LPC doubled signal after 12 h RT or 4-8°C
4	25 µg/mL at 100 ng/mL PC; 3.1 µg/mL at LPC	9 cycles F/T; At least 48h at RT At least 48h at 2-8°C.	No hook effect up to 100 µg/mL PC	Lower DT; no stability issue observed.
5	100 µg/mL at 100 ng/mL PC; 40 µg/mL at LPC	4 cycles F/T; 12h10min at RT 24h30min at 2-8°C	Observed hook effect at 10 µg/mL in screening assay. No impact as it still shows strong pos. result up to 209 µg/mL PC	Stability: LPC gave higher signal after more F/T or longer RT

Need for ADA Assay Cross-Lab Evaluation/Cross Validation

2019 FDA Guidance for Industry - Immunogenicity Testing of Therapeutic Protein Products - Developing and Validating Assays for Anti-Drug Antibody Detection

Reproducibility is an important consideration if an assay will be run by two or more independent laboratories during a study, and a sponsor should establish the comparability of the data produced by each laboratory. Comparable assay performance, including sensitivity, drug tolerance, and precision, should be established between laboratories.

ADA Assay Cross-Lab Evaluation in 3 CROs

Study design:

- Prepare the test samples by MSD personnel
  - 30 mock samples/spiked QCs, 10 samples at each of the levels at 0 ng/mL, 100 ng/mL and 3000 ng/mL PC in 10 different lots of individual normal human serum.
  - Split each sample into 4 aliquots
    - Analyze 1 set in house to confirm
- Ship 3 sets of samples (labeled with #1-30) to the testing CRO labs
- Analyze all samples (blind to CROs) as unknowns 6 times by 2 analysts in at least 3 days using the validated methods.
- Evaluate the data by MSD based on the pre-defined assessment criteria.

ADA Assay Cross-Lab Evaluation in 3 CROs (cont.)

Pre-defined assessment criteria:

- Each run has to pass the run acceptance criteria.
- Additional assessment criteria for the results from spiked samples at the different laboratories will be evaluated:
  - For individual un-spiked samples x 10 lots (0 ng/mL PC): at least 80% of samples should be screened or confirmed negative
  - For individual spiked samples at level 1 x 10 lots (100 ng/mL PC): at least 90% of samples should be screened and confirmed positive
  - For individual spiked samples at level 2 x 10 lots (3000 ng/mL PC): 100% of samples should be screened and confirmed positive
- Statistical analysis may be performed to compare the titers obtained at three labs to evaluate whether a correction factor is needed when combining data from all three labs.

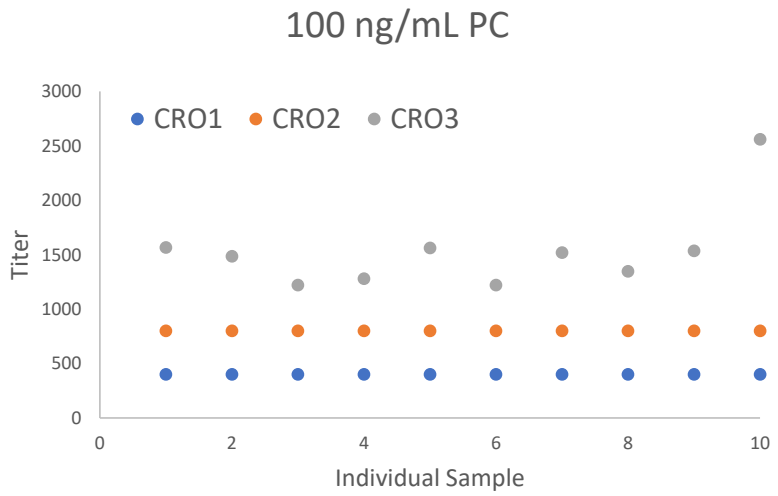
ADA Assay Cross Lab Evaluation – Screening and Confirmatory Resu

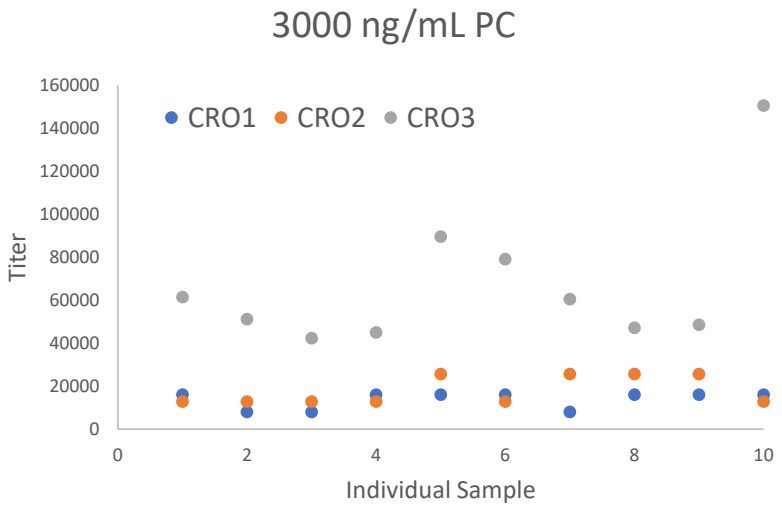
CRO Labs	Run Performance	NC (0 ng/mL)	100 ng/mL PC	3000 ng/mL PC
1	Pass	100% screened negative	100% screened and confirmed positive	100% screened and confirmed positive
2	Pass	100% screened negative	100% screened and confirmed positive	100% screened and confirmed positive
3	Pass	70% screened positive and 100% confirmed negative	100% screened and confirmed positive	100% screened and confirmed positive

- Screening and confirmatory assay results met the predefined acceptance criteria in all 3 CROs

ADA Assay Cross-Lab Evaluation – Titer Results

- Comparison of titer results from 3 labs





- Large variability of titer readout at CRO3, especially at the HPC level.
- Statistical analysis result is pending

Timespan for Assay Transfer, Validation and Cross Validation

CRO Labs	Pre-Work (SoW, reagent shipment, etc.)	Assay Development or Transfer/Testing	Validation	Validation Report	Cross Validation *
1	6 mo. (CRO evaluation)	1 mo.	1-2 mo.	2 mo.	3 mo.
2	1 mo.	5 mo.	12 mo.	9 mo.	9 mo.
3	2 mo.	4 mo.	1-2 mo.	2-3 mo.	3 mo.
4	3 mo.	2 mo.	7 mo.	3-4 mo.	NA
5	2 mo.	2 mo.	2 mo.	5 mo.	NA

*From sample receiving to validation plan, analysis and report

- Need to plan ahead for assay transfer, validation and cross validation, as it will take significant amount of time to get the assay ready for global study support.

Summary

- ADA assay transfer, validation and cross evaluation among multiple labs are challenging
  - Addressed different technical issues during the process
  - The end performance needs to be carefully evaluated; and the restrictions should be documented in the validation reports and the analytical methods that carry to the clinical study support
  - The process is time consuming and thus early planning is critical to ensure timely support to the clinical studies
  - Educating the project team on the complexity of ADA assay transfer/validation and cross validation will ensure good understanding on timespan and realistic planning on project timelines
- Given the limited guidance in the area, sharing the experiences in the industry will help establishing the best practice. We welcome all comments and suggestions.

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Questions?

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