Neutralizing Antibody (NAb) Assay Validation Testing and Reporting Harmonization Recommendations

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Background and Context

Health authorities and sponsors spend considerable time issuing and responding to filing queries due to evolving expectations and a lack of harmonized testing and reporting tools for immunogenicity assay validations.

AAPS Team	Timeframe	Constituency
ADA Harmonization (ADAH)	2017 – present	• 44 members | 29 organizations • 10 members from FDA
NAb Harmonization (NAbH)	2020 – present	• 41 members | 30 organizations • 3 members from FDA

AAPS teams convened to:

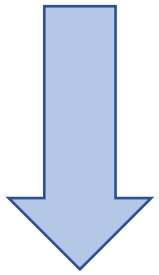
- Engage and collaborate with regulators
- Address gaps in understanding of immunogenicity assay requirements
- Develop harmonization tools for use by industry scientists to facilitate filings

Output = White papers

Session Description and Objectives

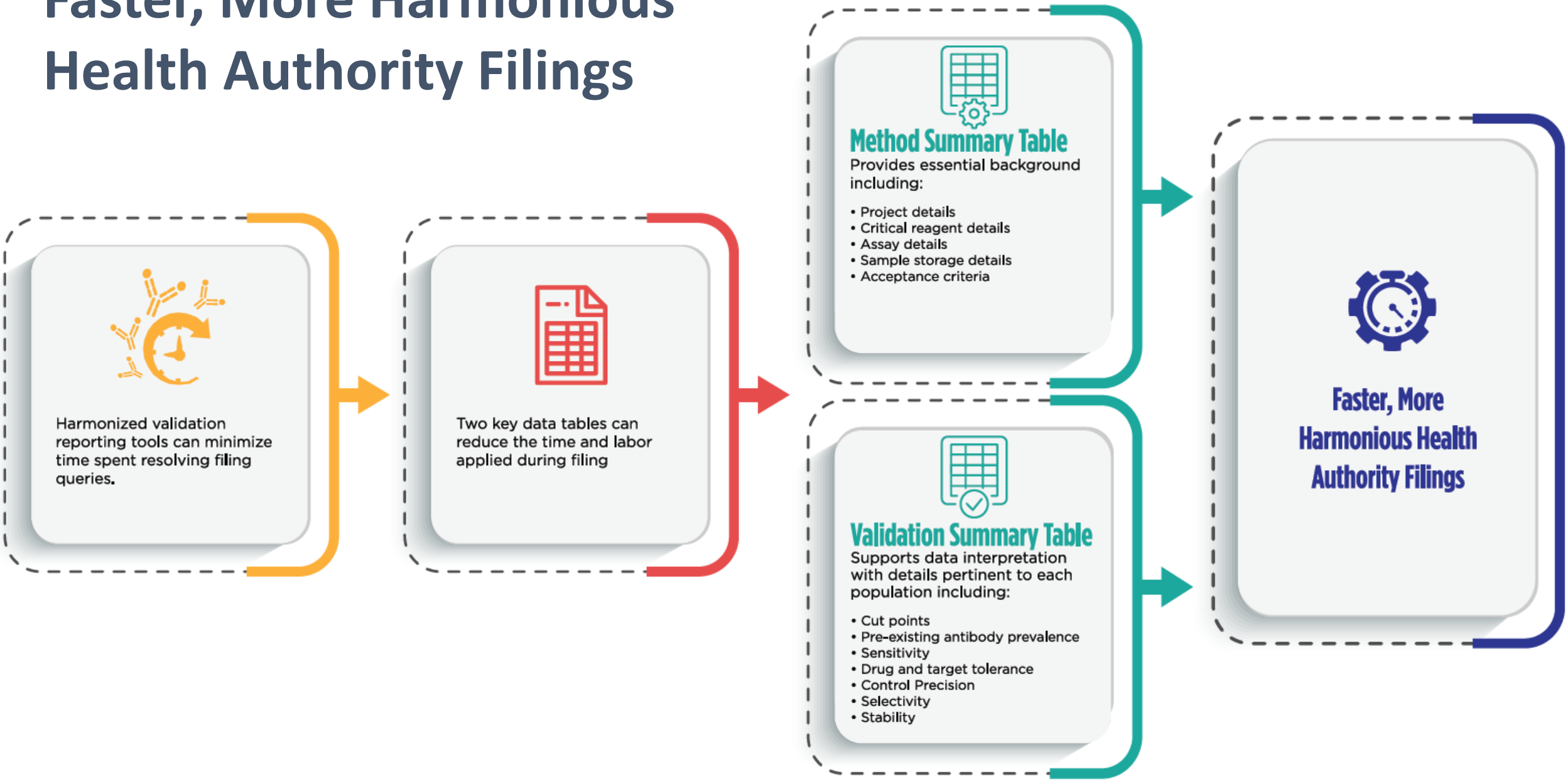
- Update on progress of NAbH team
- Promote awareness of AAPS harmonization efforts around bioanalysis of immunogenicity.
- Highlight the model framework that promotes improved consistency, clarity, and completeness of information presented in NAb assay validation reports.

Harmonized validation reporting practices



Streamlined regulatory review processes

Faster, More Harmonious Health Authority Filings



Sub-Teams and Leaders

Topic/Sub-Team	Leader(s)	Affiliation
NC Selection	Linlin Luo	Merck
Cut Point	Todd Lester	BioAgilytix
PC Selection	Swarna Ramaswamy, Jenny Hu	B2S, Amgen
Sensitivity | LPC Selection | Precision	Jason Pennucci, Fred McCush	EQRx, Pfizer
Selectivity/Specificity | Matrix Interference	Darshana Jani	Agenus
Drug Tolerance	Weifeng Xu	Merck
Target Tolerance	Bonnie Wu	Janssen
Sample Stability	Jenny Hu	Amgen
Assay Robustness	Susana Liu	Pfizer
Assay Criteria	Collective	n/a

Cut Point

Aspect	Considerations
Immunogenicity Strategy	Risk-based approach according to stage of development
Analytical Design	No one-size-fits all Balanced design Balance analytical vs biological variability
Statistics (outlier identification, cut point calculation)	Less prescriptive approaches to statistical computation of NAb assay cut points
Challenges assessing appropriateness of NAb cut point	False positives not applicable Small sample size of baseline/pre-dose samples NAb sample incidence of 0% may be justifiable

Sensitivity | LPC | Precision

Parameter	Considerations
PC Selection	• Test multiple PC in method development as needed to meet sensitivity (<1 μg/mL) and life cycle needs.
Sensitivity	• PC diluted in pooled matrix, ≥ 5 dilutions (2-3 fold) • ≥ 6 independently prepared titration series • Interpolate at assay cut point • Alternative approaches may be acceptable
LPC Concentration	• Target 1% failure rate • Alternative approaches may be acceptable

Precision	• Incorporate relevant sources of variability (i.e. analyst, run, day)
	• Normalized signal is typical; raw signal may be informative
	• Acceptance may be dependent on assay format
	  • Cell-based assays may have higher levels of imprecision
	  • Consider assay performance throughout development
	• Assay format (direct vs indirect) may dictate relevant controls
	  • PCs, drug controls, ligand controls, etc.

Selectivity/Specificity

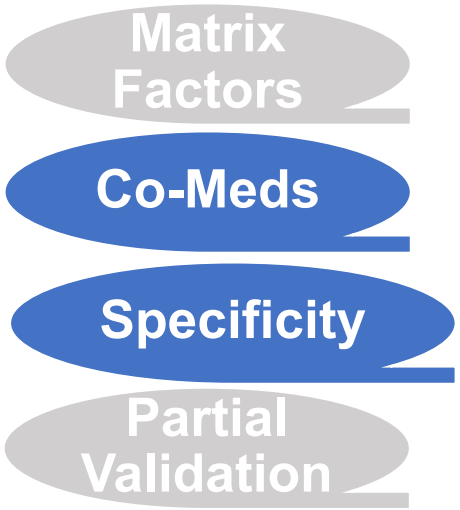
Key Assessments for Selectivity:

- **Matrix Factors**
- Co-Meds
- Specificity
- Partial Validation

Evaluation	Sample preparation			Acceptance Criteria
	Sample Type	**Spiked with**	**# of Samples**	
Selectivity	Individual Target Matrix	Unspiked and Spiked with minimum at LPC Level	10	≥80% unspiked Neg; ≥80% unspiked Pos
Hemolysis	Individual Target Matrix or Negative control (NC) spiked with <5% hemolysed blood		10 samples or 5 sets of NC	≥80% unspiked Neg; ≥80% unspiked Pos. If not passed, Reporting criteria specified in method SOP
Lipemia	Individual Target Matrix spiked with ≥300mg/dL Triglyceride level		10 samples or 5 sets of NC	
Bilirubin*	Individual Target Matrix spiked with ≥1.2 mg/dL Bilirubin level		10 samples or 5 sets of NC	

Selectivity/Specificity

Key Assessments for Selectivity:

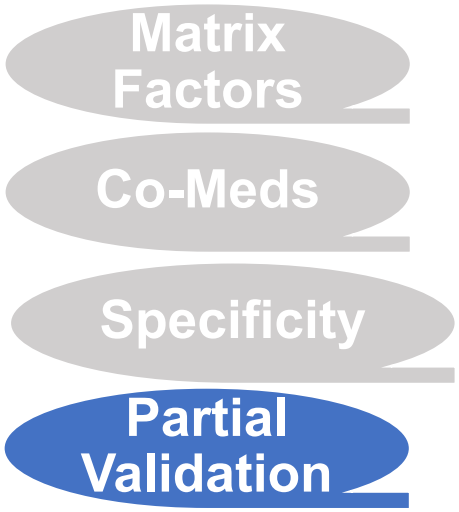


Samples to test	Spike with	Result
Test NC, LPC and HPC (n=1)	Co med spiked at expected C_{trough} and C_{max} concentrations	Report results

Assay platform	Samples to test	Spike with	Result
LBA	NC, LPC and HPC (prepared in normal or target matrix) n=1	Structurally similar, irrelavent biologic at expected C_{trough} and C_{max} concentrations	Report results
CBA	NC, LPC and HPC (prepared in normal or target matrix) n=1	Alternate stimuli at relevant concentration	Report results

Selectivity/Specificity

Key Assessments for Selectivity:



- Partial validation is warranted when
  - Different disease population
  - Change in sample collection method (anti-coagulants) or
  - Change from plasma to serum or vice versa
  - Significant change of assay component (buffer, FBS…)?
- Selectivity will be tested
  - for each new disease population
  - if In-study CP is changed
- Selectivity confirms LPC
- General selectivity acceptance criteria will be applied
- In-study baseline samples can be used for selectivity assessment

Target Tolerance

Important Considerations	Supporting Points
Impact on NAb assays	• Soluble targets i.e. ligand, soluble-shed receptor, proteolytic fragment of whole protein, other • False positive or false negative results depending on the assay platforms • May impact the accuracy of cut point assessment
Accurate measurement of target concentrations enables reliable assessment of assay target tolerance	• Multiple factors can affect free target concentration (biological, MOA, analytical) • Published target concentration may not be reliable • The recombinant target used for the assay should be close to the physiological form.
Mitigation Approaches	• Sample dilution/pretreatment with acid or heat to inactivate target • Target removal via extraction (biotin-anti-target antibody on streptavidin magnetic beads) • Blocking target with anti-target antibody
Measure and Report Target Tolerance	• For target interference generating false positives ✓Perform target titration assay in the blank serum to establish the dose response curve. ✓Tolerance level is defined as the interpolated value with the cut point. • For target interference generating false negatives ✓Target titration assay in the presence of PC at the expected sensitivity ✓Determine the level of target that reduce the assay response below the cut point
Determine the Adequacy of Target Tolerance	The target tolerance level should be higher than the physiological target concentration in sample matrix

Additional parameters in progress

- **Drug tolerance**
  - Drug tolerance is calculated by applying population-specific cut points to existing validation data
  - Ideally, method will be able to detect NAb in all study samples, despite the presence of various concentrations of drug, harmonization strategies in progress

- **Assay robustness**
  - Plate homogeneity
  - Incubation time and temperature
  - Cell propagation
  - Seeding density/viability
  - Growth media/other critical reagents

- **Stability similar to ADA**

Thank you!