USP Approaches to Quality Assessment of Biologics

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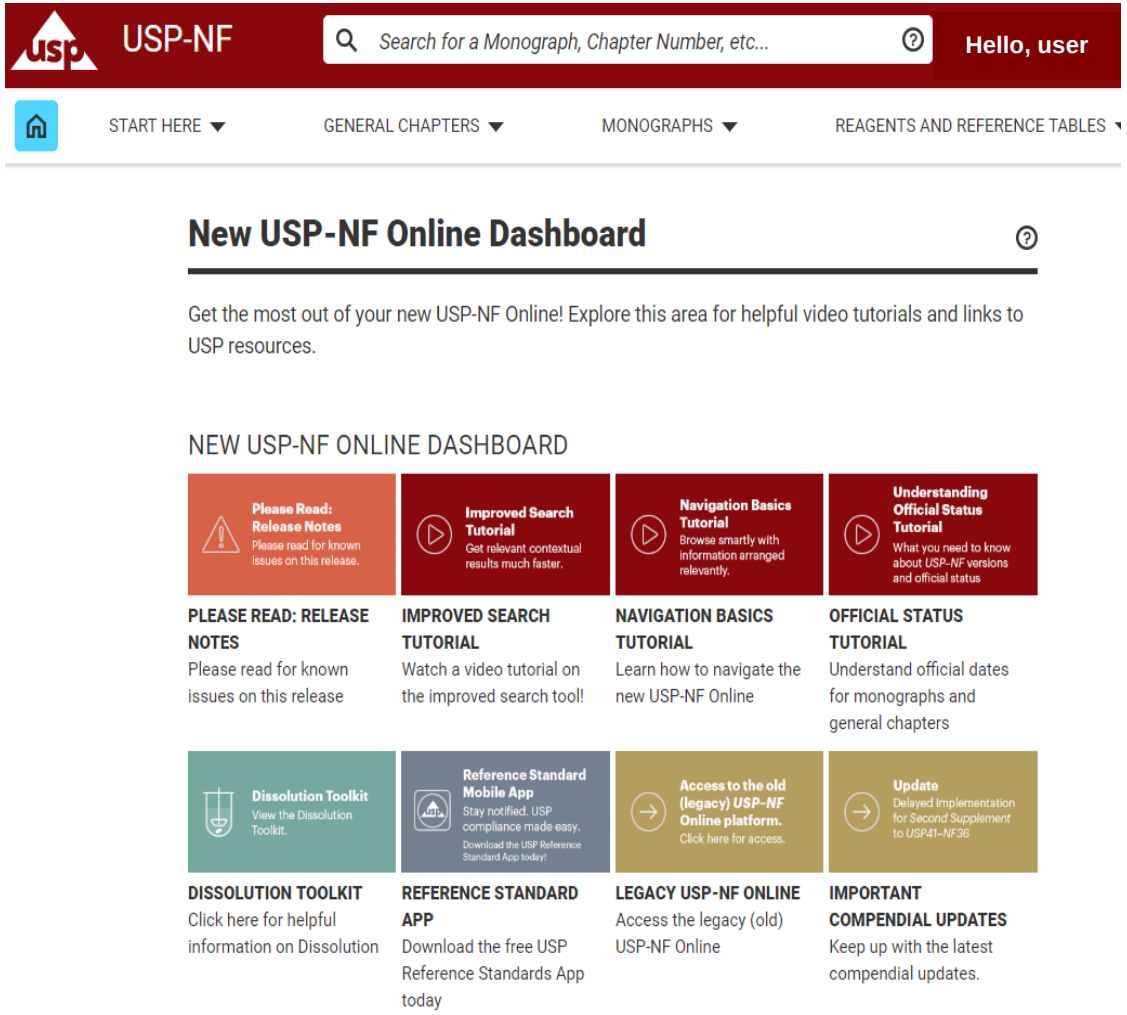
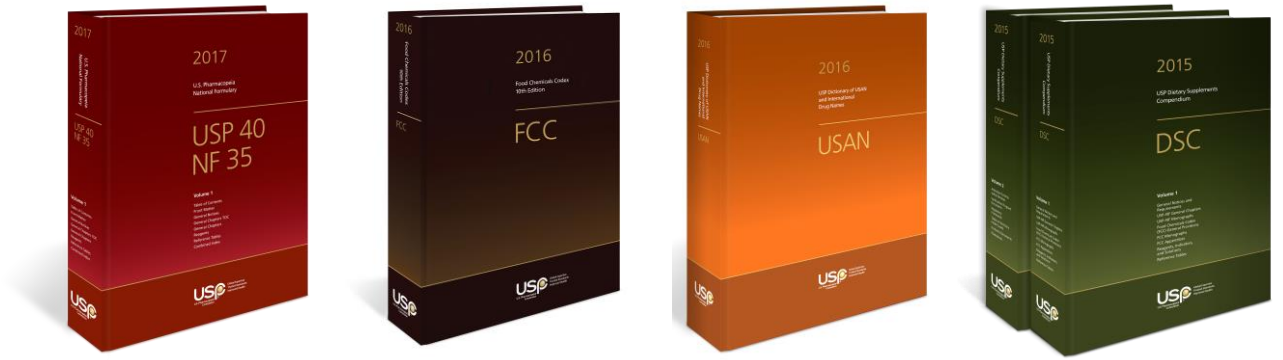
Agenda

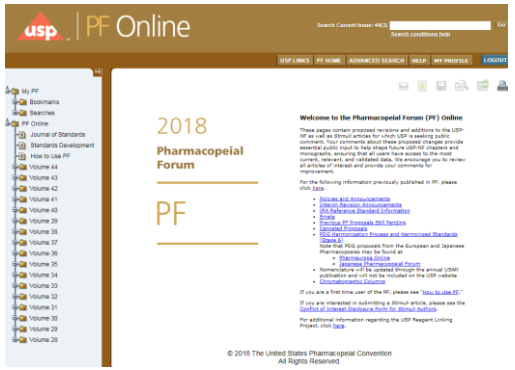
- USP approaches to biologics standards - general overview
- Case study for biologics monographs: heparins
- Standards for monoclonal antibodies
- Best practices chapters: residual host cell DNA and proteins
- Summary

USP – Public standards

Recognized in over 140 countries



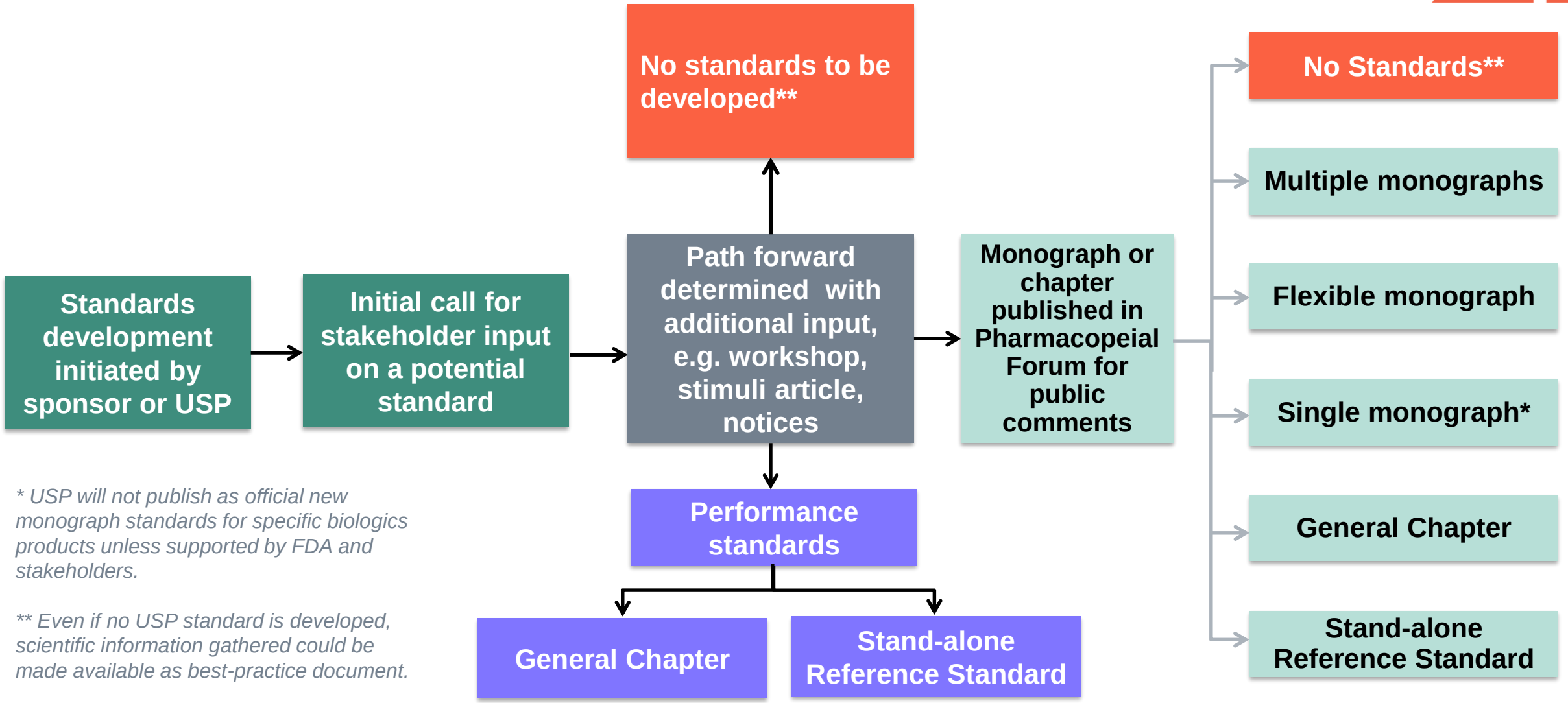




USP Standards in *USP–NF*

- Documentary Standards
  - Monographs
    - Specifications for pharmaceutical articles in commerce (from release through product shelf life)
    - Specifications – Tests, assays and acceptance criteria to demonstrate the article meets required quality standards
  - General Chapters
    - Cover broader topics and more widely applicable methods
    - Chapters from <1000> to <1999> are interpretive and provide general information and recommendations
    - Chapters below <1000> are compendially applicable and enforceable if referenced in General Notices, a monograph, or another applicable General Chapter under <1000>
    - Often describe specific procedures and Reference Standards (RSs)
- Physical Reference Materials
  - Provide traceable standards to demonstrate broad-based acceptability of procedures

Standard development for biologics licensed under the PHS Act, and early stakeholder engagement



** USP will not publish as official new monograph standards for specific biologics products unless supported by FDA and stakeholders.*

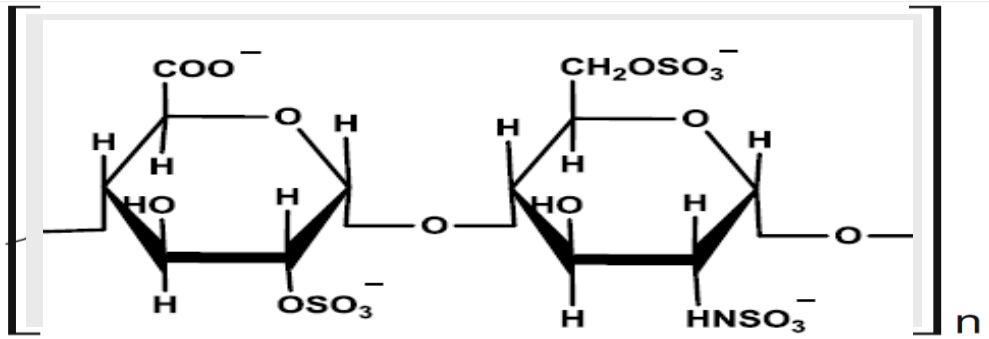
*** Even if no USP standard is developed, scientific information gathered could be made available as best-practice document.*

Biologic Monographs

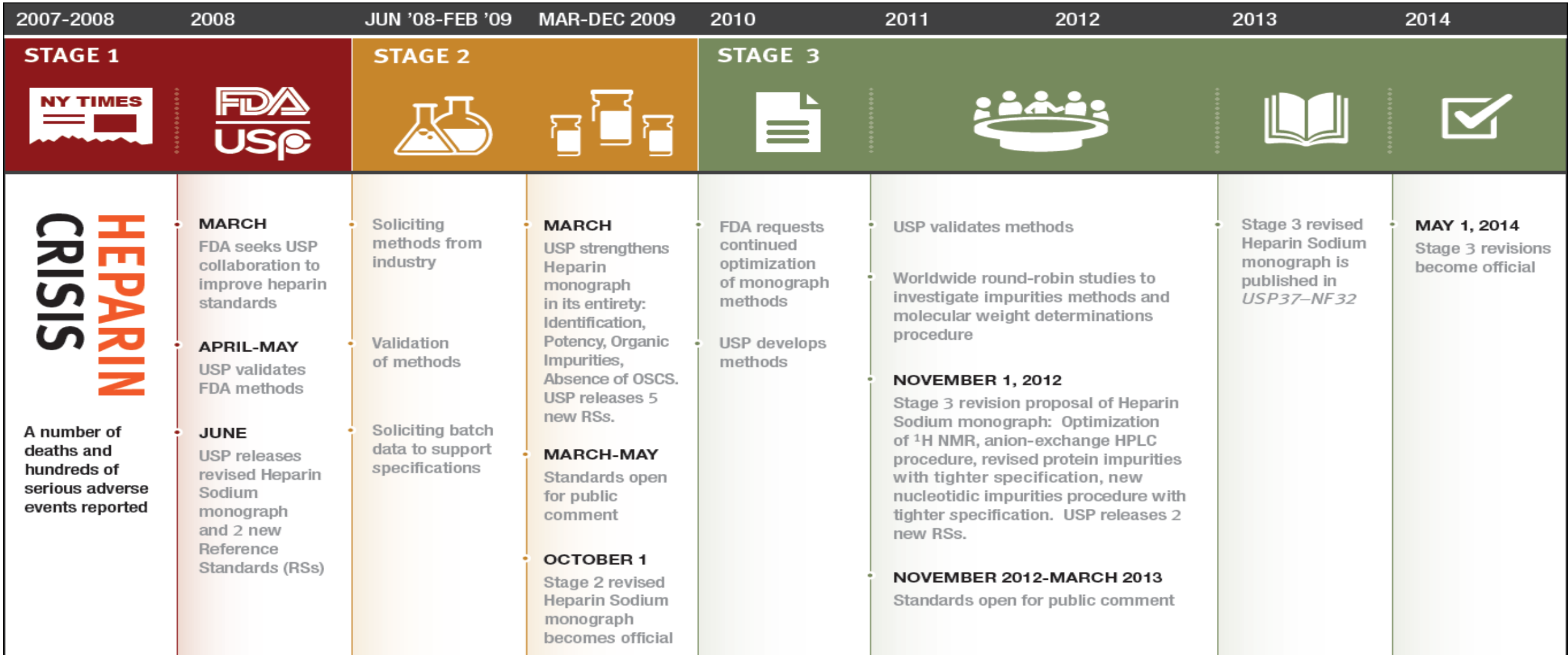
Case Study: Heparin

Heparins

- Heparin is a member of the glycosaminoglycan (GAG) family
- Heparin is a polysaccharide, polysulfated negatively charged heterogeneous mixture with molecular weight range between 2,000 to 50,000 Daltons
- The main raw material is pig intestine and majority is sourced from China
- The main disaccharide repeating unit is 2-O-sulfated iduronic acid and 6-O-sulfated, N-sulfated glucosamine comprising approximately 75%
- 5 other disaccharides units comprise the rest of the molecule
- Also the starting material for low molecular weight heparins



Heparin revision timeline



Heparin Sodium Monograph after Stage 3 revision

Identification

A. ^{1}H NMR spectrum

B. Chromatographic ID

C. Anti-Factor Xa and anti-factor IIa Ratio

D. Molecular weight Determinations

E. Identification tests-General, Sodium <191>

Assay – Anti-Factor IIa Potency

Other Components: Nitrogen Determination, Method I <461>

Impurities

- Residue on Ignition <218>
- Heavy Metals, Method II <231>
- Limit of Galactosamine in total hexosamine
- Absence of Oversulfated Chondroitin Sulfate
- Nucleotidic Impurities
- Protein Impurities

Specific Tests

- Bacterial Endotoxins Test <85>
- Loss on Drying <731>
- pH <791>
- Sterility Tests <71>

Standards for Monoclonal Antibodies

Common critical quality attributes

Biological characteristics

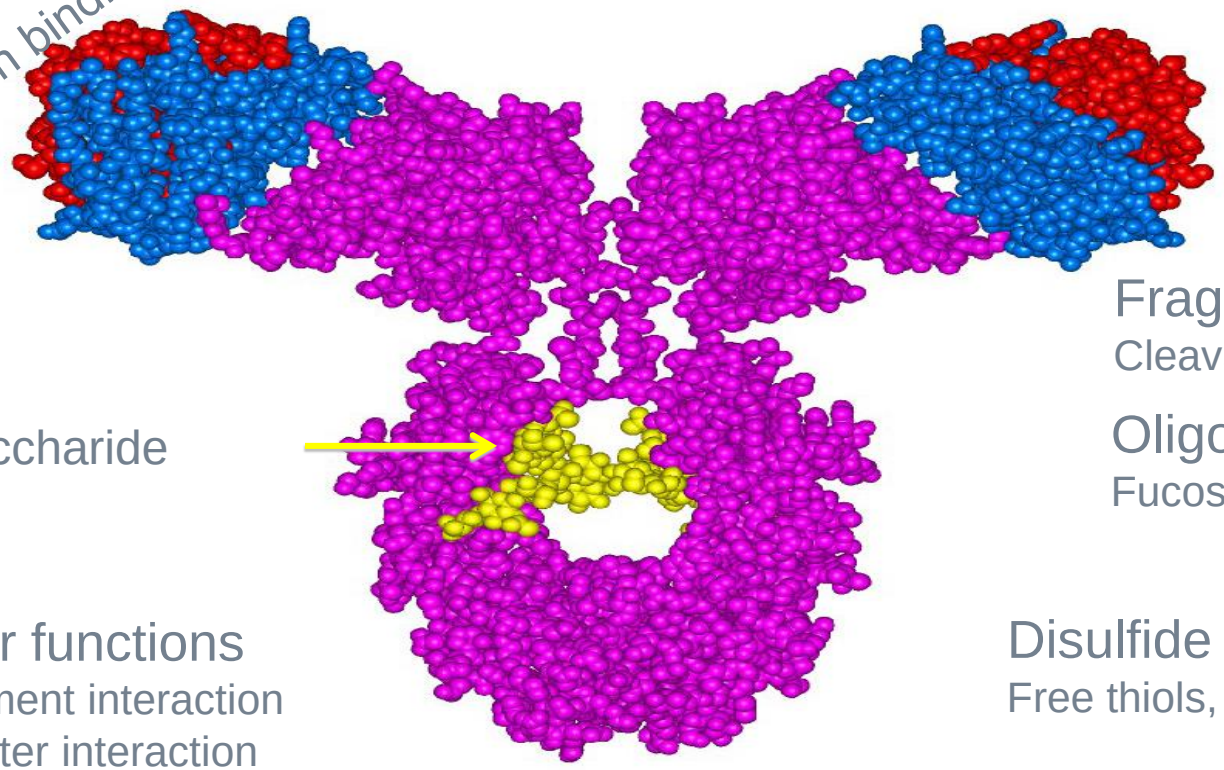
Fab

Antigen binding

Oligosaccharide

Fc

Effector functions
Complement interaction
Fc recepter interaction



Physico-chemical characteristics

N-terminal heterogeneity
Pyroglutamate formation,
other modifications

AA modifications
Deamidation, oxidation, glycation,
isomerization

Fragmentation
Cleavage in hinge region, Asp-Pro

Oligosaccharides
Fucosylation, sialylation, galactosylation

Disulfide bonds
Free thiols, disulfide shuffling, thioether

C-terminal heterogeneity
Lysine processing, proline amidation

Chapter <129> *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies*

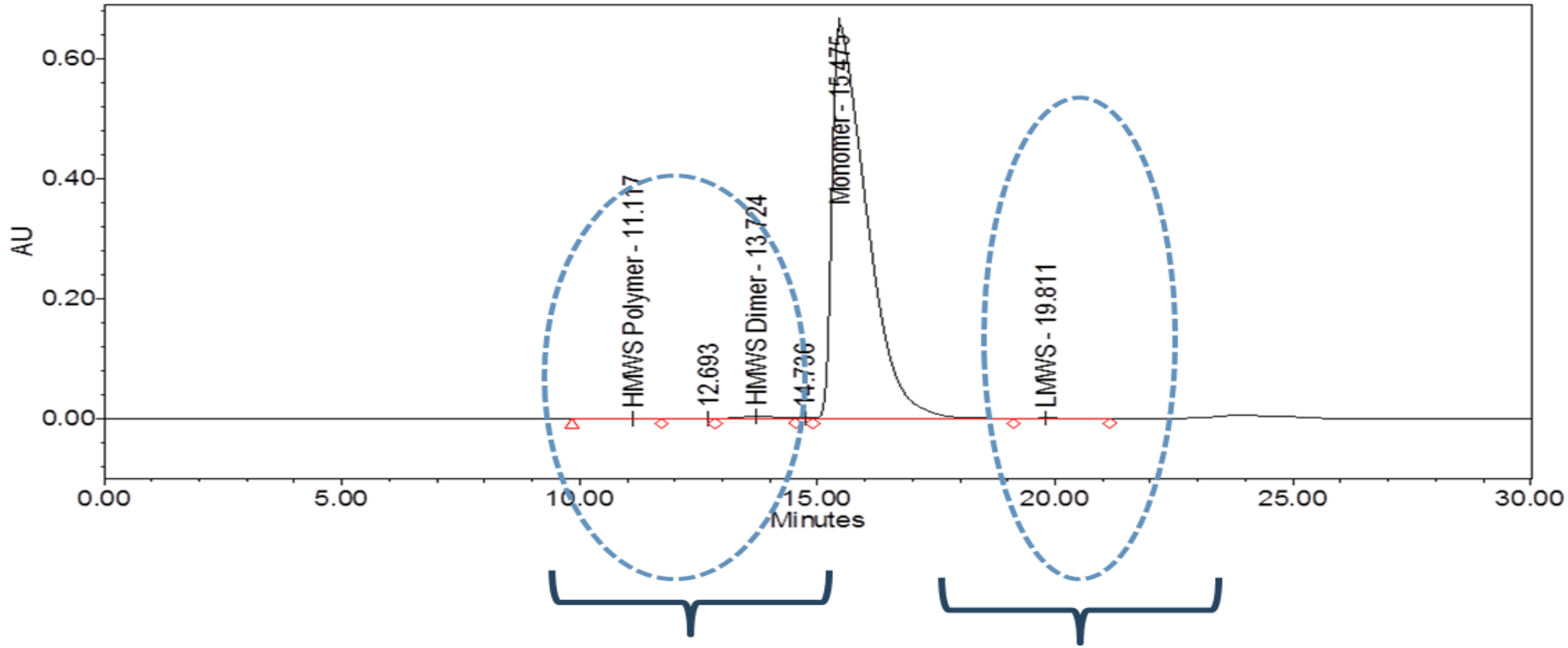
- Contains a collection of validated compendial procedures with established system suitability criteria for therapeutic monoclonal antibodies
  - Size–Exclusion Chromatography (SEC)
  - Capillary SDS Electrophoresis (reduced and non-reduced)
  - Oligosaccharide Analysis
  - Sialic Acid Analysis

- Contains a USP Monoclonal IgG System Suitability RS (catalog #1445550) to ensure suitability of the methods

- Does not contain product-specific acceptance criteria

Chapter <129> SEC-HPLC System Suitability

USP Monoclonal IgG System Suitability RS Chromatogram



HMWS

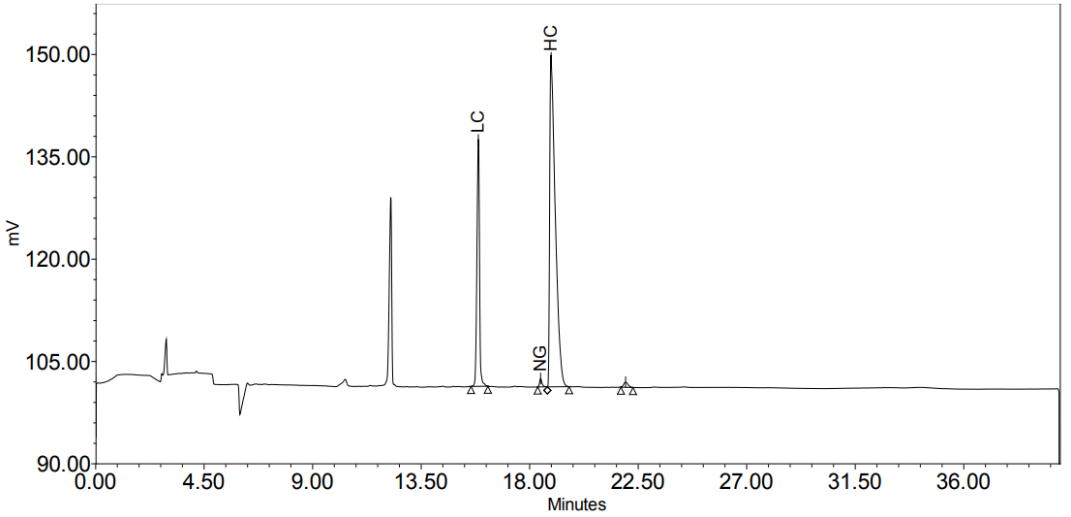
= High Molecular Weight Species

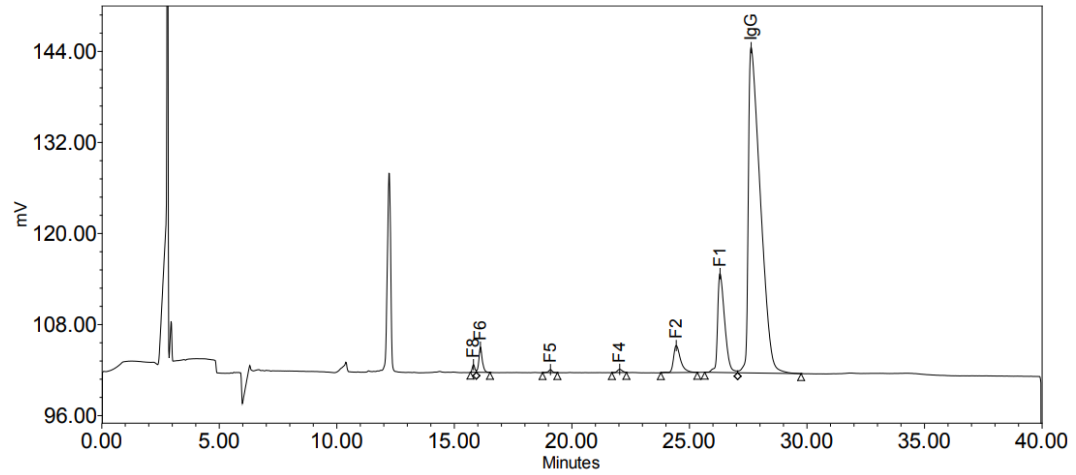
LMWS

= Low Molecular Weight Species

USP Monoclonal IgG System Suitability RS

- Reduced and non reduced mAb by CE-SDS
- Suitability requirements for USP Performance Standard
  - Reduced
    - Electropherogram consistency
    - Resolution
    - Ratio of nonglycosylated to total heavy chain
  - Non reduced
    - Electropherogram consistency
    - Resolution, amount of main, RSD





Best Practices General Chapters

Case Studies: Residual Host Cell DNA and Proteins

ICH Q6B definitions of product-related and process-related impurities in biologics

- Product-related impurities (e.g., precursors, certain degradation products) are molecular variants arising during manufacture and/or storage, which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety.
- Process-related impurities encompass those that are derived from the manufacturing process, i.e., cell substrates (e.g., host cell proteins, host cell DNA), cell culture (e.g., inducers, antibiotics, or media components), or downstream processing.

USP residual DNA testing chapters

- Chapter <1130> *Nucleic Acid-based Techniques - Approaches For Detecting Trace Nucleic Acids (Residual DNA Testing)*
  - Informational general chapter with best practices
  - Official since December 2016

- Chapter <509> *Residual DNA Testing* **NEW**
  - New chapter containing a validated method
  - 2 associated Reference Standards:
    - USP CHO Genomic DNA Reference Standard (30 ng/µL)
    - USP *E. coli* Genomic DNA Reference Standard (30 ng/µL)
  - Will publish in the Second Supplement to *USP42-NF37,* official December 1, 2019

Outline of guidance in USP Chapter <1130>

- Introduction
  - Strategies to address residual DNA
    - Validate clearance during process-validation
    - Routine monitoring of residual DNA in DS
- Sample pre-treatment
- Hybridization-based Residual DNA Assay
- DNA-binding protein-based Residual DNA Assay
- Polymerase Chain Reaction Techniques
  - Quantitative PCR (qPCR)
  - Alternate Detection Strategies
- Points to Consider

Outline of <509> *Residual DNA Testing*

- Sample Preparation (extraction)
  - Proteinase K digestion step combined with a chaotropic salt (NaI) extraction and isopropanol precipitation
    - USP survey revealed that 95% of respondents extract samples and of these 62% use chaotropic agents
- qPCR Method for DNA Detection
  - A USP global survey revealed that some form of qPCR is used by 82% of respondents - by far the most commonly used technique for detecting DNA
  - Includes
    - Primer and probe sequences for CHO and *E. coli*
    - System Suitability Requirements
    - Acceptance Criteria for Accuracy and % RSD
      - Limit to be defined in product monograph

Impact of host cell proteins

- Residual HCPs have the potential to affect product quality, safety, and efficacy, including:
  - Immune responses to HCPs
  - Unwanted bioactivity (homology to endogenous human proteins)
  - Enzymatic activity that impacts the drug substance or excipients (e.g., lipases for polysorbate), affecting stability, potency
- Risks can vary based on many factors, including:
  - Dose (mg biologics/kg body weight)
  - Route of administration
  - Frequency of dosing (acute or chronic indications)
  - Number of biotherapeutics a patient takes
  - Patient population (immune-compromised, etc.)

Challenges in HCP analysis

- HCPs vary according to the cell substrate, as well as upstream and downstream manufacturing processes
  - HCPs can vary in pI (~3–11), hydrophobicity, and molecular weight (from ~5 kDa to at least ~250 kDa)
  - Host cell substrates vary, from bacteria, to yeast, to mammalian or insect cells
- Low levels of residual HCPs in a large excess of protein product
- The population of HCP species may change during process development
- Not all HCPs are immunogenic so may not be detected by immunoassays
- If a particular HCP is enriched during product purification, then a false negative or low dose value may result (hook effect)
- Challenging critical reagent development and characterization process to ensure detection of most HCPs

GC <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals

- Official in USP41-NF36 1S (December 2015)
- Covers:
  - Immunoassay Methods
    - Reagents
    - Method Development
    - Qualification
    - Validation
  - Supporting / Orthogonal Technologies
    - Electrophoresis Methods (1D and 2D SDS-PAGE and CE-SDS)
    - Western Blot
    - Chromatographic Methods
    - Mass spectrometry Methods
- No Reference Standards

Development of performance standards

- Performance Standards
  - Physical reference standards which support biologics analytical testing throughout the product lifecycle
  - Used to ensure and demonstrate methods and process performance
  - Broadly applicable to product families or classes as opposed to a specific drug substance or drug product
- Examples
  - Oligosaccharide Mixtures (A-D) for glycan analysis
  - Monoclonal Antibody IgG System Suitability Standard for characterizing mAbs (SEC and CE)
  - BSA (Bovine Serum Albumin) for protein quantification, system suitability (for proteins which don't have a specific reference standard)

HCP related standards were identified as high priority at roundtable discussions with industry experts

Summary

- USP continues to modernize its monographs and develop other compendial standards in collaboration with stakeholders
- USP will expand its collection of high quality Reference Standards that support characterization of biologics throughout the drug development pathway
- USP's Biologics team will engage outside experts to prioritize standards that are most needed by manufacturers and regulators, including those for advanced therapies
- Follow our activities at: https://www.usp.org/biologics

2020–2025

call for candidates

Join us on the Journey

Collaborate with highly dedicated leaders from science, medicine, healthcare practitioners, industry and academia to help us establish standards that make it possible for 2 billion people around the world to have access to quality medicines, dietary supplements and foods.

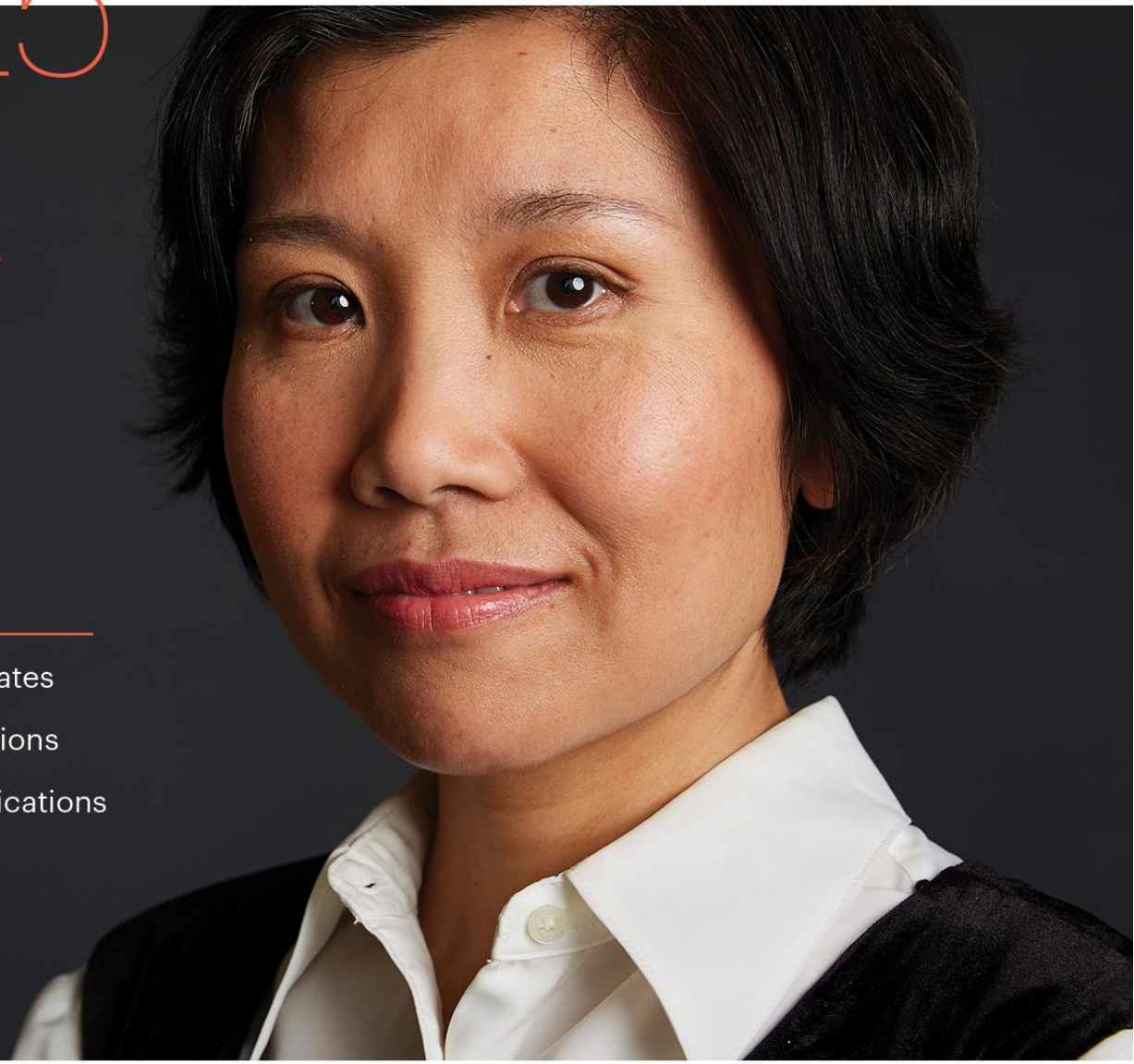
Important dates:

Jul 2018: USP launched the 2020-2025 Call for Candidates

Jan 2020: Deadline for Expert Committee chair applications

May 2020: Deadline for Expert Committee member applications

Jul 2020: 2020–2025 Council of Experts and Expert Committees begin their work



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For additional information visit **callforcandidates.usp.org** or contact **USPVolunteers@usp.org.**

Questions

Empowering a healthy tomorrow

Thank You

Empowering a healthy tomorrow