

# Evolving USP Standards to Enhance Quality Assessment of mAbs and AAV

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# Mission

**To improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods.**

# USP strategic priorities for 2025-2030 cycle



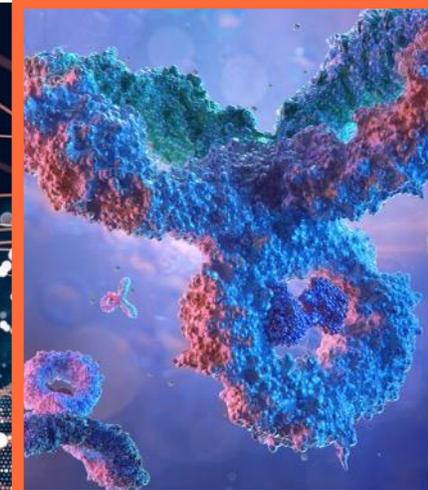
**Increase the volume of the world's most relied upon quality-assured medicines**



**Solve cross-cutting quality challenges to improve health outcomes**



**Strengthen upstream resilience of the global pharmaceutical supply chain**



**Expand availability of and access to quality-assured biologics products**



**Accelerate adoption of digital tools for quality assurance of medicines**



**Enable more environmentally sustainable pharmaceutical manufacturing**



# Development of USP Standards



## Documentary Standard

USP Receives Method Donation

Development of Compendial Method

Review by Expert Committee

90-day Review in USP-PF

Review/Approval by Expert Committee

Publish in USP-NF



## Physical Standards

Ideas from Stakeholders

Development of Standard

Collaborative Study

Review/Approval by Expert Committee

Release of Product

# USP Biologics strategic plan



**Engagement and scientific connectivity with biologics stakeholders across industry, government, regulators, and academia**



**Adjacencies such as education, and training**



**Documentary standards targeted at advancing quality topics broadly**



**Technical collaborations and partnerships**



**Reference materials and reference standards to support demonstrating analytical performance**



**Global leader in biologics quality**

- Enhancing quality from R&D through manufacturing and distribution
- Anticipating and tackling critical quality issues

# USP biologics modalities



## Topics



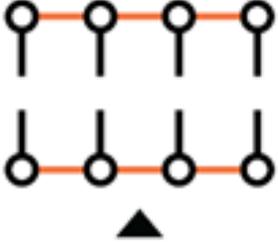
**Monoclonal Antibodies (mAbs)**



**Peptides**



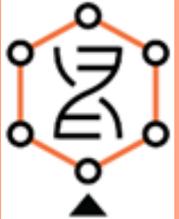
**Proteins**



**Oligonucleotides**



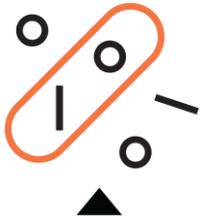
**Heparins**



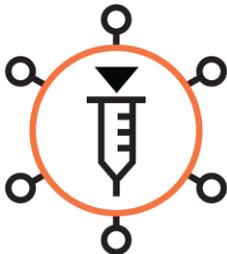
**Cell and Gene Therapies (CGT)**



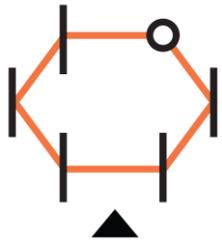
**Microbiology**



**Antibiotics**



**Vaccines**



**Carbohydrates**

# Critical quality attributes (CQAs)



- ▶ **Proper CQA assessment is essential** for product safety, efficacy, and consistency
- ▶ **Appropriate analytical methods**, which often require standards, need to be implemented for accurate analysis of CQAs

## **From ICH Q8(R2):**

- ▶ A **physical, chemical, biological or microbiological property** or characteristic that should be **within an appropriate limit, range, or distribution** to ensure the desired product quality.
- ▶ Potential drug product CQAs **derived from the quality target product profile and/or prior knowledge** are used to guide the product and process development.
  - Relevant CQAs comes from risk assessments and can evolve as more is learned about process and product

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# USP's mAb Standards and Tools

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# CQAs for monoclonal antibodies (mAbs)



## Biological characteristics

## Physico-chemical characteristics

### **N-terminal heterogeneity**

Pyroglutamate formation,  
other modifications

### **Amino acid modifications**

Deamidation, oxidation, glycation,  
isomerization

### **Fragmentation**

Cleavage in hinge region, Asp-Pro

### **Oligosaccharides**

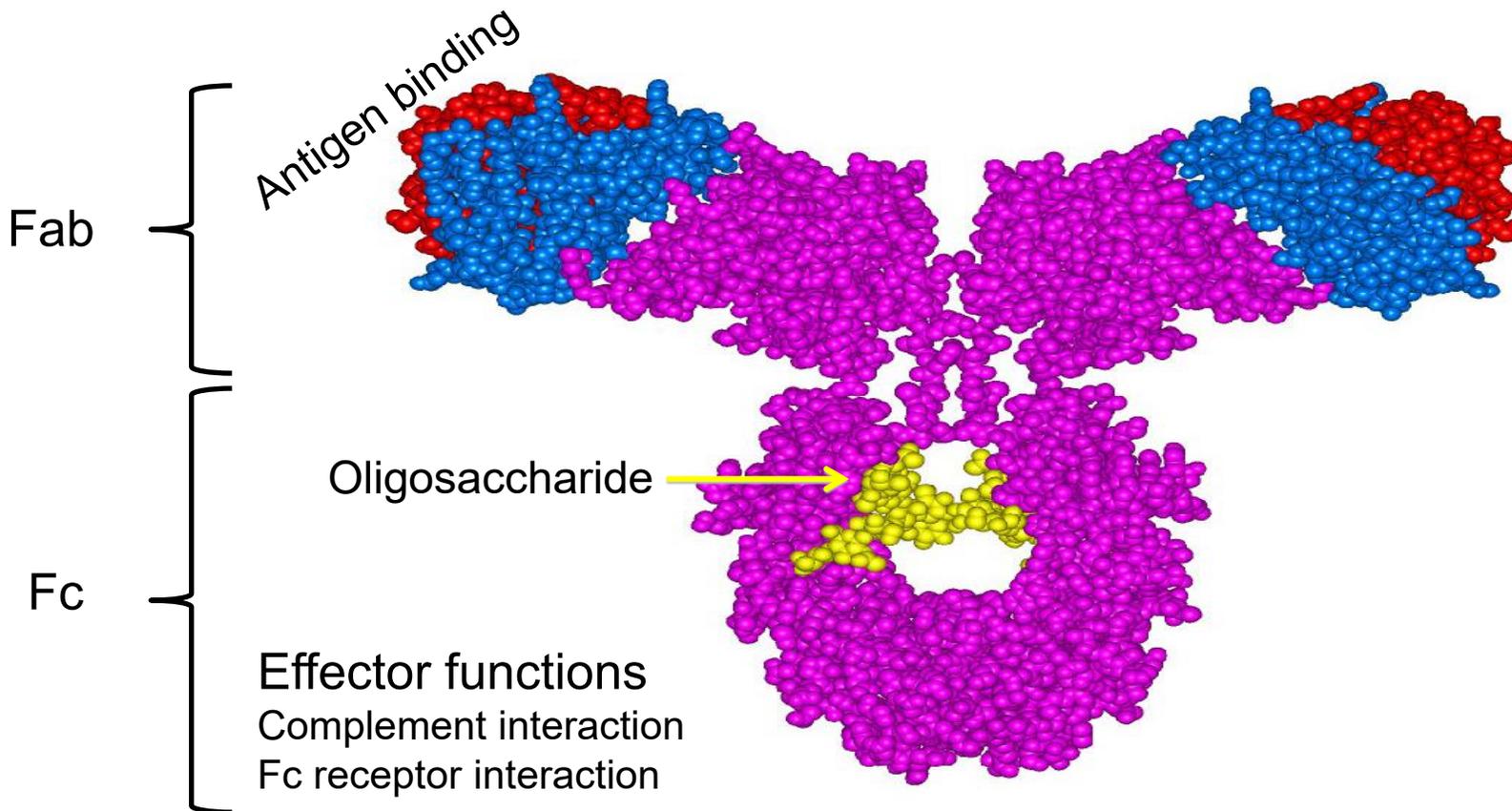
Fucosylation, sialylation, galactosylation

### **Disulfide bonds**

Free thiols, disulfide shuffling, thioether

### **C-terminal heterogeneity**

Lysine processing, proline amidation





## Documentary Standard

<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies

- ▶ SE-(U)HPLC
  - Methods for measuring the percentage of monomer and high-molecular-weight species
- ▶ CE-SDS (reduced and nonreduced)
  - Methods for quantitative estimation of product-related impurities
- ▶ Oligosaccharide Analysis
  - Analysis of N-Linked Oligosaccharides and Sialic Acid
  - Two method options:
    - CE-LIF
    - LC with fluorescence detection



## Physical Standards

Catalog #: 1445550

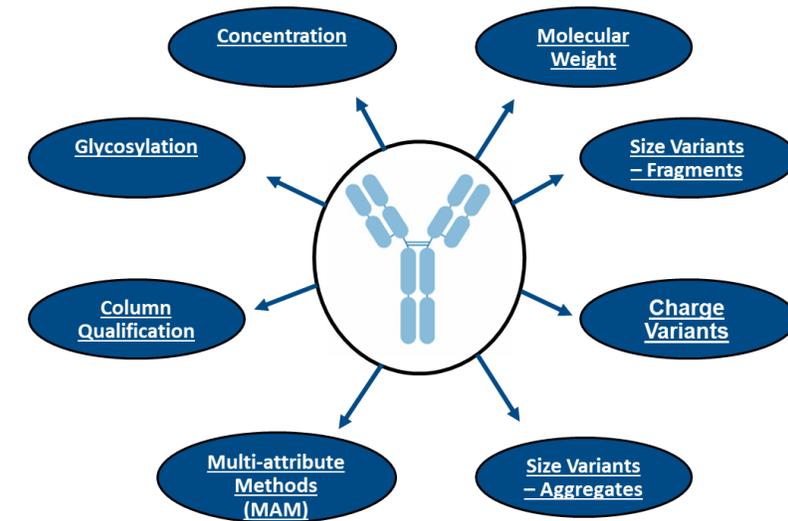
- ▶ Monoclonal IgG System Suitability
  - 2 mg/vial (lyophilized)
  - IgG1 expressed in CHO
  - Meets system suitability requirements for <129>



# Multiple uses of USP mAb standards



- ▶ **Challenge:** Need for reliable standards to assess a plethora of quality attributes
- ▶ **USP Solution:** Four distinct mAb standards (IgG1 and IgG4)
  - Expressed in CHO, produced by typical manufacturing conditions
  - Characterized for multiple CQAs
  - Sequence, formulation, and extinction coefficient provided



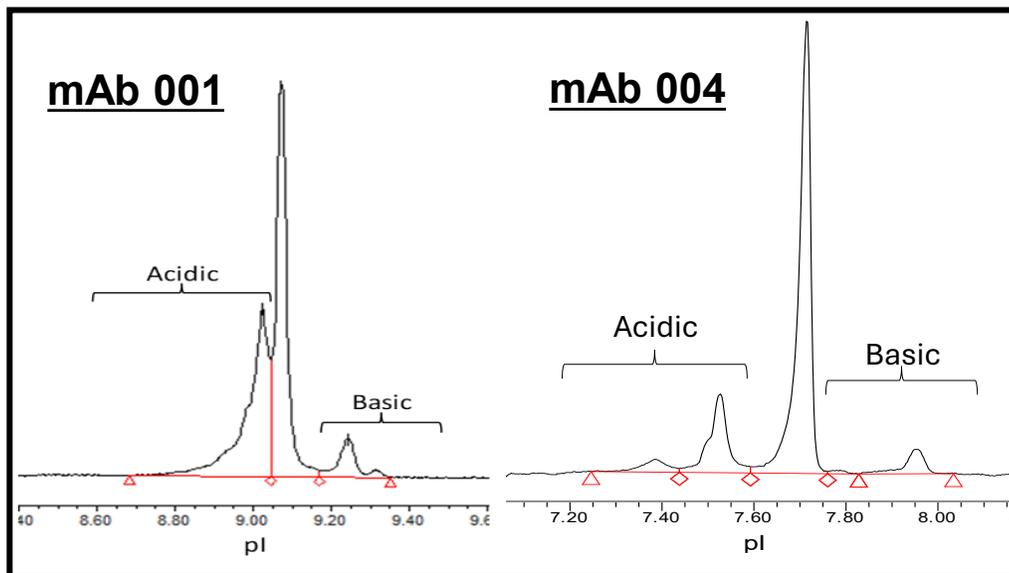
	USP mAb 001	USP mAb 002	USP mAb 003	USP mAb 004
<b>USP Catalog #</b>	1445539	1445547	1445595	1457499
<b>MW</b>	~147 kDa	~150 kDa	~146 kDa	~146 kDa
<b>Theoretical pI*</b>	8.7	8.1	8.1	7.8
<b>IgG</b>	IgG1	IgG1	IgG1	IgG4

\*Calculated using ProtParam (ExpASy)

# Charge variant profiles of USP mAbs



- ▶ **Challenge:** Need for standards to monitor charge variant separations
- ▶ **USP Solution:** Four mAb standards with varying pIs and charge variant profiles
  - USP is also currently developing a **degraded mAb standard**
    - Contains enriched acidic forms and can be used for CEX column qualification or cIEF and icIEF

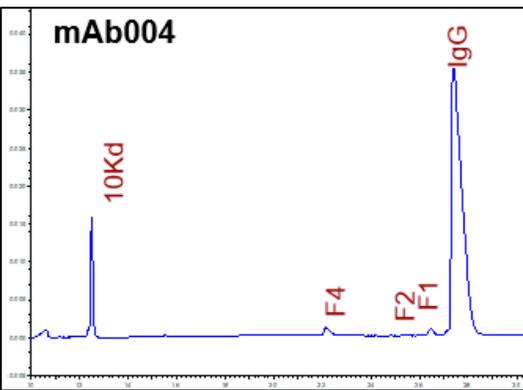
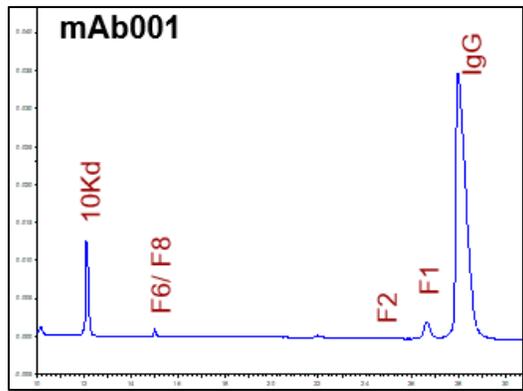


Material	Experimental pI	Acidic	Main	Basic
mAb 001	9.2	38%	54%	8%
mAb 004	7.7	25%	67%	7%

# Size variant profiles of USP mAbs



- ▶ **Challenge:** Need for standards to monitor methods used for fragment analysis
- ▶ **USP Solution:** Develop standards with different isotypes, purities, and impurity profiles

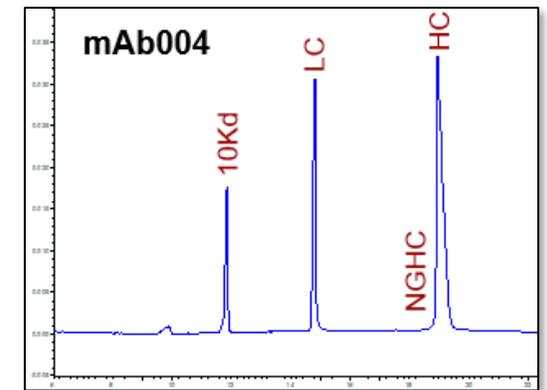
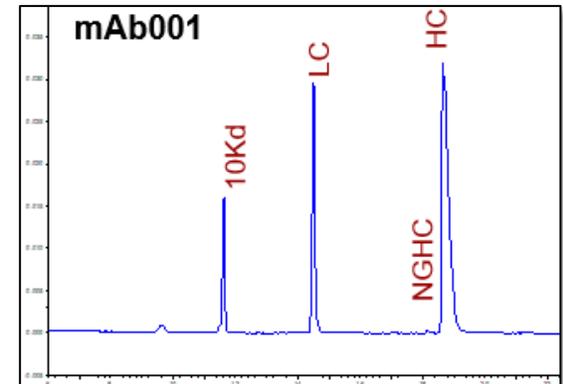


## CE-SDS (non-reducing)

USP Standard	IgG main peak (%)
mAb 001	95
mAb 004	96

## CE-SDS (reducing)

USP Standard	Species (% TDA)			
	LC	NGHC	HC	Total impurity peaks
mAb 001	34.1	0.3	65.4	0.3
mAb 004	33.4	0.2	67.5	0.2

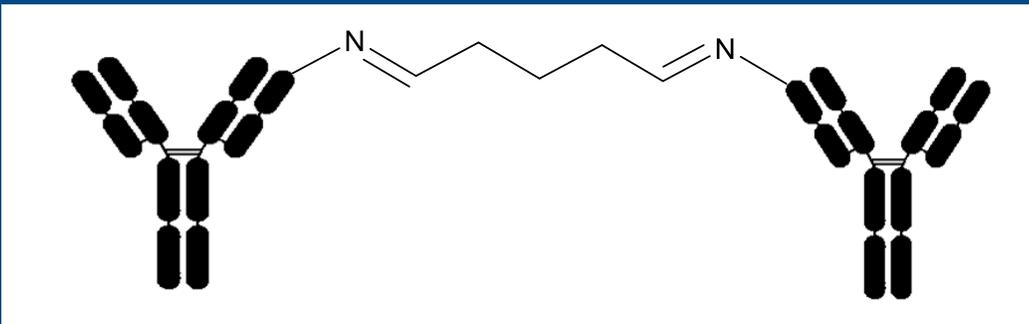


# HMW mAb standard for aggregation analysis



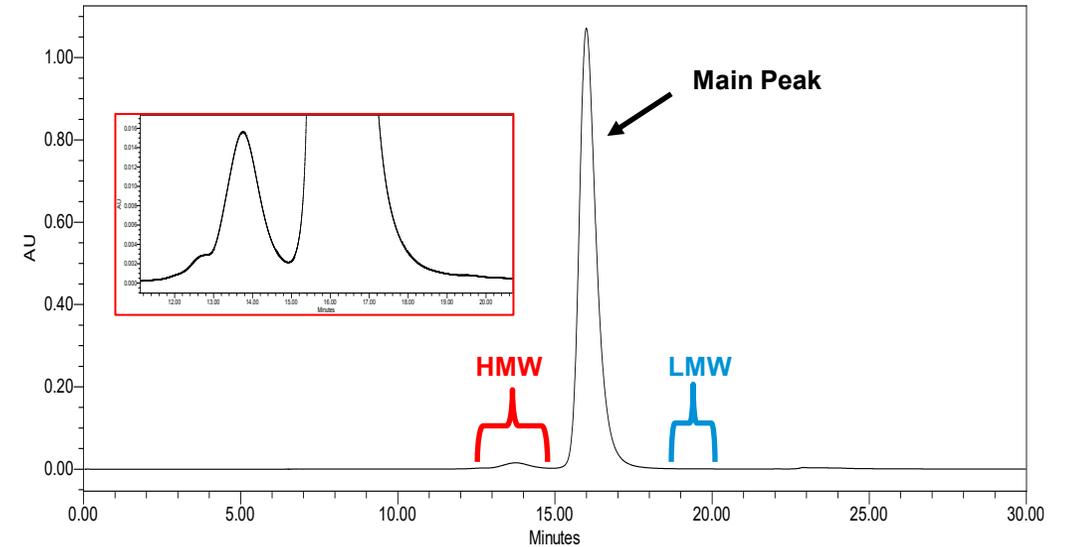
## Physical Standard

- ▶ Cross-linked USP mAb002
  - Contains ~2.5% dimer
- ▶ Can be used for development and column qualification using:
  - SE-(U)HPLC
  - SEC-MALS
- ▶ Expected release: Spring 2026



\*Figure for illustration purposes only

## SE-HPLC Results



Result	% HMWS	% Main Peak	%LMWS
mAb 002	0.8	99.2	<0.1
HMW mAb	2.4	97.6	<0.1

# Compendial methods for glycan analysis



- ▶ USP offers 3 distinct compendial methods for glycan analysis
- ▶ As methodology has continued to evolve, USP continues to add relevant methods

## USP <212>

### *Oligosaccharide Analysis*

- **N-linked glycan profiling**
  - HPLC
    - **HILIC** or **HPAEC** Separation
      - 2-aminobenzamide (**2-AB**) Labeling
  - CE-LIF
    - **APTS** Labeling
  - Oligosaccharide standard mixtures

## USP <129>

### *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies*

- **N-linked glycan profiling**
- HPLC
  - **HILIC** Separation and Anthranilic acid (**2-AA**) labeling
- Capillary electrophoresis (CE-LIF)
  - **APTS** labeling
- **Sialic Acid Analysis**
  - HPAEC-PAD

## USP <210>

### *Monosaccharide Analysis*

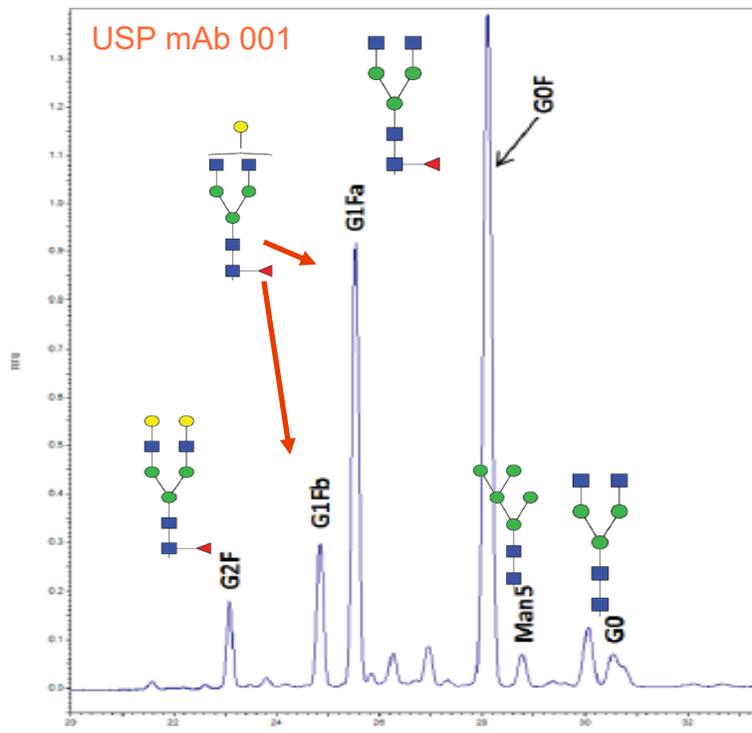
- **Sialic acid analysis**
  - Enzymatic and chemical release methods
  - Two chromatographic methods
    - **RP-HPLC** with **DMB** labeling
    - **HPAEC-PAD**
  - Sialic acid standards

# Case study – N-Glycan profiling

## N-Glycan profiling by <212> Oligosaccharide Analysis

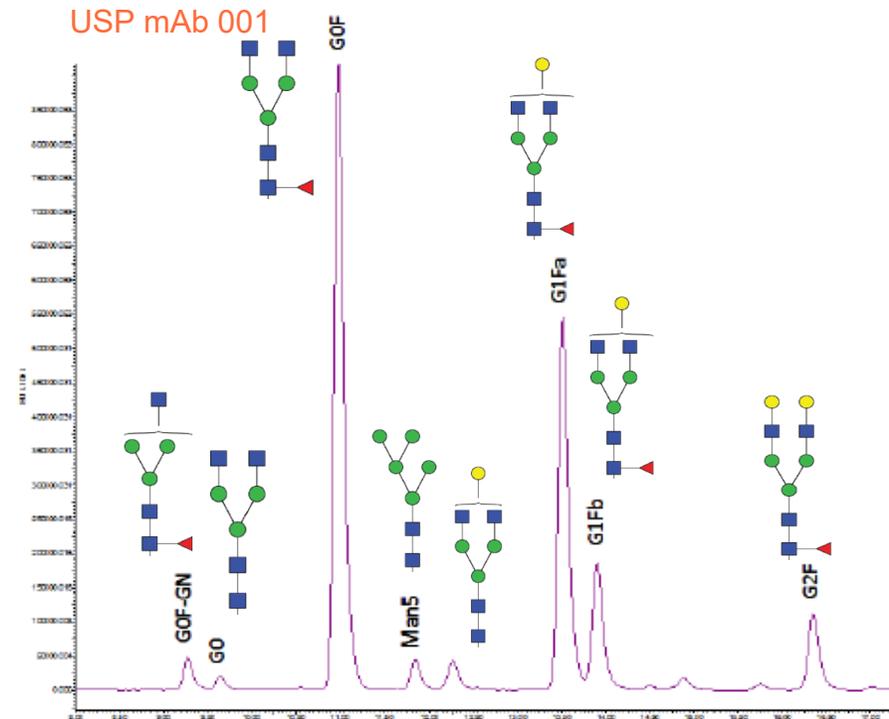
### ▶ CE-LIF Analysis

- Enzymatic release (PNGase)
- Fluorophore label (APTS)



### ▶ HILIC-FLR-MS\*

- Enzymatic release (PNGase)
- Fluorophore label (2-AB)



\*In-house method based on <212>, incorporating MS detection for glycan species confirmation.

## Regulatory Guidance\*

- ▶ WHO (Technical Report, 878):
  - Suggests a limit of <10 ng/dose
- ▶ FDA (CMC Information for INDs):
  - Suggests a limit of <10 ng/dose with DNA size <200 base pairs
- ▶ EMA (Guideline on Quality of Gene Therapy):
  - No inclusion of known oncogenic/tumorigenic sequences

\*Based on risks associated with the cell source, drug product, and administration, limits can vary.

## USP Solutions

### Documentary Standards

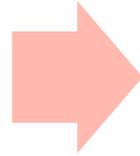
- <1130> *Nucleic Acid-Based Techniques – Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)*
  - General Chapter with best practices
- <509> Residual DNA Testing
  - Procedural chapter with validated extraction and qPCR procedures

### Physical Standards

- E. Coli Genomic DNA Reference Standard
  - Catalog #: 1231557
- CHO Genomic DNA Reference Standard
  - Catalog #: 1130710

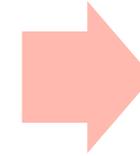
## Challenges

- ▶ Recombinant proteins are expressed in host cells
- ▶ Residual HCPs have the potential to affect the product quality, safety, and efficacy



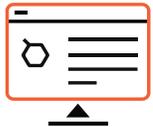
## Regulatory Guidance

- ▶ No specific limits exist in regulatory guidance
- ▶ ICH Q6B states that these impurities should be minimized



## USP Solutions

- ▶ Documentary standards that provide best practices for HCP analysis
- ▶ CHO-based HCP standards for accurate HCP quantification



## Documentary Standards

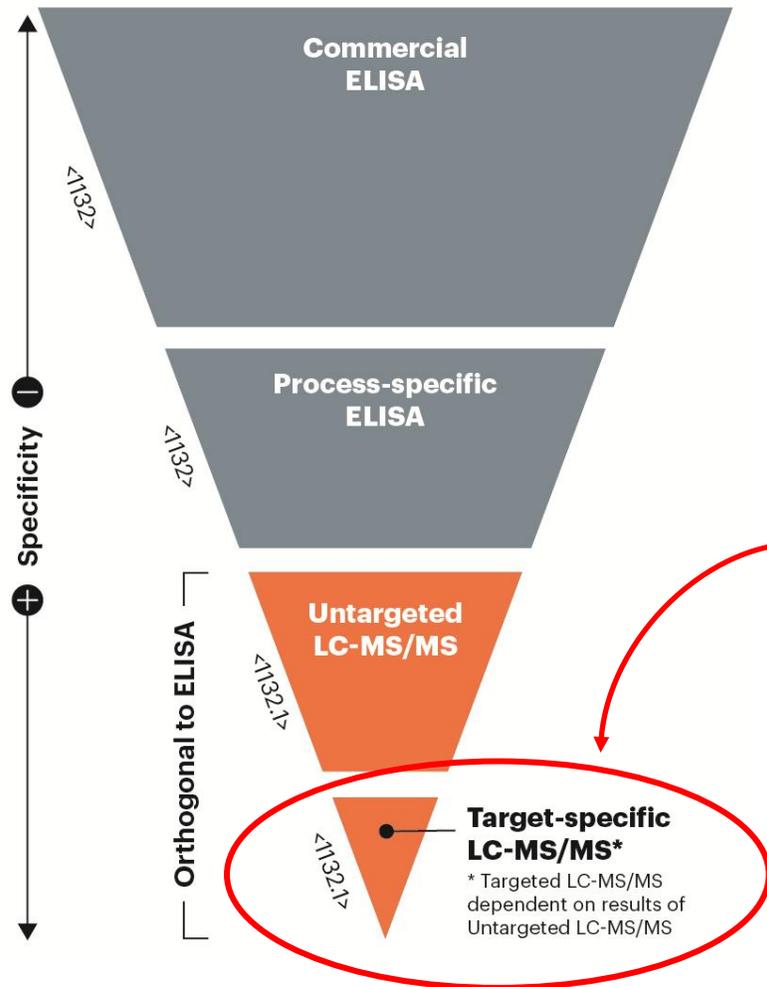
- ▶ <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals
  - Informational chapter with best practices with ELISA focus
- ▶ <1132.1> Residual Host Cell Protein Measurement in Biopharmaceuticals by Mass Spectrometry
  - Best practices for HCP MS, including sample preparation, LC separation, and mass spectrometry analysis



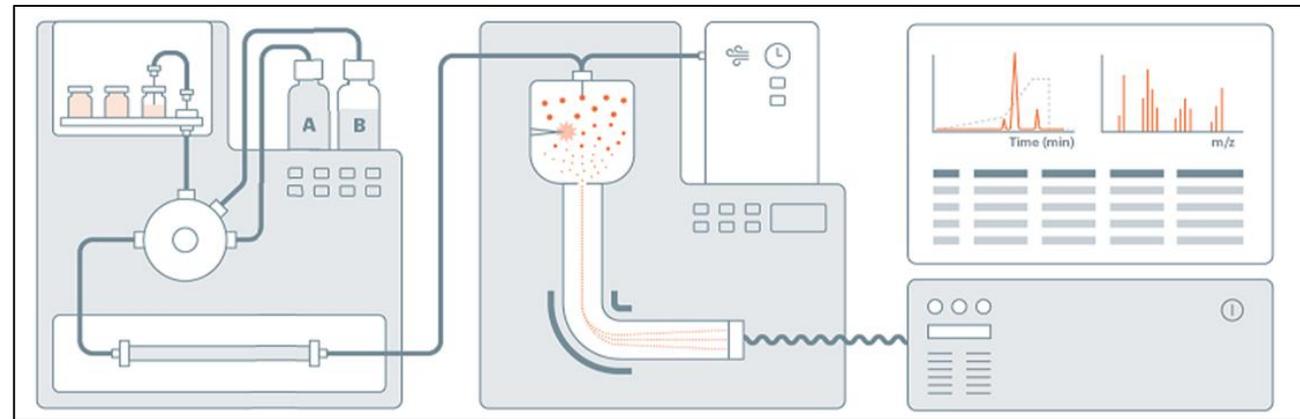
## Physical Standards

- ▶ **Purified proteins for high-risk HCPs**
  - Recombinant CHO PLBL2
- ▶ **Sample matrix**
  - Pooled CHO Null Cell HCCF
- ▶ **Stable isotope labeled peptides**
  - SIL peptides for high-risk CHO HCPs

# Targeted approach for high-risk HCPs



- ▶ LC-MS(MS) enables highly targeted HCP analysis that was previously unattainable with ELISA.
- ▶ Efforts can now focus on high-risk HCPs
- ▶ USP has prioritized development of standards for problematic HCPs in CHO



# USP Biologics mAb analytical guide



**In-Process Testing**  
(Process Monitoring & Control)

**Release & Stability**  
(Specifications)

**Extended Characterization**  
(Product Quality Monitoring)

**Release & Stability**

- Compendial Tests
- Size Variants
- Charge Variants
- Glycosylation
- Potency & Biological Activity
- Process Related Impurities
- Stability
- Safety
- Excipients
- Concentration

**In-Process Testing**

- Concentration
- Process Related Impurities
- Charge Variants
- Size Variants
- Glycosylation
- Safety

**Extended Characterization**

- Concentration
- Compendial Tests
- Size Variants
- Charge Variants
- Glycosylation
- Potency & Biological Activity
- High Order Structure
- Immunogenicity Testing

**Circular Dichroism**

**Fourier-Transform Infrared Spectroscopy (FTIR)**

**Dynamic Light Scattering**

**Check for yourself!**

## USP Resources

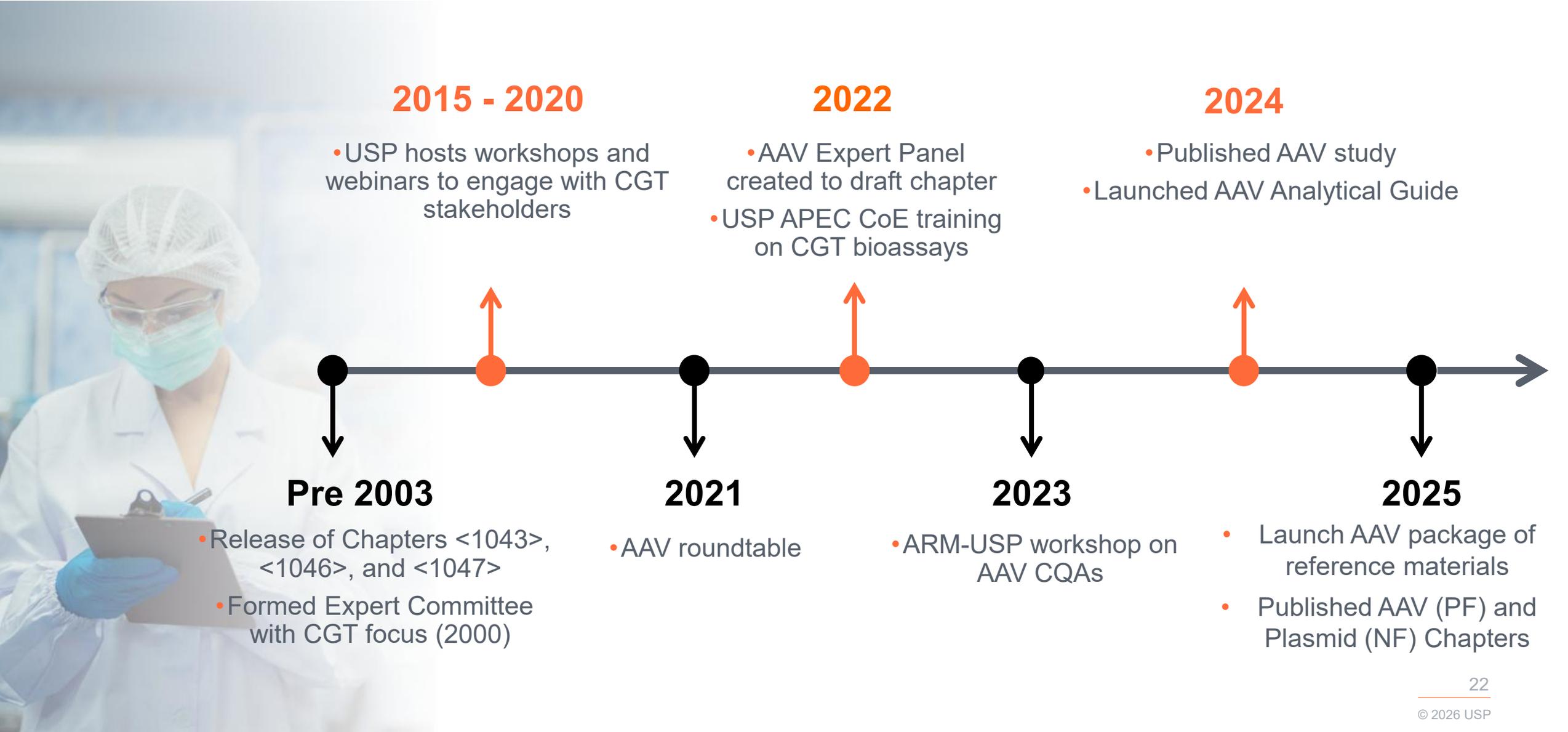
- ▶ Reference Standards
- ▶ Documentary Standards
- ▶ USP Educational Contents
- ▶ Other Resources

2

## USP's AAV Standards and Tools

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# USP's trajectory in the CGT field



## 2015 - 2020

- USP hosts workshops and webinars to engage with CGT stakeholders

## 2022

- AAV Expert Panel created to draft chapter
- USP APEC CoE training on CGT bioassays

## 2024

- Published AAV study
- Launched AAV Analytical Guide

## Pre 2003

- Release of Chapters <1043>, <1046>, and <1047>
- Formed Expert Committee with CGT focus (2000)

## 2021

- AAV roundtable

## 2023

- ARM-USP workshop on AAV CQAs

## 2025

- Launch AAV package of reference materials
- Published AAV (PF) and Plasmid (NF) Chapters

# USP documentary standards for CGT



## Existing Chapters

<1046> Cell-based Advanced Therapies and Tissue-Based Products

<1047> Gene Therapy Products  
(under revision)

<1043> Ancillary Materials for Cell, Gene, and Tissue-Engineered Products



## New\* Chapters

\*Initiated by USP Expert Committee serving from 2020 - 2025

**<1040> Quality Considerations of Plasmid DNA as a Starting Material for Cell and Gene Therapies<sup>1</sup>**

**<1067> Best Practices in Manufacturing of Recombinant Adeno-Associated Viral (rAAV) Vector<sup>2</sup>**

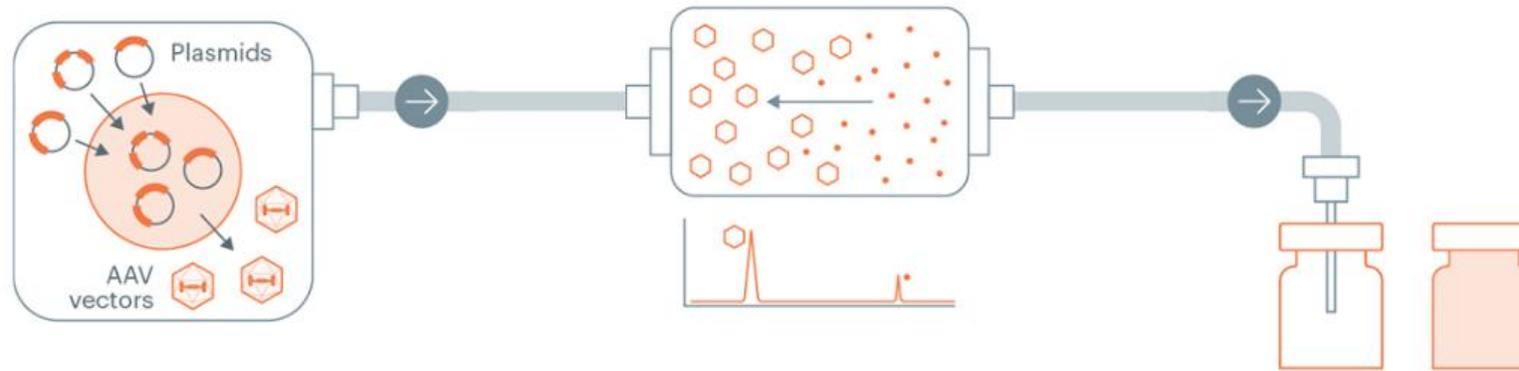
**<89.3> Endonuclease (*Serratia marcescens*)<sup>2</sup>**

**<TBD> Best Practices for the Production and Characterization of Lentivirus for Cell and Gene Therapy<sup>3</sup>**

<sup>1</sup>Published in USP-NF - to be official on 01Feb2026

<sup>2</sup>Published in USP-PF; <sup>3</sup>In development

# Initial focus for AAV standard development



## Upstream

### Raw Materials

- Requires novel raw and starting materials
- Control critical for safety

## Downstream

### Process Impurities

- Critical to remove problematic materials
- Residual impurity testing is often difficult

## Formulation

### Quality Attributes

- Lack of harmonized characterization methods
- Lack of quality viral vector standards

# USP standards to ease analytical challenges



## Raw Materials



Plasmid  
Qualification



Endonuclease  
Qualification

## Process Impurities



Residual Host Cell  
DNA



Residual Plasmid  
DNA

## Quality Attributes



AAV Empty:Full  
Ratio



Capsid  
Quantification



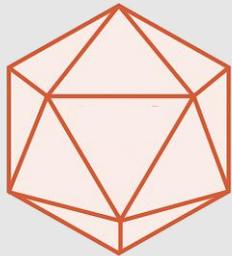
Genome Titration

# AAV standards for empty-full analysis



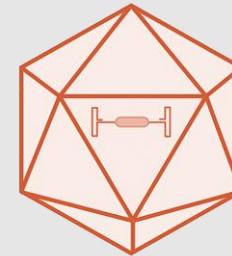
- ▶ **Challenge:** Previously available AAV reference materials do not meet the demands of users
- ▶ **USP Solution:** AAV reference standards for use as standard or control for empty/full analysis
  - The physical properties include high volume, high concentration, and high purity
  - The standards characterized in multi-lab study using multiple empty/full methods

## AAV8 (Empty Capsids)



3.1E+12 cp/mL  
300  $\mu$ L  
 $\leq 10\%$  Full Capsids

## AAV8 (Full Capsids)



4.7E+12 cp/mL  
300  $\mu$ L  
 $\geq 90\%$  Full Capsids

# AAV8 empty/full data



- ▶ All AAV data collected from a multi-lab study using five methods
  - Results showed a general alignment between methods for the two products

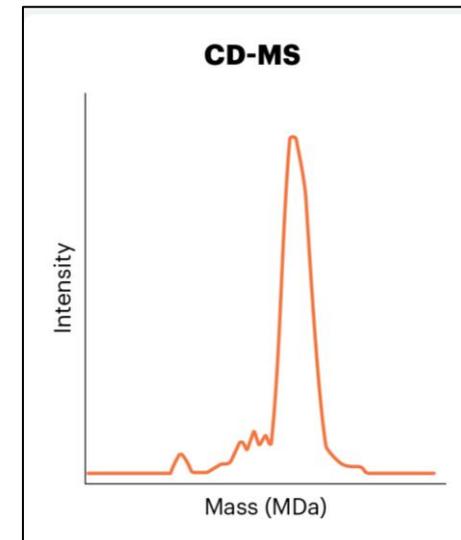
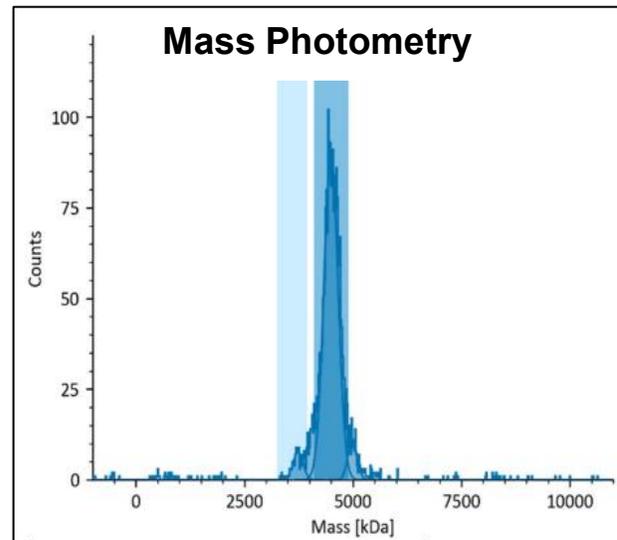
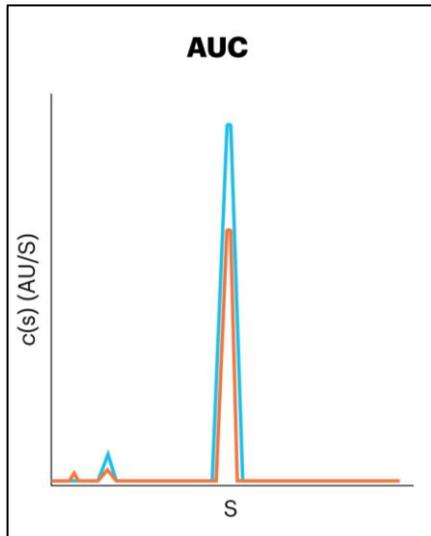
Testing Method	AAV8 (Full Capsids)		AAV8 (Empty Capsids)	
	% Full	% Empty	% Full	% Empty
<b>SEC-MALS</b>	98	2	2	98
<b>Mass Photometry</b>	91	6	5	95
<b>CDMS</b>	80	4	1	99
<b>AUC</b>	91	3	1	91
<b>UV (A260/A280)</b>	95	5	-	100

# AAV8 partial-filled AAV data



- ▶ It is also critical to measure partial-filled AAV

Sample	Mass Photometry	Charged Detection Mass Spectrometry	Analytical Ultra Centrifugation
AAV8 (Full)	3.2%	15.7%	6.4%
AAV8 (Empty)	1.4%	1.0%	2.9%

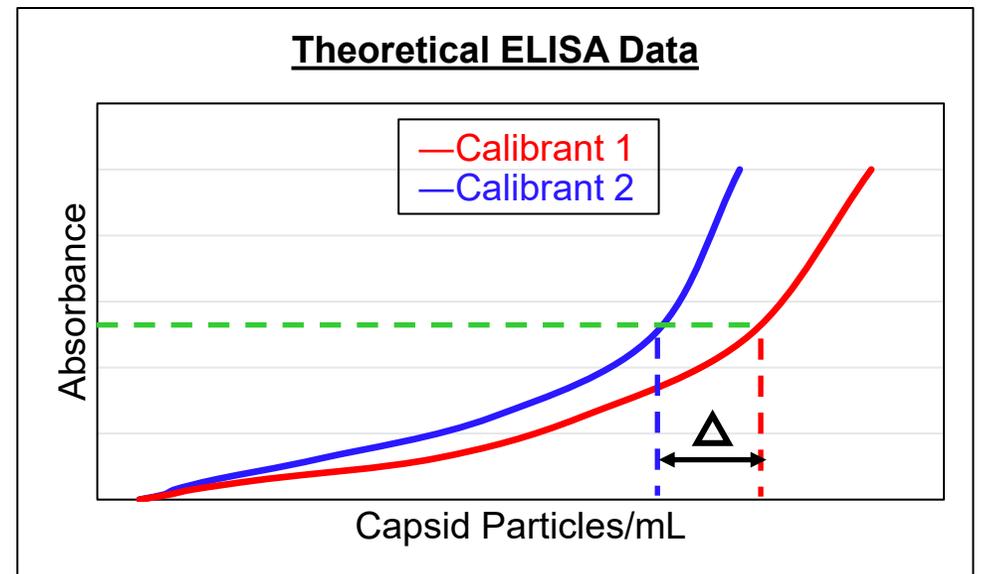


# Improving accuracy in AAV ELISA tests



- ▶ **Challenge:** Inaccurate quantification of calibrants frequently undermines ELISA accuracy
- ▶ **USP Solution:** AAV reference materials for use as control or calibrant
  - Quantify each standard using SEC-MALS (absolute direct measurement)

Feature	USP Reference Material
Serotypes	1, 2, 5, 8, and 9
Capsid Titer Method	SEC-MALS
Lot-to-Lot Variability	Low



# AAV9 capsid titer case study

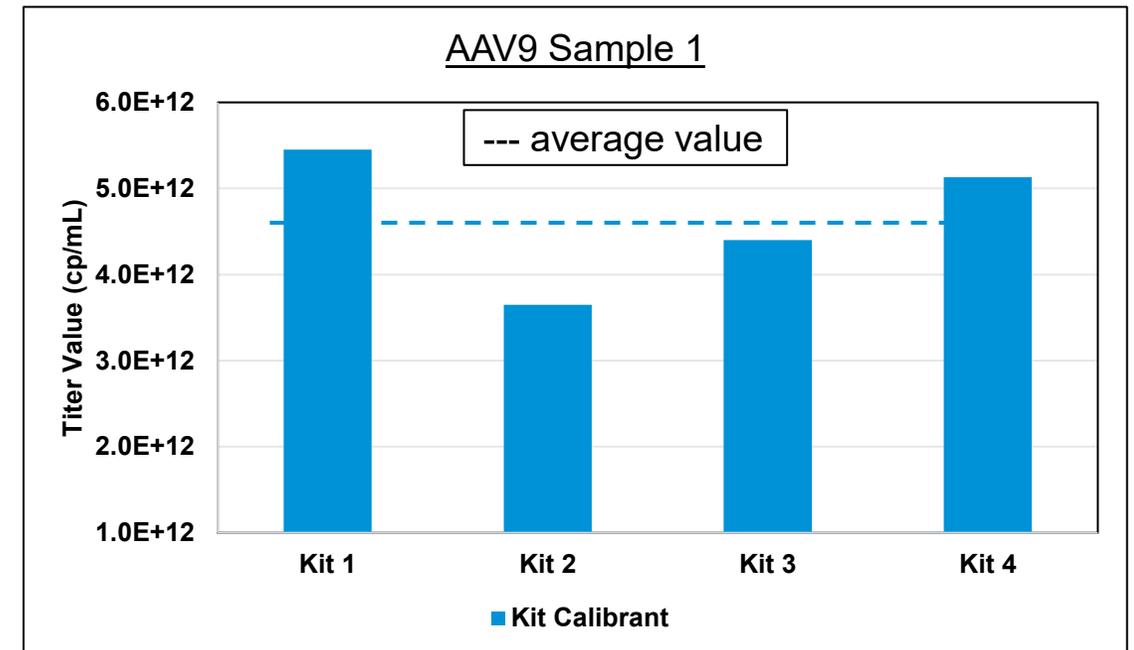


- ▶ **Experiment:** compared 4 ELISA kits using both the kit calibrant and USP AAV9 as calibrant
- ▶ **Results:** USP reference material increases accuracy and precision of ELISA tests

AAV9 Sample 1						
	Kit 1	Kit 2	Kit 3	Kit 4	Average	CV
<b>Kit Calibrant</b>	5.5E+12	3.7E+12	4.4E+12	5.1E+12	4.7E+12	17.2%
<b>USP AAV9 Calibrant</b>						

AAV9 Sample 2						
	Kit 1	Kit 2	Kit 3	Kit 4	Average	CV
<b>Kit Calibrant</b>	5.1E+13	3.0E+13	4.4E+13	4.4E+13	4.2E+13	20.4%
<b>USP AAV9 Calibrant</b>						



# AAV9 capsid titer case study

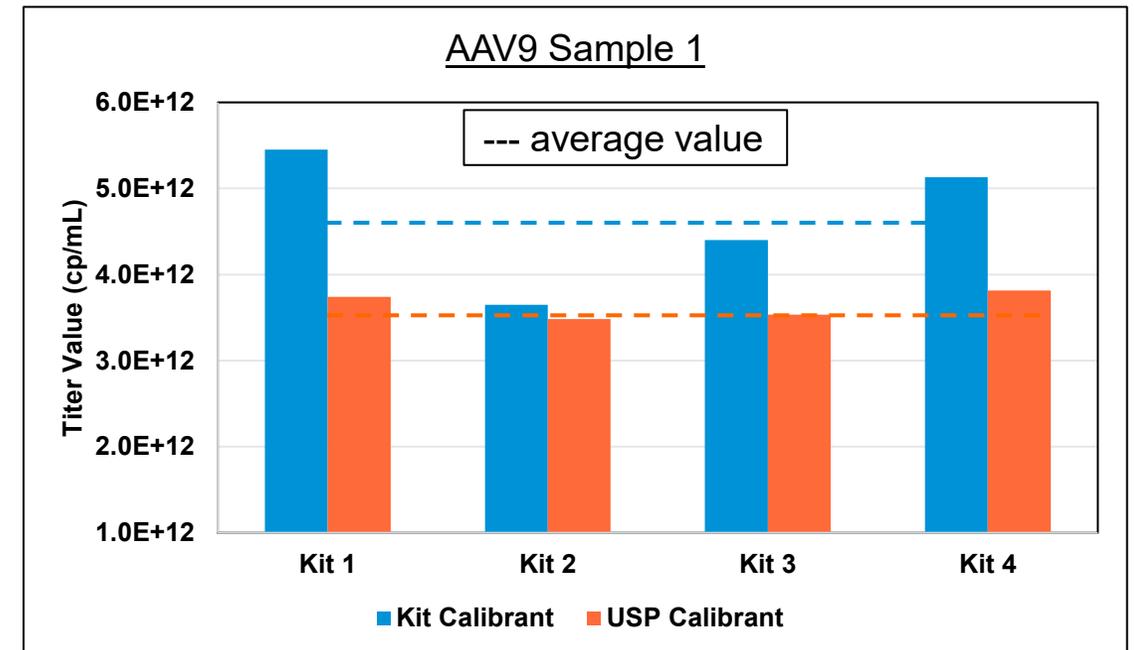


- ▶ **Experiment:** compared 4 ELISA kits using both the kit calibrant and USP AAV9 as calibrant
- ▶ **Results:** USP reference material increases accuracy and precision of ELISA tests

AAV9 Sample 1						
	Kit 1	Kit 2	Kit 3	Kit 4	Average	CV
<b>Kit Calibrant</b>	5.5E+12	3.7E+12	4.4E+12	5.1E+12	4.7E+12	17.2%
<b>USP AAV9 Calibrant</b>	3.7E+12	3.5E+12	3.5E+12	3.8E+12	3.6E+12	4.4%

AAV9 Sample 2						
	Kit 1	Kit 2	Kit 3	Kit 4	Average	CV
<b>Kit Calibrant</b>	5.1E+13	3.0E+13	4.4E+13	4.4E+13	4.2E+13	20.4%
<b>USP AAV9 Calibrant</b>	3.5E+13	3.4E+13	3.4E+13	3.3E+13	3.4E+13	3.1%



# More study details available in App Note



Application Note

## Improving accuracy and precision of AAV ELISA measurements using USP AAV Capsid Titer Analytical Reference Material



### Introduction

Gene therapy represents one of the fastest growing biotherapeutic modalities worldwide, with AAV at the forefront. This rapid growth is constantly challenging CMC (Chemistry, Manufacturing, and Controls) approaches, from manufacturing through release testing. Having appropriate analytical methods to evaluate the critical quality attributes (CQA) of AAV throughout the production process is required. One essential CQA is AAV particle concentration, which is often referred to as either capsid titer or particle titer. It is imperative to monitor the number of AAV particles throughout the entire purification process to ensure manufacturing efficiency and consistency and to monitor product quality. For this reason, capsid titer measurements are routinely performed at multiple steps throughout the production process.

assays, a calibrant is used to generate a standard curve, which is subsequently used to quantify the target analyte. In most ELISAs, the concentration of the calibrant is determined via a direct protein concentration method and, in some cases, may be adjusted to align with reference materials from previous lots to ensure consistency, even at the expense of accuracy. However, these methods are difficult to implement with AAV particles, making it more difficult to properly determine the absolute concentration of AAV. Nonetheless, there are methods available that can indeed directly measure the capsid titer value of AAV. Unfortunately, few of the ELISA kit manufacturers use direct measurements to set the value of their capsid calibration standard. This has severely hampered the accuracy of ELISA values for AAV titration, as is shown in our data below. To help address this problem, USP has developed four capsid titer reference materials (AAV1, AAV5, AAV8, and AAV9) that can either be run alongside the

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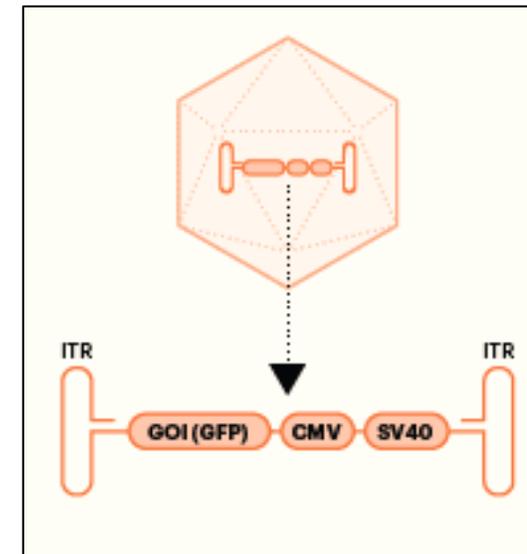
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# AAV genome titer reference material



- ▶ **Challenge:** Lack of AAV calibrants or controls for genome titer (plasmids known to be inadequate)
- ▶ **Solution:** An AAV particle for use in qPCR and dPCR genome titer tests
  - The vector genome contains five common PCR targets (SV40, ITR2, CMV, CBA, and GFP)
  - dPCR used for quantification in multi-lab study

Target Region	Genome Titer (vg/mL)
SV40	7.0E+10
ITR2	8.3E+10
CMV	4.1E+10
CBA	4.8E+10

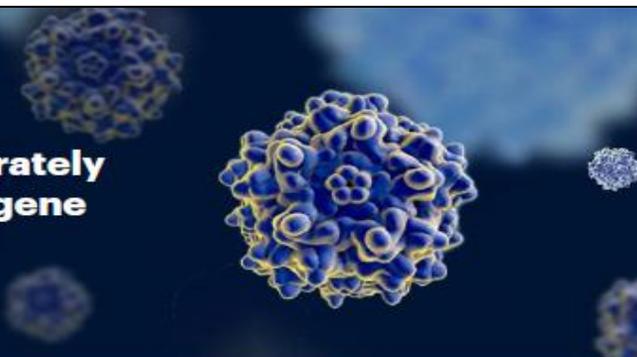


# Learn more in our Application Note



**Application Note**

**USP reference material to accurately measure genome titers of AAV gene therapy products**

A 3D illustration of several Adeno-Associated Virus (AAV) particles, which are spherical and composed of a protein shell and a genome. They are shown in various orientations and sizes against a dark blue background.

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**Introduction**  
Vector genome titer (i.e., the amount of transgene in an AAV particle) is a critical quality attribute for AAV products and an important in-process test to help monitor and control the manufacturing process. Digital or quantitative polymerase chain reaction (dPCR or qPCR, respectively) are industry standards for accurate and reliable genome titer

**Intended use of reference material**  
To meet the demand for high-quality AAV genome titer reference materials, the United States Pharmacopeia (USP) has developed [USP Quantitative AAV Titration PCR Control \(Catalog #: 1012106\)](#), an analytical reference material designed to improve the accuracy and reliability of genome titer measurements. This reference material is intended for

Explore the full application note



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## Application Note key takeaways:

- Plasmid DNA is an insufficient control/calibrant for AAV genome titer
- USP dPCR and qPCR method procedures, primers, and probes shared in the document
- USP reference material can serve as control for dPCR or calibrant for qPCR

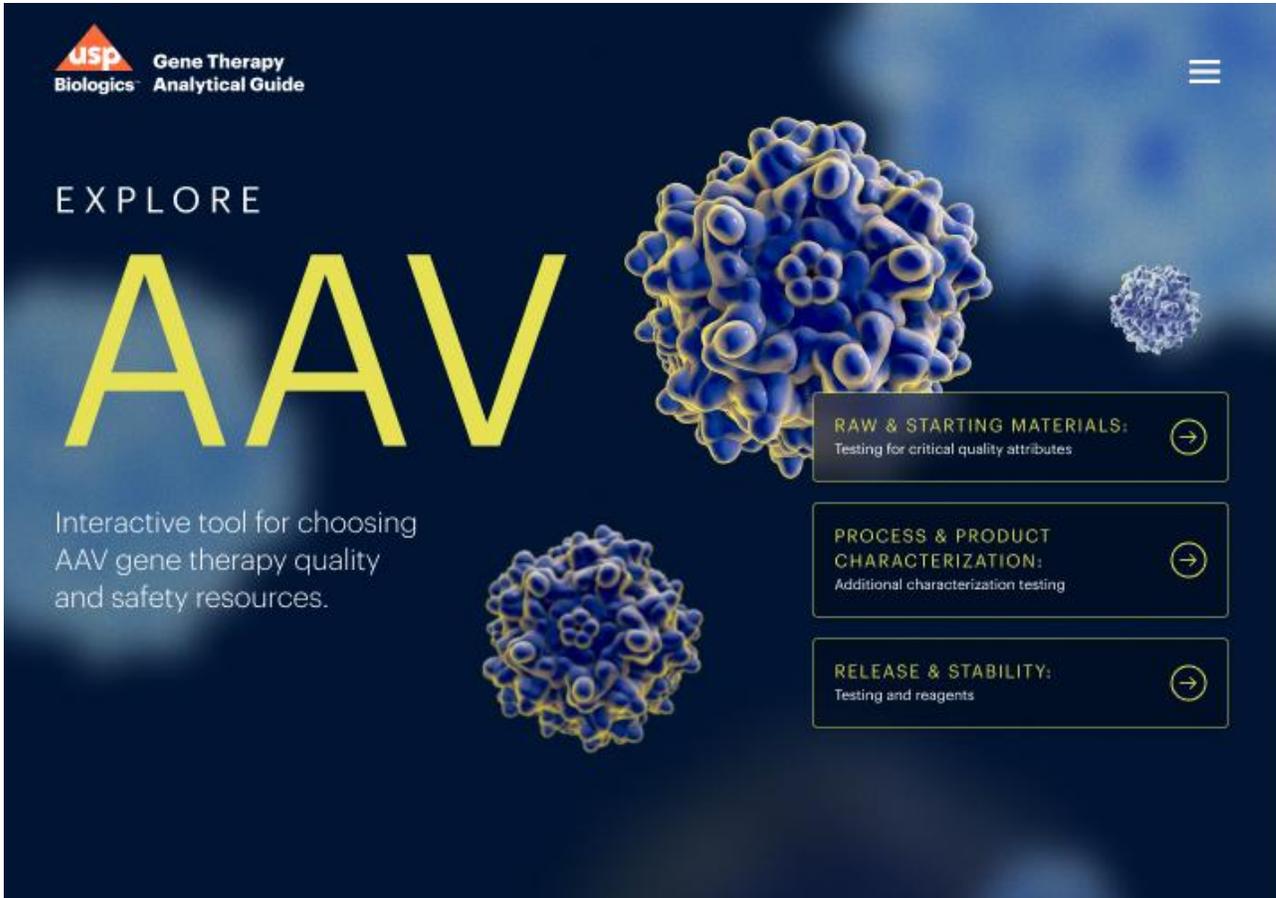
# Compiled AAV standards and resources



Product	Catalog Number	Associated Resource
AAV8 Empty/Full Standards	<u>1000302</u> , <u>1000301</u>	<u>App Note</u>
AAV 1, 5, 8, and 9 Capsid Titer	<u>1800237</u> , <u>1800239</u> , <u>1800240</u> , <u>1800241</u>	App Note
Genome Titer	<u>1012106</u>	<u>App Note</u>
Residual Plasmid	<u>1544900</u>	<u>App Note</u>
Residual HEK293 DNA	<u>1592106</u>	<u>App Note</u>
Endonuclease	<u>1235470</u>	USP Chapter <89.3> (PF(51)5)
Plasmid Topology	1544906 - 1544912	<u>Webinar</u>

- Slides available upon request
- Visit [www.usp.org/biologics/aav](http://www.usp.org/biologics/aav) for updates

# USP “Gene Therapy Analytical Guide”



▶ <https://genetherapyanalyticalguide.usp.org>

- Provides appropriate analytical tests and USP standards in one easy-to-access location

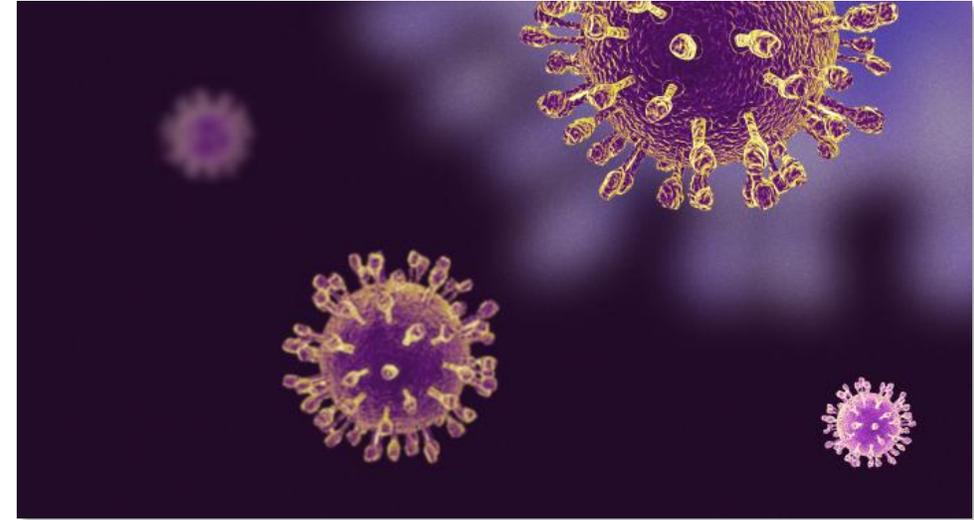


# Ongoing work in CGT



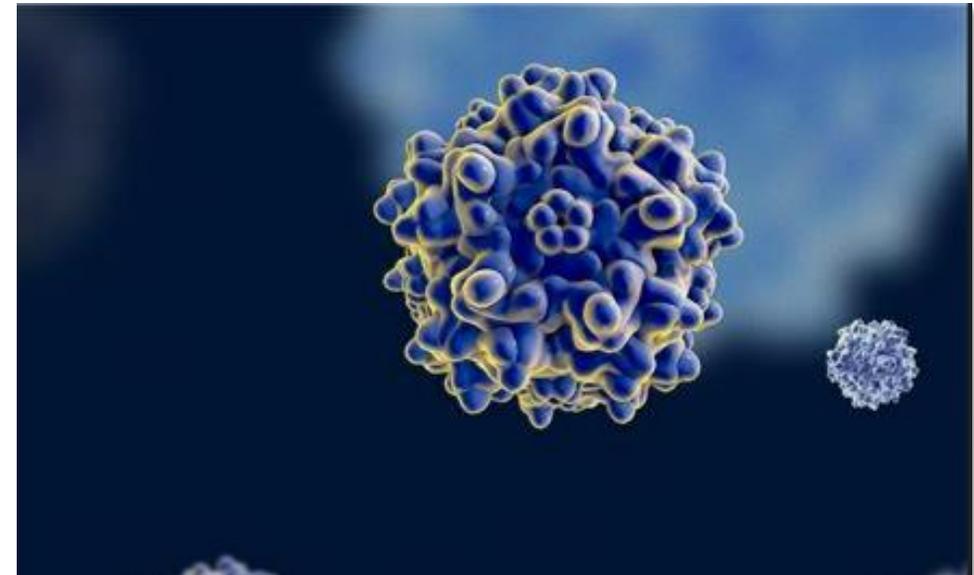
## LVV and Cell Therapy

- ▶ Lentivirus Expert Panel drafting chapter
- ▶ Developing LVV Reference Standard for physicochemical characterization
- ▶ Working on other cell therapy related products



## AAV

- ▶ Additional empty/full standards with different serotypes
- ▶ Active working group to develop compendial AAV methods





- ▶ USP offers many opportunities for stakeholder engagement
- ▶ Donate methods and/or material to support standard development
- ▶ Review of Chapters and Stimuli Articles on Pharmacopeial Forum
  - <https://www.uspnf.com/pharmacopeial-forum>
- ▶ Notices to be posted on USP web site
  - <https://www.usp.org/biologics>

# Stay Connected

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