USP Standards to Support Host Cell Protein Analysis by Mass Spectrometry

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Outline

- HCP Documentary Standards
  - GC <1132>
  - LC-MS Chapter
- HCP Physical Standards
  - Peptides
  - Proteins
  - Next Steps

Documentary Standards

Host Cell Protein in USP-NF

- Individual monographs
  - e.g., INSULIN LISPRO

- **HOST CELL PROTEIN:** The residual host cell protein content is NMT 10 ng/mg, determined by a validated method or demonstrated by a validated process.

- USP-NF in <1132> *Residual Host Cell Protein Measurement in Biopharmaceuticals*
  - Official in USP 41-NF 36
  - Contains Immunoassay Methods, Reagents, Method Development, Qualification, and Validation
  - Includes Limited Discussion on Supporting / Orthogonal Technologies
    - Electrophoresis Methods (1D and 2D SDS-PAGE and CE-SDS), Western Blot, Chromatographic Methods, and Mass spectrometry Methods
  - No associated Reference Standards

- Proposed >1000 chapter in development by Host Cell Protein Expert Panel
  - Working Title: *Identification and Quantitation of HCP Impurities in Biological Products* ***using Mass Spectrometry***
  - Contain general guidance and best practices
  - Submission to PF expected in early 2023

HCP Expert Panel Membership

- Expert Volunteers:
  - Ned Mozier, Chair, Pfizer, US
  - Severine Clavier, Sanofi, France
  - Annick Gervais, UCB, Belgium
  - Suli Liu, Biogen, US
  - Jingjie Mo, Johnson & Johnson, US
  - Rosalynn Molden, Just-Evotec, US
  - Veronika Reisinger, Novartis/Sandoz, Austria
  - Kevin Van Cott, University of Nebraska – Lincoln and Haemtech, US
  - Donald Earl Walker, Nektar Therapeutics, US
  - Fengqiang Wang, Merck, US
  - Stefanie Wohlrab, Roche Diagnostics, Austria
  - Ying Zhang, Pfizer, US
  - Yiwei Zhao, Alkermes, US
- Government Liaisons:
  - 2 from FDA
- USP Scientific Liaisons:
  - Niomi Peckham
  - Ying Han

Vision of the Expert Panel

- Provide guidance on how to initiate measurement of HCP using MS and how to ensure an organization's characterization and development strategy is comprehensive.
  - Our goal is to provide a consistent guide for use across the biopharmaceutical industry
- The goals of the chapter are:
  - Contain general guidance and best practices
    - Principles of techniques, but not the detail of a Standard Operating Procedure
  - Establish terminology
  - Avoid information that can become outdated rapidly
  - Include reference to emerging modalities
  - Technology sections will provide flexibility for instrumentation
- Sections should serve as high-level guides and provide minimum requirements

USP General Chapter Development Process

1 – Expert Committee members are selected for a 5-year term. They are not representative of companies.

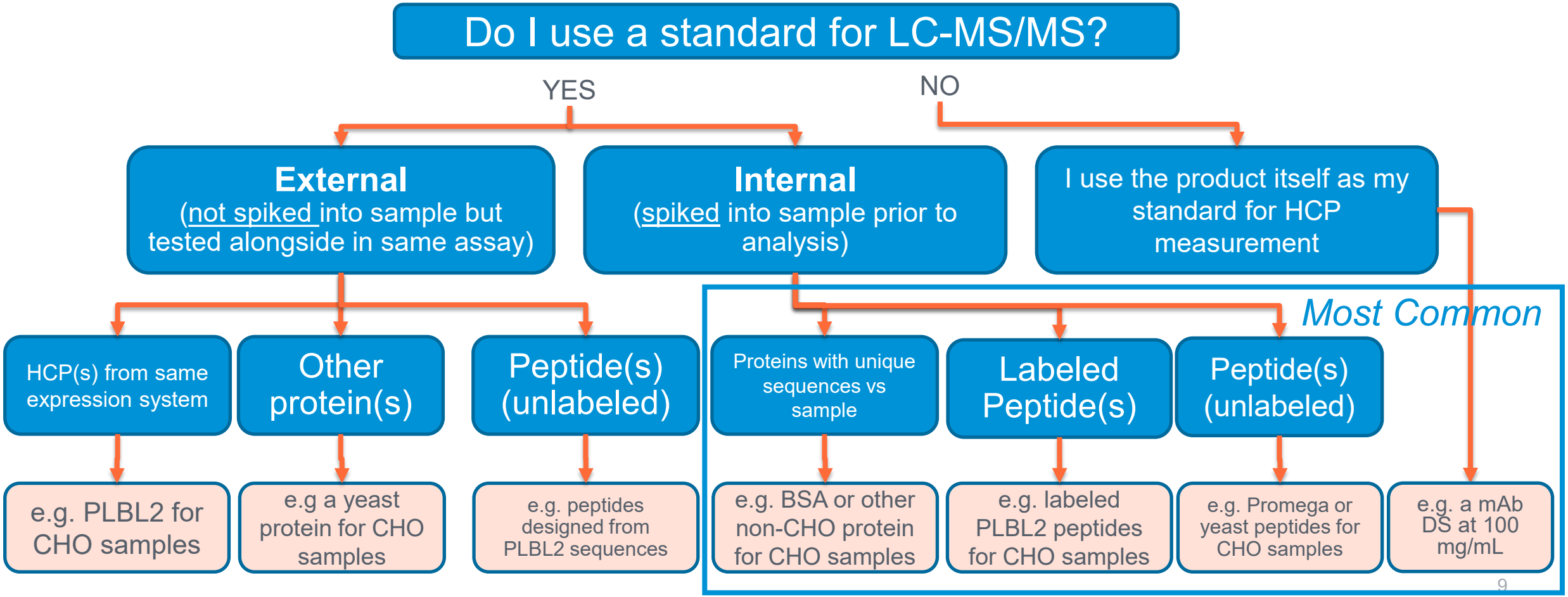
2 – Expert Panel members are selected for a specific task. They may represent their own interest. They are advisors of the Expert Committees on one specific topic

FAQs for HCP Analysis by Mass Spectrometry

- What parameters impact qualification and quantification results of HCP using LC-MS/MS?
  - Sample Preparation, Standards, Acquisition Methods and Data Processing
- What are the main applications of LC/MS-MS for HCP analysis?
  - Process Clearance, Identify HCPs in DS, Characterize ELISA Reagents, Facilitate Risk Assessment
- At different stages of development, what workflows and sample types are appropriate?
- What do terms like Identification, Qualitative, Quantitative, Semi-Quantitative, Relative Quantitation and Absolute Quantitation practically mean and how do they differ?
- How should HCP content be reported and what units are appropriate?
- How should I compare HCP data from LC/MS-MS to that from ELISA?

Use of Standards in LC-MS/MS

The HCP EP asked members to rank how often their organization used these approaches for standards in analysis of HCP by LC-MS/MS.



Physical Standards

Development of HCP Reference Standards

Proposed Standards

- Focused on individual proteins or peptides corresponding to HCPs of concern
  - HCPs that have been reported to impact patients or product
  - High abundance HCPs commonly detected in product and/or late process steps
  - Verified against published benchmarking studies
- Support identification and quantitation
  - Mass spectrometry is first priority
  - Potentially support immunoassays for individual HCPs

Reference Standard Format: Protein vs Peptide

PEPTIDE HCP STANDARDS

- Advantages
  - Easier and faster to produce
  - Easy to generate in stable isotope labeled format
- Disadvantages
  - Limits application to MS
  - Does not account for losses during sample preparation
  - Requires multiple peptides for each protein

PROTEIN HCP STANDARDS

- Advantages
  - More accurate quantitation since standard undergoes same sample preparation
  - Could support wider range of applications
- Disadvantages
  - Limited sources of purified CHO proteins
  - Limited availability of HCP clones

Peptide Standards in Development

- First group (Production)
  - CHO Host Cell Proteins
    - 3 target proteins
  - 3 peptides per target protein under evaluation for use in identification and quantitation applications
- Second group (Pilot)
  - 20 peptides under evaluation
  - 7 additional target CHO proteins
  - final selections to be made after proof-of-concept functional testing
- Peptide specifications
  - stable isotope labeled Lys or Arg
  - high purity (>95%)
  - minimum of 2 peptides per target
  - sequences selected based on publications and feedback from stakeholders
- Form Factor
  - dissolved peptide (liquid)
  - stored frozen ≤-65°C
- Formulation
  - USP Solubility and Stability studies to confirm solvent

Peptide Analytics

- Production Scale materials
  - Characterization of solid by vendor
    - Peptide content by AAA
    - Purity by HPLC-UV (> 95%)
    - Identity by LC-UV-HRMS
    - Sequence Verification by MS/MS
    - Residual Water (Karl Fischer)
    - Inorganic Content (Anions and Cations)
- Pilot Scale materials
  - Characterization by vendor
    - Purity by HPLC-UV (>95%)

https://andrews-glass.com/wp-content/uploads/2021/02/HighRecoveryGlass.pdf

Proof of Concept Study

- Objectives
  - Evaluate utility of peptides for absolute quantitation
    - Spike samples with peptides and compare to external standards
  - Confirmation of use for Production Scale
    - Identity and Quantitation
  - Proof of Concept for Pilot Scale
    - Selection of final peptides

- Evaluate detectability and linearity of peptides in relevant CHO matrices
  - CHO HCCF (null and producing)
  - Protein A eluate
  - IEX eluate
- Standard (denaturing) and native digests
  - Native digests on Protein A and IEX eluates
- Multiple workflows
  - DDA
  - SWATH

Proof of Concept Study

- Preliminary data
  - Standard (denaturing) and Native digests, DDA workflow
    - Are peptides detected?

	Peptide	HCCF, Null Cells Lot A	HCCF, Null Cells Lot B	HCCF, Null Cells Lot C	HCCF, Producing Cells	Protein A	IEX	Protein A	IEX
Digest		Standard	Standard	Standard	Standard	Standard	Standard	Native	Native
Production Target 1	1	+	+	+	+	+	+	+	+
	2				+			+	+
	3			+	+			+	+
Production Target 3	4	+	+	+	+	+		+	
	5	+	+	+	+	+		+	
	6	+	+	+	+	+		+	
Pilot Target 4	10	+	+	+	+	+	+	+	+
	12	+	+	+	+	+	+		
	13	+	+	+	+	+		+	+
Pilot Target 10	27								
	28	+			+			+	
	29								

Proposed Collaborative Study & Round Robin

- Collaborative Study will focus on peptide analytics
  - USP and external laboratories
  - Vialed final product (liquid)
    - AAA for concentration
    - HPLC-UV HRMS for purity
  - *Certificate will include physicochemical analysis only*
- Proposed Round Robin
  - Can occur in parallel with Collaborative Study
  - Functional testing only
    - Can include a pre-defined method and/or participant methods

HCP Standards in Development

- Recombinant CHO Phospholipase B-like 2 protein (PLBL2) [HIS]
  - C-terminal 6-HIS tagged
  - Expressed in CHO
  - Formulated in 1X PBS at 2 mg/mL
  - Store at ≤ -65°C
  - Donated Material
    - *Thank you!*

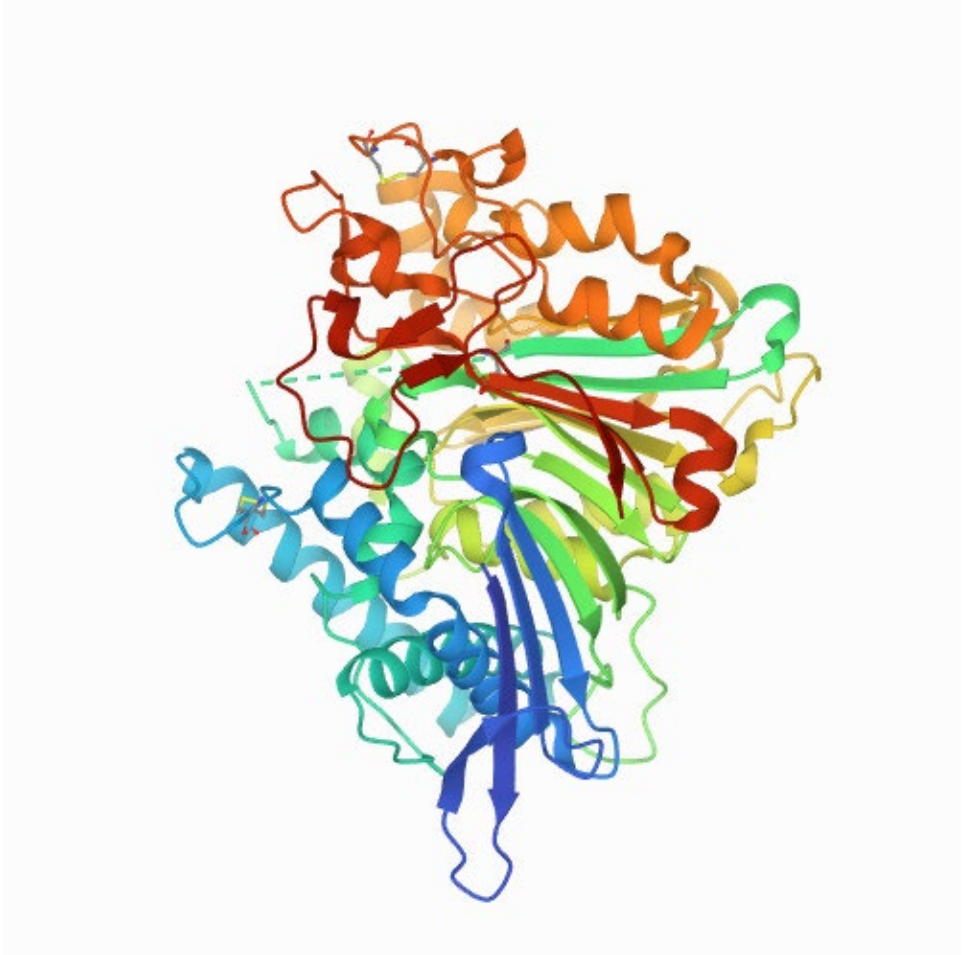


Image from rcsp.org, Crystal structure of the lysosomal 66.3 kDa protein from mouse solved by S-SAD, http://doi.org/10.2210/pdb3FBX/pdb

PLBL-2 Development

Characterization

- Peptide Map by RP-HPLC-MS
  - > 75% sequence coverage
- SEC-HPLC
  - > 90% main peak
- SDS-PAGE (reducing)
  - Major bands at ~ 66 kDa, 42 kDa, and 30 kDa
- Anti-HIS Western Blot
  - Confirmed HIS tag
- Functional Testing by LC-MS/MS
  - Standard digest (denaturing), DDA
    - Work in progress

Next Steps

- Collaborative Study will focus on protein analytics
  - Concentration by UV_{280}
  - SEC-HPLC
  - SDS-PAGE
  - *Certificate will include physicochemical analysis only*
- Round Robin
  - Can occur in parallel with Collaborative Study
  - Functional testing only
    - Quantitative application
    - Can include a pre-defined method and/or participant methods

Summary

- USP is developing new standards to support HCP analysis by mass spectrometry
  - New Expert Panel is drafting a general chapter (>1000) on best practices for MS-based HCP analysis
  - Physical standards are under development for individual high-risk HCPs
    - Stable isotope labeled peptides
      - 9 peptides to 3 protein targets
      - Physicochemical characterization underway
      - Proof of Concept study underway
      - Collaborative Testing and Round Robin in planning stage
    - Purified proteins
      - PLBL-2
      - Physicochemical characterization and functional testing underway
      - Collaborative Testing and Round Robin in planning stage