USP Standards and Tools to Establish System Readiness and Facilitate Implementation of the Multi-Attribute Method

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Agenda

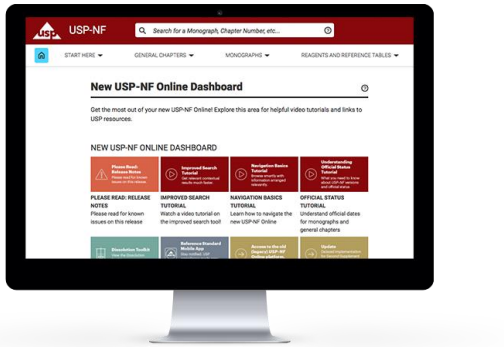
- USP Overview
- Introduction to MAM and Project Scope
- Establishing System Readiness
- Analytical Method Transfer and Assay Performance across Labs
- Additional USP Resources

1 USP Overview

USP Overview

- Founded in 1820, United States Pharmacopeia (USP) has provided public standards for medicines to protect patient safety and improve public health for over 200 years.
- USP is an independent, scientific nonprofit organization focused on building trust in the supply of safe, and quality medicines.
- USP standards are used in over 150 countries and enforced in over 40 countries; public standards are available to verify the quality and safety of medicines.
- USP works globally to help ensure medicines are stored, transported, and administered properly.

Understanding USP Standards

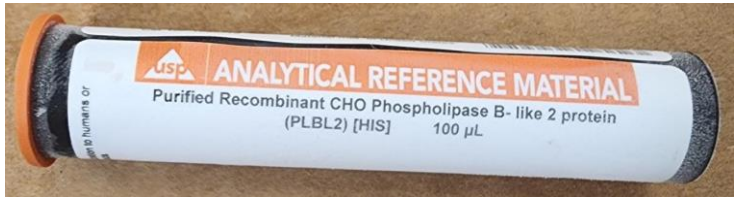


Documentary standards

- Monographs: Specifications for DS and DP
- <1000 general chapters: Procedure and validated methods
- >1000 general chapters: Informational

Reference standards

- USP offers >3500 reference standards, including DS, DP, impurities, reagents, etc.
- Tested in multi-lab studies
- Approved by the appropriate USP Expert Committee



Analytical Reference Materials (ARMs)

- Fit-for-purpose assessments
- Details on testing/application on Product Information Sheet or application notes
- Potential uses: Assay control, control material for method development, standardization testing across laboratories, method transfer

Introduction to MAM and Project Scope

Comparison of Common PQAs Measured by MAM *vs.* Conventional Methods

- MAM is technology that allows a scientist to investigate multiple quality attributes in a single method
- LC-MS-based peptide mapping approach has emerged as the most mature and widely used platform for MAM
- Advantages of MAM
  - Improved efficiency by replacing multiple technologies
  - More specific information on site of modification
  - Alignment with QbD concepts

mAb Product Quality Attribute		MAM	Conventional Method				
		Pep Map LC-MS	SEC	IEX/cIEF/ icIEF	rCE-SDS	nrCE-SDS	Glycan by HILIC
Identity		+	-	+/-	-	-	-
Soluble aggregates		-	+	-	-	+/-	-
Fragments/Clips		+	+/-	-	+	+	-
Amino acid mutation/Mis-incorporation		+	-	-	-	-	-
Cys related modifications	Unpaired Cys	+	-	+/-	-	-	-
	Disulfide isoform	+	-	-	-	-	-
	Thioether	+	-	-	+/-	-	-
Glycosylation	*N*-linked glycosylation	+	-	+/-	-	-	+
	Non-glycosylated	+	-	-	+	-	-
	O-Linked glycosylation (Ser, Thr)	+	-	+/-	-	-	-
Isomerization (Asp)		+	-	+/-	-	-	-
Oxidation (Met, Trp)		+	-	-	-	-	-
Hydroxylysine		+	-	-	-	-	-
Charge variants	Deamidation (Asn, Gln)	+	-	+	-	-	-
	Glycation	+	-	+	-	-	-
N-Terminal modifications	Signal peptide	+	-	-	-	-	-
	N-Terminal pyroGlutamate	+	-	+	-	-	-
C-Terminal modifications	Lys deletion	+	-	+	-	-	-
	Amidation	+	-	+	-	-	-

"+" : application can be used *"-" : application not commonly used* *"+/-" : application may be used*

Table adapted from USP General Chapter *<1060> Mass Spectrometry Based Multi-Attribute Method for Therapeutic Proteins*

<1060> Mass Spectrometry-Based MAM for Therapeutic Proteins

- Based on stakeholder input, USP established an Expert Panel to draft a chapter on MAM
- Scope
  - Best practices chapter (> 1000)
  - Focus on MS peptide-based workflow
    - Brief mention of intact and subunit workflows
  - Description of key components
  - Considerations from characterization, process development and QC
- Draft chapter was published in Pharmacopeial Forum (PF) 49(5) for public comment
  - MAM Expert Panel has addressed all comments
  - <1060> expected to be available in USP-NF 2025 Issue 2

Name	Organization
Edward Chess (Chair)	Consultant
Rachel Chen	Biogen
Disha Dadke	Aurobindo Biologics
Andrew Dawdy	Pfizer
Anita Krishnan	Biocon Biologics
Zhirui (Jerry) Lian	Eli Lilly
Benjamin Moore	Travere Therapeutics
Yuko Ogata	Pfizer
Da Ren	BioTherapeutics Solutions
Lei Wang	Takeda
Christopher Yu	Genentech
Ying Zhou	Teva

Project Background and Objectives

A 2019 publication by FDA staff* outlined 4 considerations for adoption of MAM in QC:

1) Risk assessment
2) Method validation
3) New peak detection capability and specificity
4) Performance vs. conventional methods

- Collecting data to support bridging from conventional techniques to MAM is a significant investment
- This study will provide a publicly available dataset and a roadmap to inform transitioning to MAM

* S Rogstad *et al Analytical Chemistry* **2019** *91* (22), 14170-14177 DOI: 10.1021/acs.analchem.9b03808

- Cooperative agreement with FDA under a BsUFA-funded research grant
- Objectives
  - Assess the performance of the MS-based MAM versus conventional QC methods to identify differences in PQAs
  - Correlate changes in those PQAs with bioactivity, binding affinity, and structure
- Considerations for Study Design
  - Selected Adalimumab and Etanercept as examples of mAb and fusion protein therapeutics
  - Used USP mAb 001 RS as control and system readiness tool

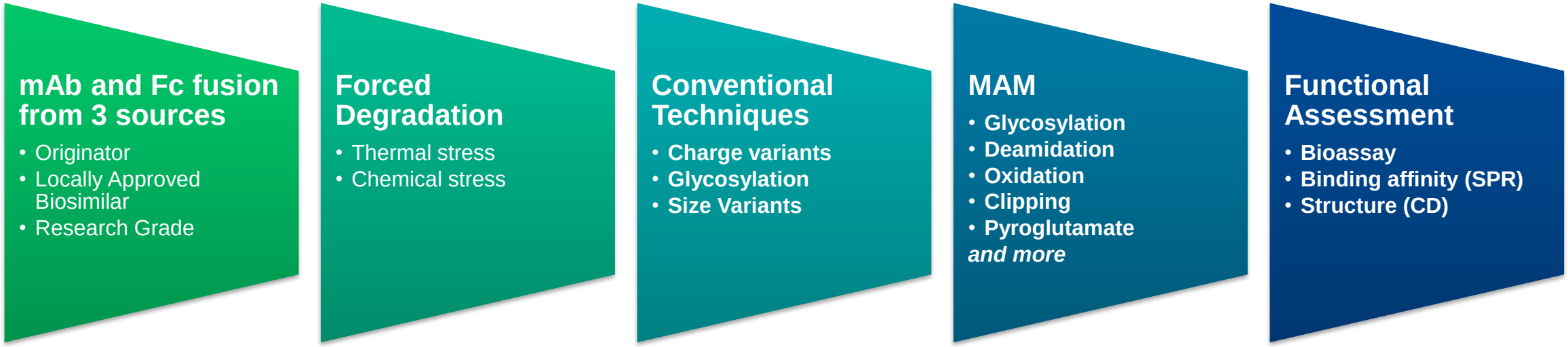
Establishing System Readiness

Overview from using USP mAb 001 as a system readiness standard for analysis of *adalimumab and etanercept* *

* *This project is supported by the Food and Drug Administration of the US DHHS under FAIN U01FD008862 totaling 1,530,721 dollars and is fully funded by FDA and DHHS. The contents are those of the author(s) and do not necessarily represent the official views of, nor an endorsement of FDA, DHHS, or the United States Government.*

Cooperative Project with FDA*

BsUFA III Pilot Research Program



- Selected Adalimumab and Etanercept as model systems for mAbs and Fc fusion proteins due to availability of biosimilar and research grade products
- Leverages multiple sources and forced degradation to generate a wide range of modifications for comparison of method performance

Expected Outcomes

- Public dataset comparing performance of MAM vs conventional methods
  - Advantages/ disadvantages of MAM vs. conventional QC methods
  - Assessment of correlation of changes in PQAs with function and structure
- A roadmap to facilitate adoption

What is System Readiness?

Facilitates the transition from method development to cGMP

- From <1060> *Mass Spectrometry-Based MAM for Therapeutic Proteins*
  - In general, **“system readiness”** indicates the analytical system is functioning and passes predefined criteria, which makes it ready for analysis.
  - The term “system readiness” is often used in the **non-cGMP stage for system suitability** during analytical method development.
  - In cGMP stage testing, in addition to the system readiness check, a **formally defined system suitability test** will need to be established.
- Metrics should be accurate and simple!

<1060> Common Metrics	
• Total Ion chromatogram (TIC) signal intensity	• Integrated peptide area
• Mass Accuracy	• Met oxidation (a measure of artifactual oxidation)
• MS Resolution	• In-source fragmentation
• Retention Time	• MS/MS fragment ion intensity (if applicable)
• Chromatographic resolution	• MS/MS fragment ion mass accuracy (if applicable)

System Readiness

Considerations for MAM System Readiness Standards

MAM Standard	Advantages	Disadvantages
Commercial Peptide Mix	• Easy sample preparation / no enzymatic digestion involved • Simpler and may be more consistent measure • Application across multiple projects – facilitates large body of system readiness data	• No measure of sample preparation quality • May not be as representative of the final sample (e.g., N-glycosylation, oxidation hotspots, deamidation hotspots) • Cost
Commercial Protein Standard	• May be more representative of sample • Can assess quality of enzymatic digestion along with system • Application across multiple projects – facilitates large body of system readiness data	• Requires enzymatic digestion of sample – may be less reliable measure of system itself • May not capture system's ability to measure attribute types specific to a project (e.g., a conjugated site on a protein) • Cost
In-House-Manufactured Protein Standard	• Opportunity to access quality of enzymatic digestion along with system • Application across multiple projects – facilitates large body of system readiness data	• Requires enzymatic digestion of sample • Does not allow for evaluation of the exact data processing method used for the project-specific samples • May require a different LC-MS method than that used for the project-specific MAM assay • No vendor Certificate of Analysis - QA burden on user
Project-Specific Reference Material	• Provides most complete assessment of the exact MAM assay, including project-specific attributes • Opportunity to access quality of enzymatic digestion along with system	• Requires enzymatic digestion of sample – may be less reliable measure of system itself • No vendor Certificate of Analysis - QA burden on user

Adapted from draft of USP General Chapter *<1060> Mass Spectrometry Based Multi-Attribute Method for Therapeutic Proteins*

USP mAb 001 RS as a tool for System Readiness

IgG1 monoclonal antibody Reference Standard

- 10 mg/ml mAb product
- Well characterized
- Publicly available

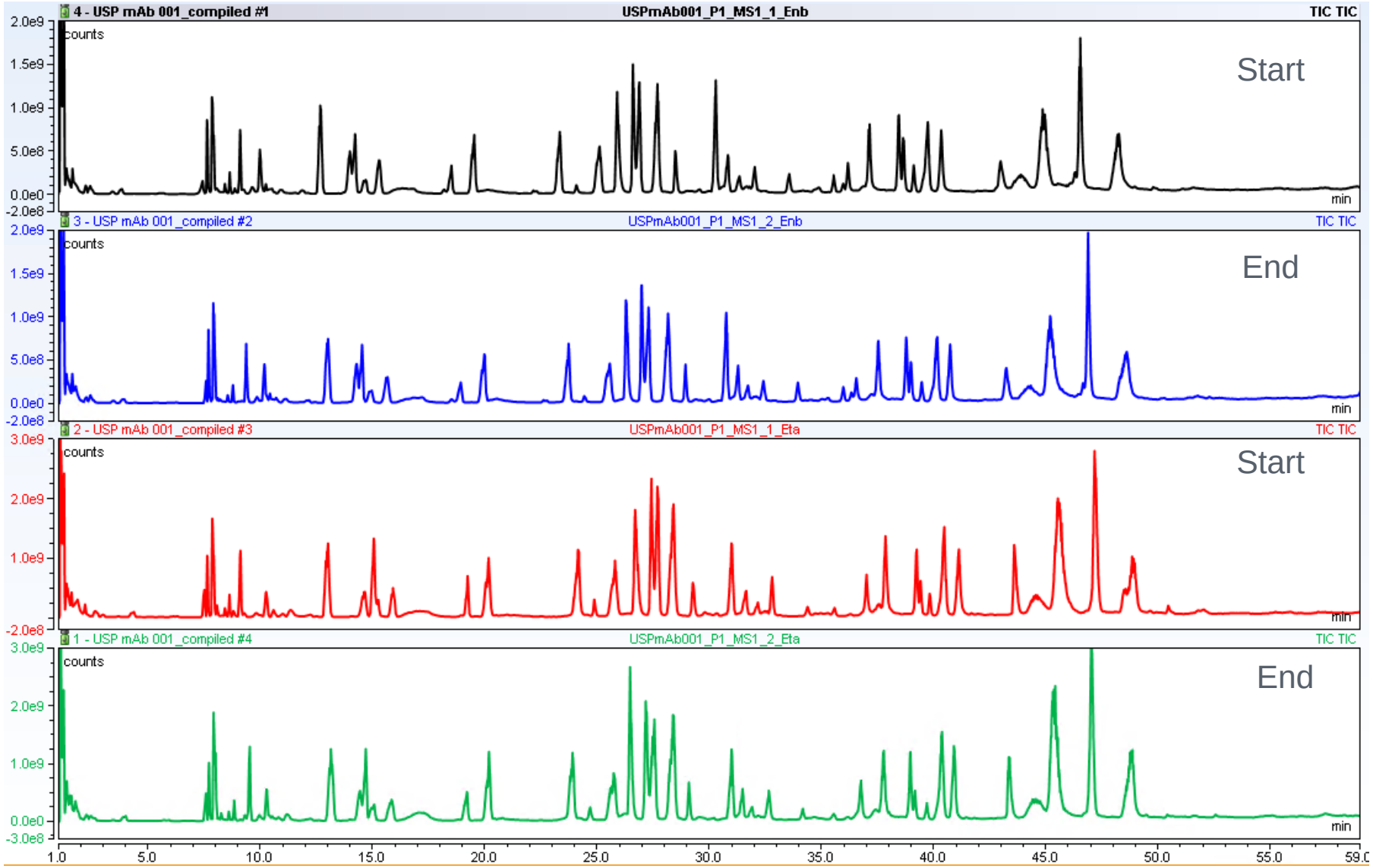
Representative of sample type and contains common PQAs

- N-glycosylation
- Oxidation hotspots
- Deamidation hotspots
- C-term Lys
- N-terminal pyro cyclization
- DP clipping site

Fit-for-purpose as a System readiness standard and digestion control

- Peptides generated are suitable for system readiness attributes
- Sequence coverage > 97% for light and heavy chains
- Used in previous internal multi-lab studies

Representative Tryptic Digest Base Peak Chromatograms of USP mAb 001 Reference Standard



- Different analysts
- Different days
- Different sample lots
- Different reagent lots

N=2

Profiles are highly similar

Sequence of USP mAb 001 Reference Standard and selected peptides for MAM system readiness

Heavy Chain

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWVKQT PGRGLEWIGA IYPGNGDTSY
NQKFKGKATL TADKSSSTAY MQLSSLTSED SAVYYCARST YYGGDWYFNV WGAGTTVTVS
AASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS
SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKAE PKSCDKTHTC PPCPAPELLG
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR
WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K

Light Chain

QIVLSQSPAI LSASPGEKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT SNLASGVPVR
FSGSGSGTSY SLTISRVEAE DAATYYCQQW TSNPPTFGGG TKLEIKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Sequence Coverage	
Heavy Chain	100 %
Light Chain	100 %

- Selected peptides for system readiness are underlined
- 3 peptides from heavy chain are also present in Adalimumab and Etanercept sequences
  - TTPPVLDSDGSFFLYSK
  - DTLMISR
  - TPEVTCVVVDVSHEDPEVK

System Readiness Criteria

Determined using USP mAb 001

- TIC signal: Chromatogram signal 1e8
- Mass accuracy: between -5 to 5 ppm
- Integrated component area: >1e7
  - More intense peptide area: > 1e8
- Retention time: 2 min range
- DTL**M**ISR oxidation: Between 2 - 4%
- Bracketing injections, evaluate at T=0 and end of run. Minimal difference expected

Other system readiness criteria for bracketing standards to consider and set acceptance criteria

- Select one consistent peptide peak, P1 (e.g PENNY peptide)
- Compare peak area
- Compare retention time difference
- Select another consistent peptide, P2 and determine Relative Retention Time (RRT)

Keynote: System readiness criteria should be set based on your system and data collected

All system readiness parameters pass after 60 hrs analysis run time in Lab A

Parameter	Peptide	Eval. Result	Operator	Ref. Value (1)	Ref. Value (2)	Test Result
TIC	Check Standard	**3.23E+09**	**>=**	1.00E+08		Passed
		2.84E+09				
Mass Accuracy (ppm)	(+2) TTPPVLDSDGSFFLYSK	**-0.4**	**between**	-5	5	Passed
		-1.0				
	(+2) Q[Pyroglutamate]IVLSQSPAILSASPGEK	**-0.6**				
		-1.5				
	(+3) TVAAPSVFIFPPSDEQLK	**-0.1**				
		-0.8				
	(+2) DSTYSLSSTLTLSK	**-1.0**				
		-1.2				
	(+3) VDNALQSGNSQESVTEQDSK	**-1.2**				
		-1.4				
Component area	(+2) Q[Pyroglutamate]IVLSQSPAILSASPGEK	**3.97E+07**	**>=**	1.00E+07		Passed
		4.03E+07				
	(+3) TPEVTC[Carbamidomethylation]VVVDVSHEDPEVK	**6.92E+08**		1.00E+08		
		6.81E+08				
	(+3) TVAAPSVFIFPPSDEQLK	**1.70E+08**		1.00E+07		
		1.71E+08				
	(+2) DSTYSLSSTLTLSK	**5.73E+07**				
		5.72E+07				
	(+3) VDNALQSGNSQESVTEQDSK	**9.68E+07**				
		9.61E+07				

For Eval.Result: **Bold** font= Result from initial bracketing std run Normal font= Result at end of run

All system readiness parameters pass after 60 hrs analysis run time in Lab A

Parameter	Peptide	Eval. Result	Operator	Ref. Value (1)	Ref. Value (2)	Test Result
Retention Time (min)						
	(+2) TTPPVLDSDGSFFLYSK	**40.8**	between	40	42	Passed
		41.0				
	(+3) TPEVTC[Carbamidomethylation]VVVDVSHEDPEVK	**28.2**		27	29	
		28.5				
	(+3) TVAAPSVFIFPPSDEQLK	**44.3**		43	45	
		44.6				
	(+2) DSTYSLSSTLTLSK	**27.3**		26	28	
		27.7				
	(+3) VDNALQSGNSQESVTEQDSK	**9.4**		8	10	
		9.3				
% M256 Oxidation						
	DTLMISR	**2.67**	between	2	4	Passed
		2.56				

For Eval.Result: **Bold** font= Result from initial bracketing std run Normal font= Result at end of run

- All 17 executed test cases passed at start and end of run sequence

Analytical Method Transfer and Assay Performance across Labs

Considerations for Adoption of MAM in QC

System Readiness is appropriate in a non-cGMP environment, additional work is needed to support adoption of MAM in QC:

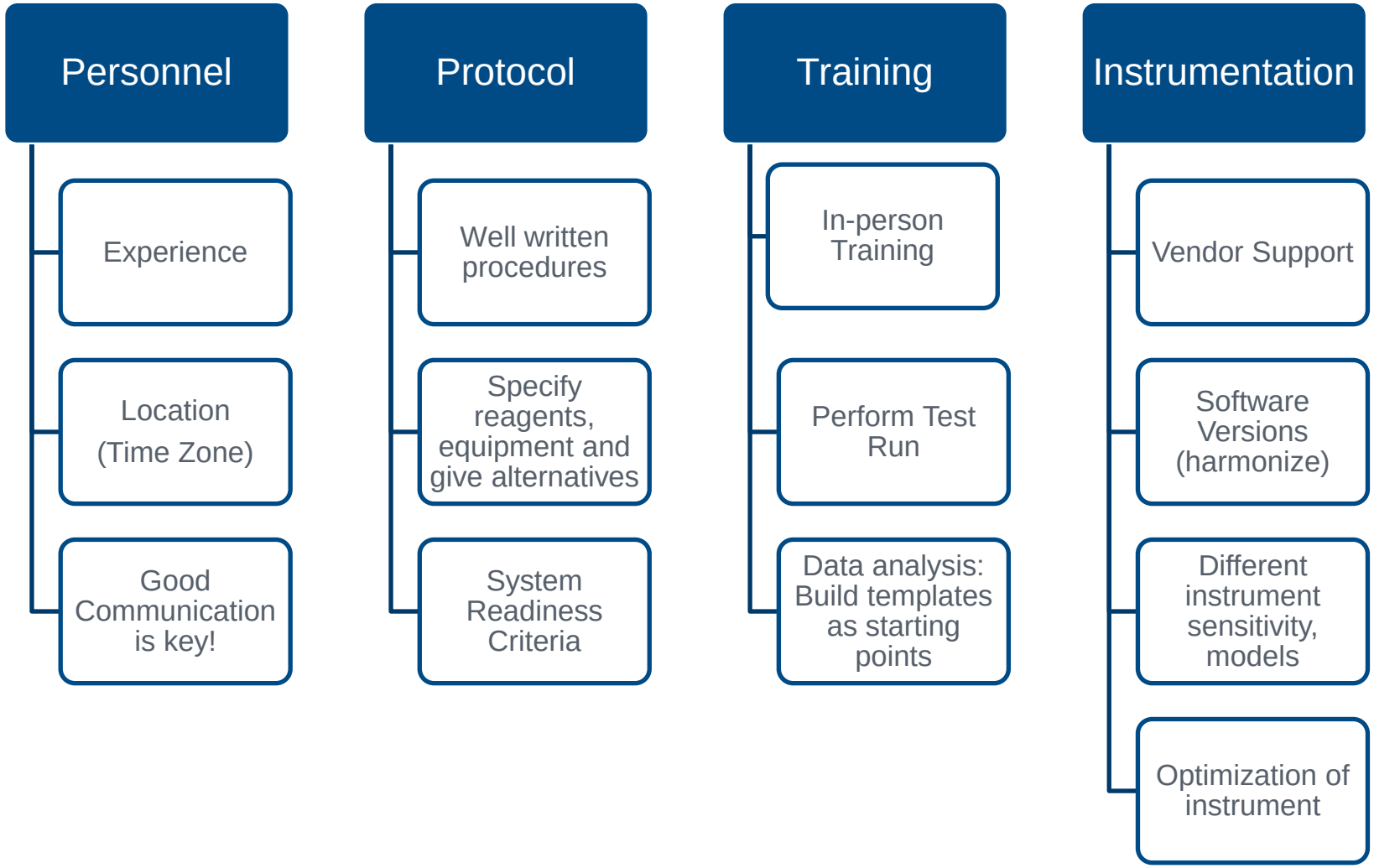
- Qualification
- Validation
- Robustness
- System Suitability
- Analytical Transfer
- Lifecycle Management

see <1060> for additional details

- While full qualification and validation was out of scope for this study, an Analytical Transfer was conducted to provide assessment of additional factors:
  - Robustness of sample preparation
  - Differences in instrumentation
  - Site and personnel differences

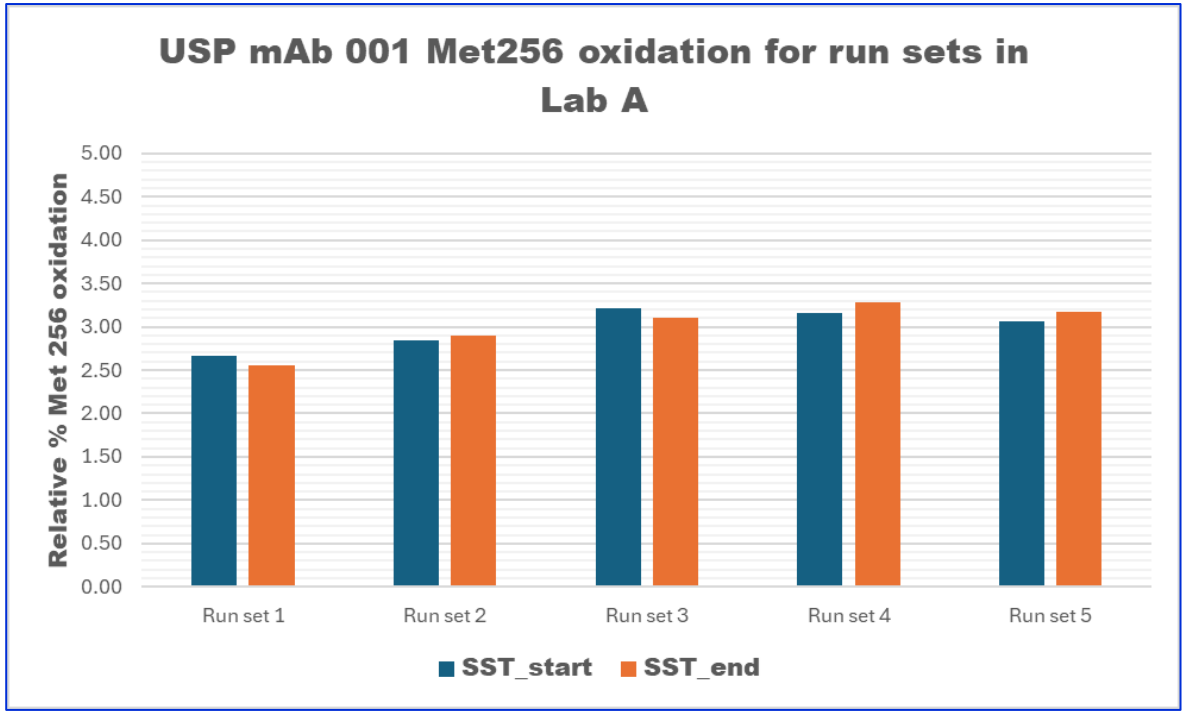
Analytical Method Transfer Considerations

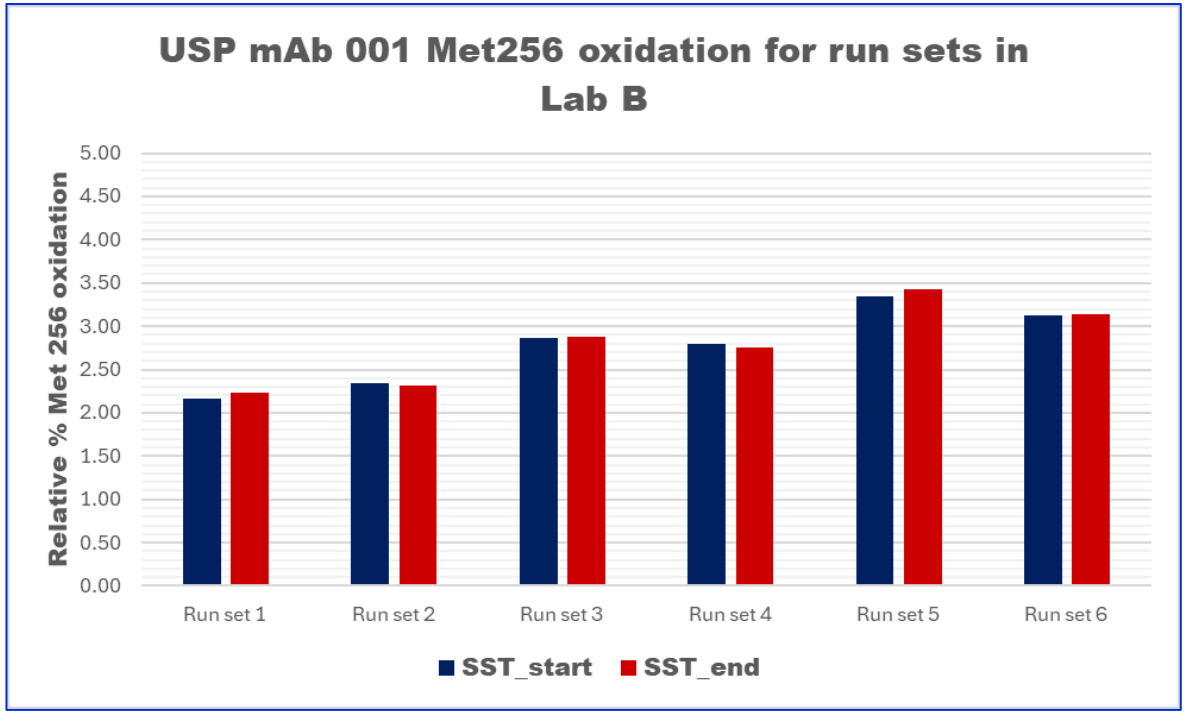
Method was transferred to USP Lab in Hyderabad India from Rockville MD



- Not a formal Analytical Transfer, but the same principles apply
- Receiving laboratory was able to successfully run the method and obtain comparable results

Impact of Run Time on M256 Oxidation Criteria

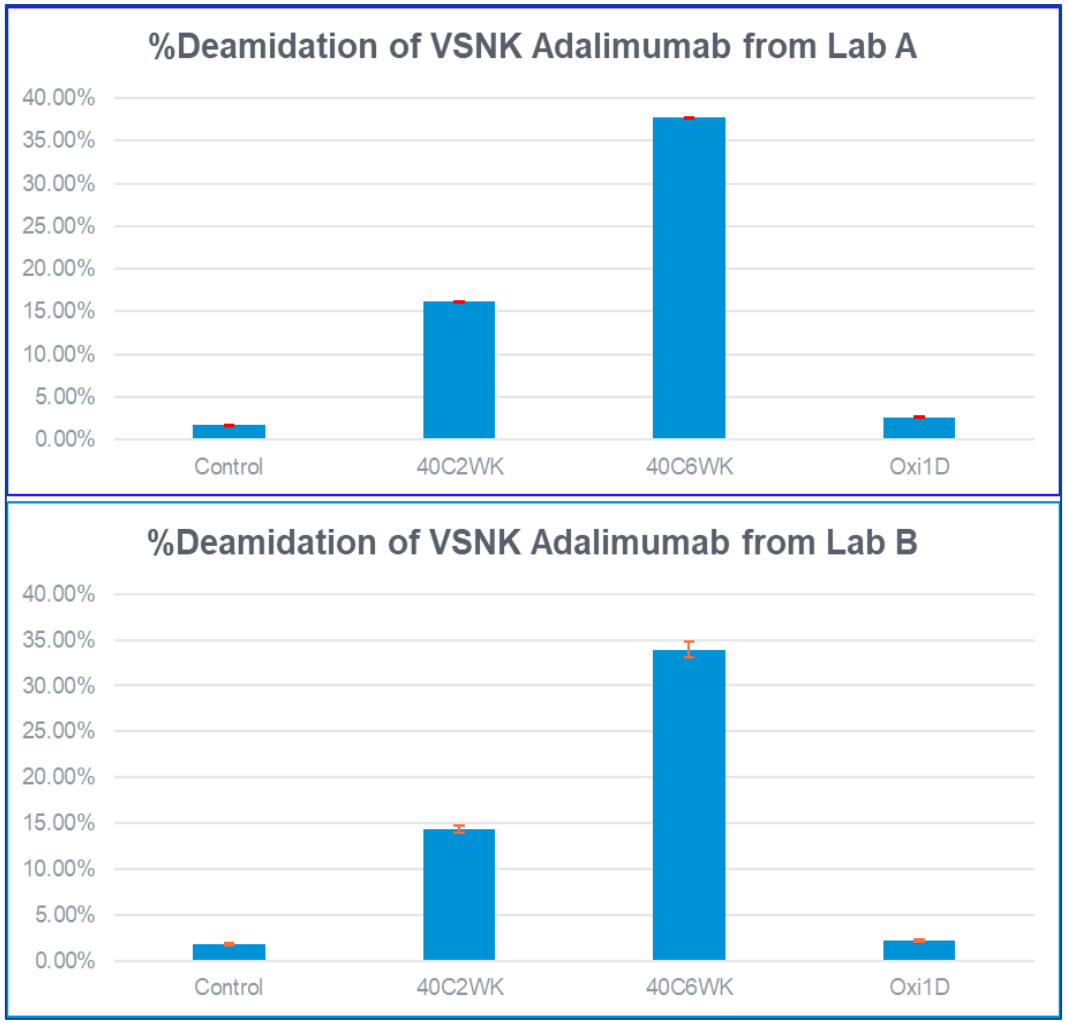


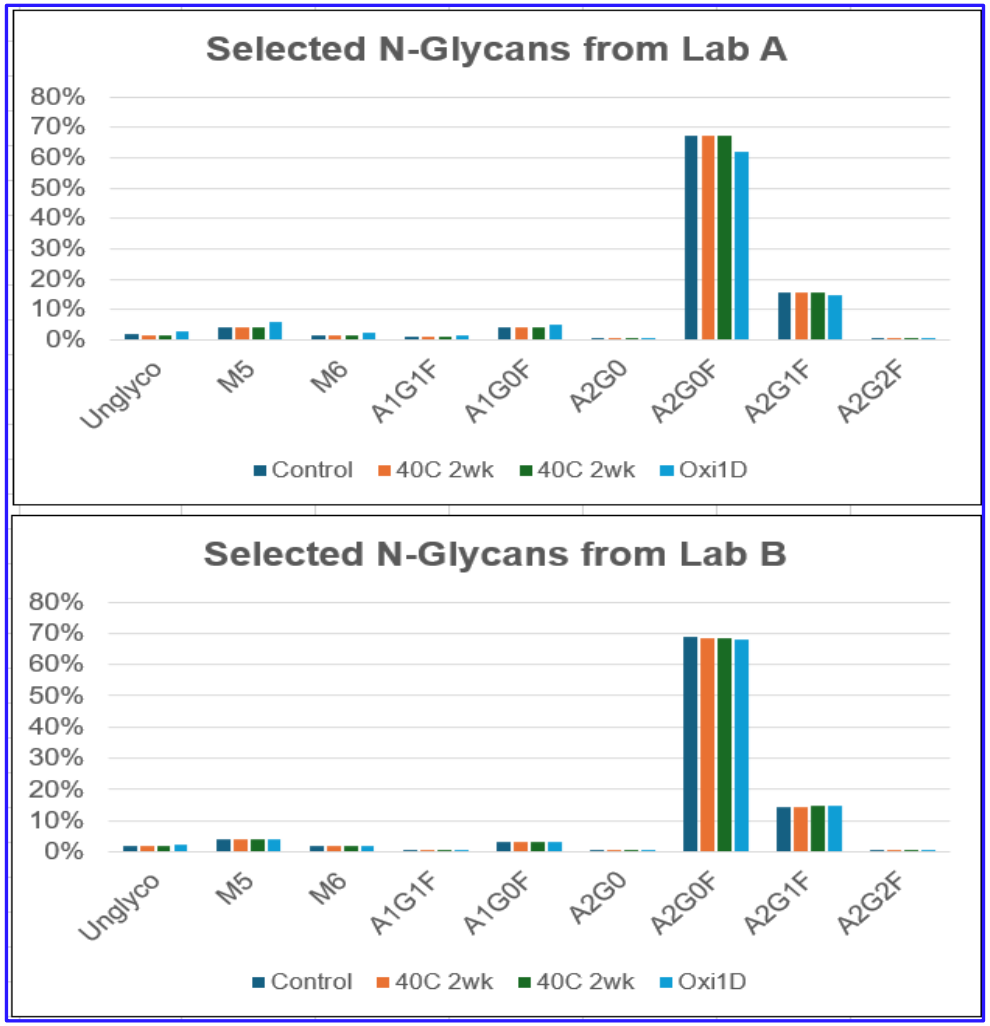


Artifactual oxidation measure

- No significant increase of Met256 oxidation from start to end of runs. Time difference between start and end sample run was 60 hours.
- Met 256 oxidation levels are between 2-4 % , met system readiness criteria for both lab on different instruments, analysts, reagents, days and location

MAM Analysis Data from Lab A and Lab B for Adalimumab

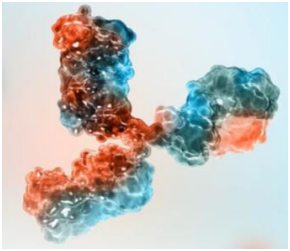


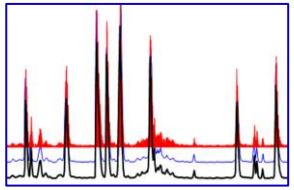


- VSNK deamidation trends comparable between Lab A and Lab B for Adalimumab. Thermal stress induced deamidation
- N-Glycans trends comparable between Lab A and Lab B for Adalimumab. Stress induced variability is minimal

Lessons Learned and Challenge

- **Lessons**
  - A well written sample preparation protocol is critical
  - Performing test run is useful for baseline information and source condition optimization.
  - Plan sufficient time for test run and in-person training
  - Clean instrument and prior calibration saves time
  - Instrument sensitivity:
    - Optimization of LC-MS conditions might be needed, plan time for this
    - Adjust system readiness criteria to match specific instrument capability
    - Be mindful of software bias/limitations
- **Challenge**
  - Data analysis of Etanercept is ongoing. Challenges noted are analysis of sialylated glycopeptides and O-glycans.





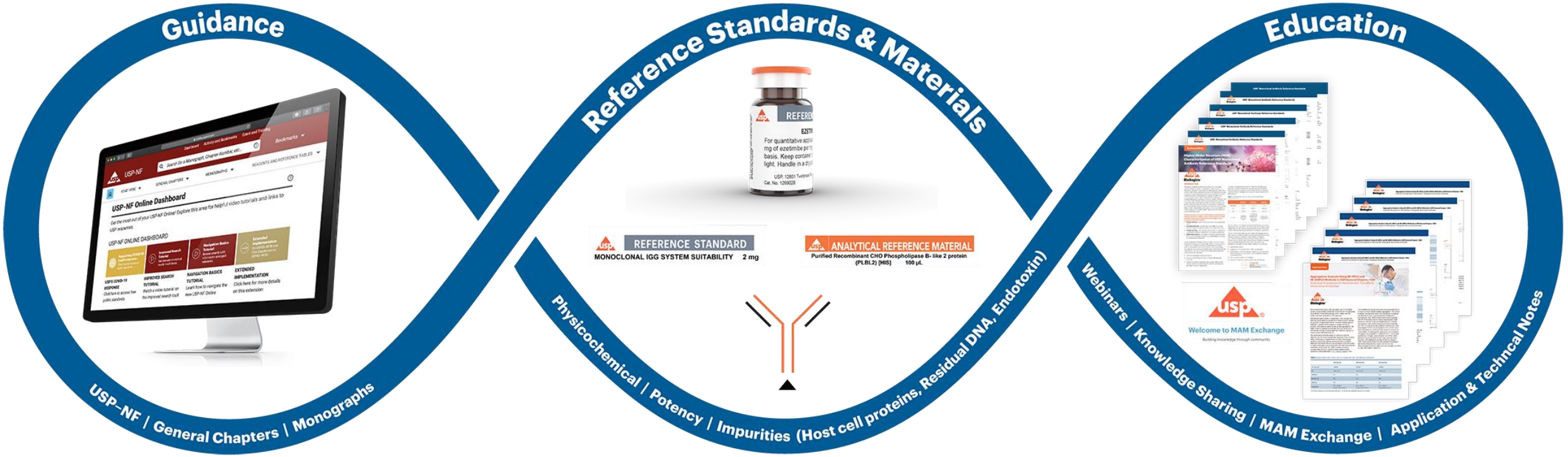
Summary and Next Steps

- Followed best practices described in *<1060> Mass Spectrometry-Based MAM for Therapeutic Proteins* to establish System Readiness
  - Used USP mAb 001 Reference Standard as a system readiness standard for MAM applications and benchmarking
  - Peptides selected for performance monitoring cover the retention time range where selected PQAs eluted off the column

- Next Steps for comparison of MAM vs Conventional Methods
  - Complete comparison of MAM vs conventional methods, including:
    - Ability to detect differences between products and changes upon forced degradation
    - Specificity
  - Extend analysis to include functional and structural changes
    - Cell-based bioassay, binding by SPR, structure by CD
    - Correlation of differences in function/structure with MAM/conventional methods

Additional USP Resources

Additional USP Resources



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