USP® and ATCC® Resources that Support Monitoring of Impurities in Biologics

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January 23, 2024

Outline

- Introduction to the ATCC-USP partnership
- Residual DNA testing support
- Residual host cell protein standards
- Microbiology solutions

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1 INTRODUCTION TO THE ATCC-USP PARTNERSHIP

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Over 200 Years of building trust in the US and beyond

ATCC® – Life science innovations that touch people

- Founded in 1925 - we have been supplying scientists with essential scientific resources, services, and standards for nearly a century
- ATCC® is ISO 9001 and ISO 13485 certified and ISO/IEC 17025 and ISO 17034 accredited
- Leading global supplier of authenticated cell models and viral and microbial standards
- An innovative R&D company that provides better models
  - Gene editing, microbiome, NGS, primary cells, and advanced cell models
- Services provider
  - Customer base in diagnostics, drug discovery, and applied markets; cGMP and Biorepository Services
- Patent repository consists of >90% of all USA bio-patents

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A collaboration to enable innovation in biologics

For ATCC and USP, advancing quality assessment tools is the priority

- Both non-profit organizations have built a legacy of delivering high-quality materials and standards
- A multi-year strategic collaboration was signed in 2023 to jointly provide robust and innovative solutions for biologics
- The first materials support testing clearance of host cell DNA

https://www.usp.org/biologics/atcc-usp-collaboration

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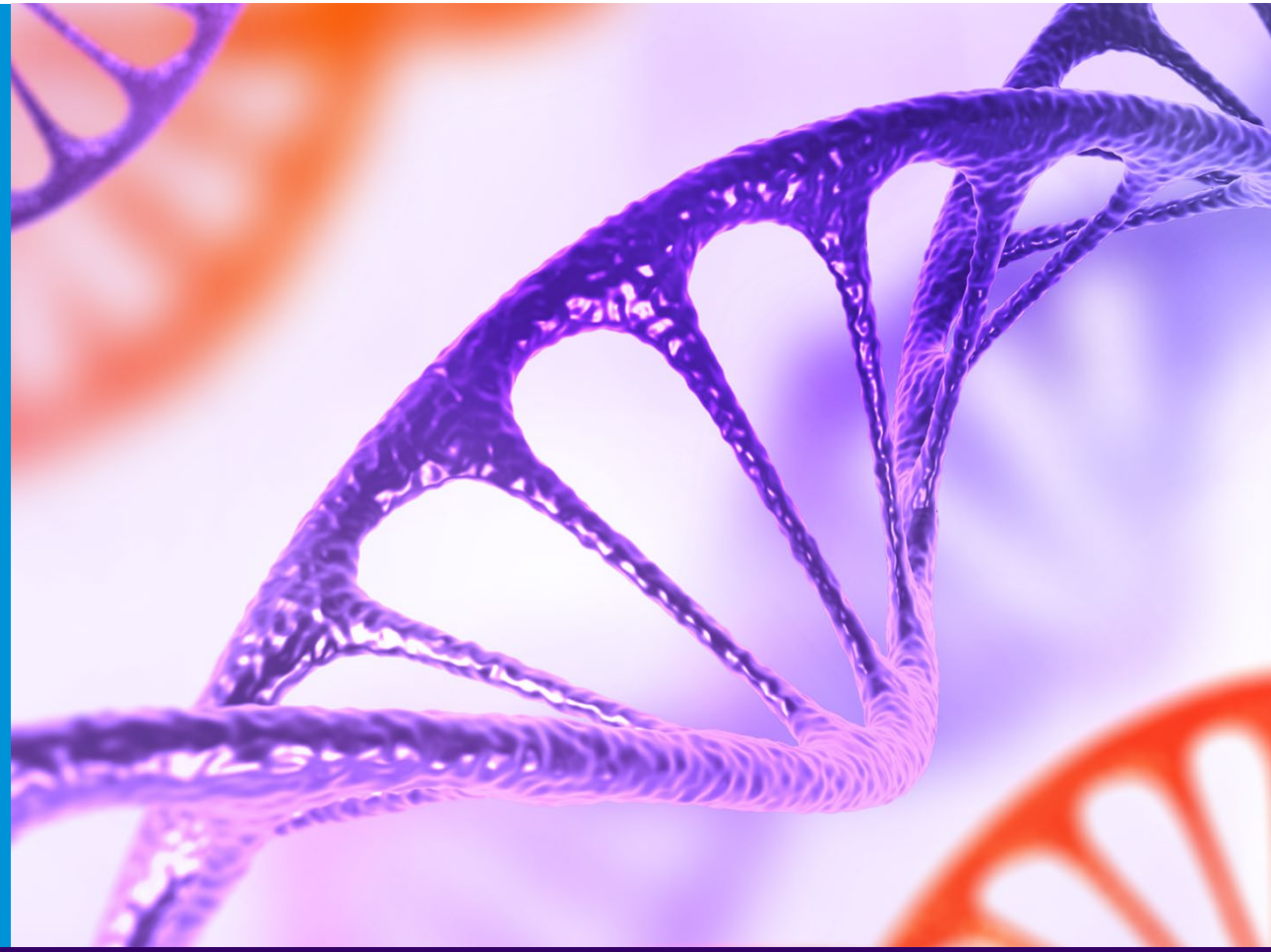
Perspectives from our leadership

- “By combining our strengths, we can better support institutions and stakeholders that engage in R&D, process development and release of biologics, as well as provide them with quality products that meet their needs,” said Raymond H. Cypess, D.V.M., Ph.D., chairman and CEO of ATCC. “This collaboration also offers solutions that customers can rely on to mitigate their regulatory acceptance risks and accelerate commercialization of new therapies.”

- “Our goal, together with ATCC, is to provide robust and innovative solutions that ensure our customers feel confident that the answers they get are reproducible and accurate, and ultimately can provide safe biologic therapies to patients who depend on them,” said Ronald T. Piervincenzi, Ph.D., CEO of USP. “This agreement begins with the first of many potential product areas for our organizations to provide standards and other solutions.”

https://www.usp.org/news/atcc-and-usp-enter-strategic-collaboration-to-support-global-quality-production-of-biologics



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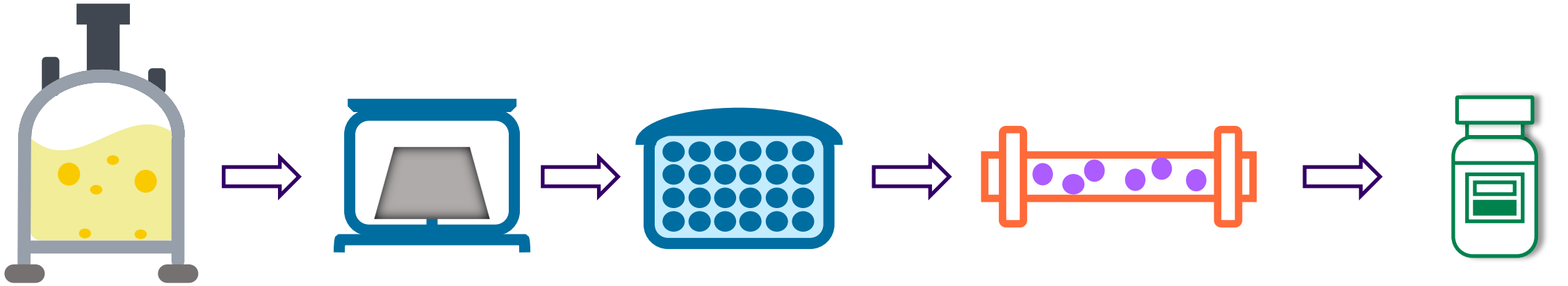
Bioproduction: testing for impurities and contaminants is critical throughout the process



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RESIDUAL DNA TESTING SUPPORT

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Regulatory Guidance on Residual DNA Limit

- WHO : <10 ng/dose

WHO Technical Report Series, 878, Annex 1

- FDA : Less than 10 ng/dose and the DNA size to blow approximately 200 base pairs

Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) Guidance for Industry, Jan 2020

- EMA: There is no inclusion of known oncogenic/tumorigenic sequences

Guideline on the Quality, Non-clinical and Clinical Aspects of Gene Therapy Medicinal Products, March 2018

- However, based on the risks associated with the cell source, drug product and its administration, these limits can vary. Therefore, residual DNA specifications should be established in collaboration with appropriate regulatory agencies

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ATCC-USP product offering/solutions

Quantitative Genomic DNA (gDNA) isolated from cell lines used in the manufacturing of biologics – vaccines, therapeutics, etc.

Market	SKU	Product name	Species	Originated from
Cell and Gene Therapy	1592106	Quantitative **HEK-293** Genomic DNA	Human Embryonic Kidney (*Homo sapiens*)	ATCC® CRL-1573™
	1592170	Quantitative **Sf9** Genomic DNA	Fall Armyworm Ovary (*Spodoptera frugiperda*)	ATCC® CRL-1711™
Vaccines	1592111	Quantitative **MDCK** Genomic DNA	Madin-Darby Canine Kidney (*Canis familiaris*)	ATCC® CCL-34™
	1592112	Quantitative **MRC-5** Genomic DNA	Human Lung, Medical Research Council (*Homo sapiens*)	ATCC® CCL-171™
	1292190	Quantitative **Vero** Genomic DNA	African Green Monkey Kidney (*Cercopithecus aethiop*)	ATCC® CCL-81™
Vaccines & Proteins	1592100	Quantitative **BHK-21** Genomic DNA	Syrian Golden Hamster kidney (*Mesocricetus auratus*)	ATCC® CCL-10™

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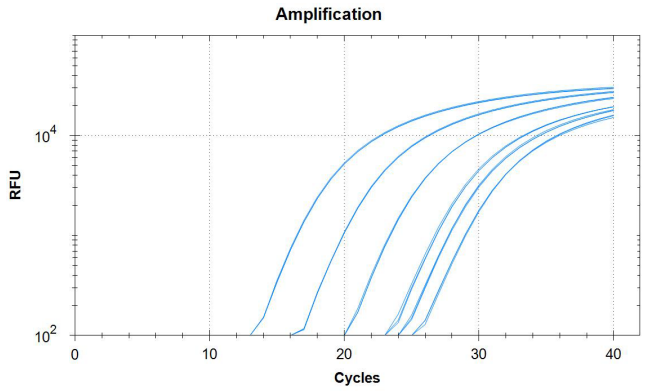
Vero gDNA Performance as Analytical Reference Material in qPCR

Amplification



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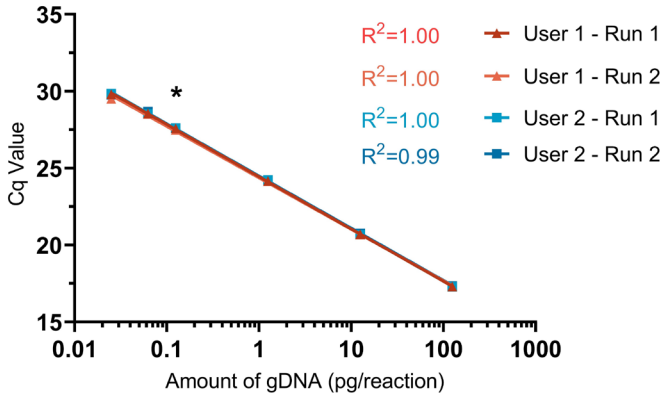

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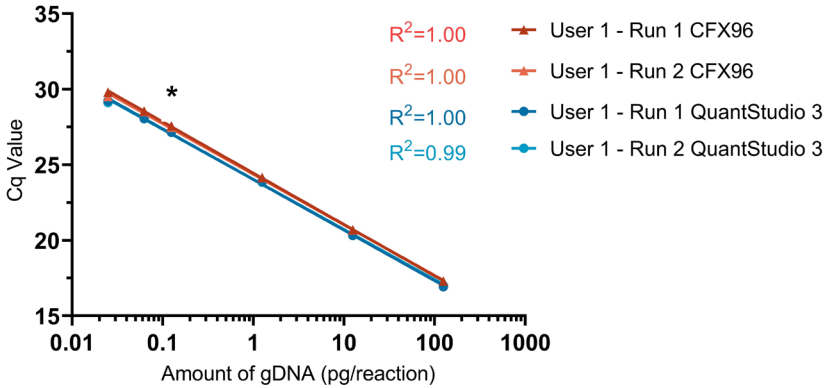

Publication	Assay Target Seq.
*** Vernay O., et al. J. Vir. Meth., 2019.**	**α-satellite**
André M., et al. Biologicals. 2016	18S rRNA
Zhang W., et al., J. Pharm. Biom. Anal. 2014	Alu
Funakoshi K., et al., Nature Sci Rep. 2017	Alu

* Published Lower Limited of Quantitation (LOQ)

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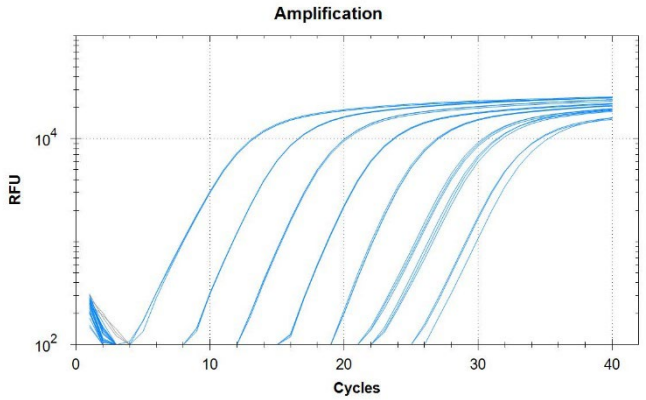
HEK-293 gDNA Performance as Analytical Reference Material in qPCR

Amplification



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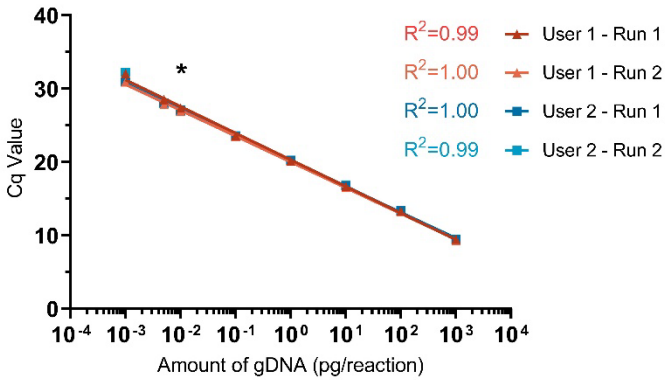

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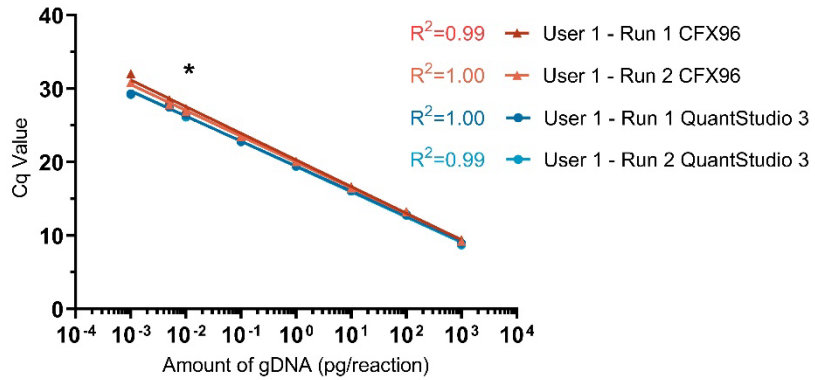

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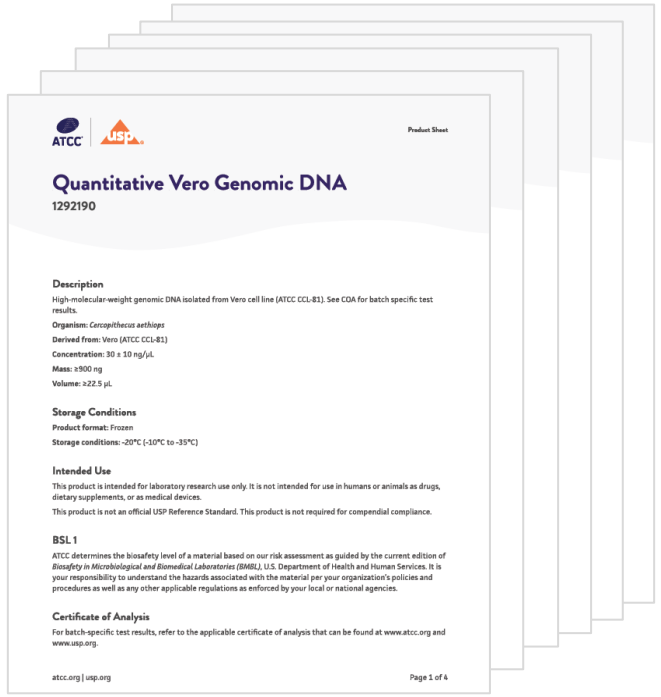
Technical and Support Documents

Application Notes

Product Sheets



*Materials available on **atcc.org** and **usp.org** (scan respective QR code to access)*

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USP Residual DNA Testing Standards

Documentary standards

- <1130> Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)
  - Informational General Chapter with best practices
    - Hybridization-based residual DNA assay
    - DNA-binding protein-based residual DNA assay
    - PCR
  - No associated reference standard
- <509> Residual DNA Testing
  - Official on December 01, 2019
  - A procedure chapter containing validated extraction and qPCR methods
  - Two associated RSs

USP Reference Standards

- *E. coli* Genomic DNA Reference Standard (Catalog # 1231557)
- CHO Genomic DNA Reference Standard (Catalog # 1130710)

***E. coli* gDNA RS**

CHO gDNA RS

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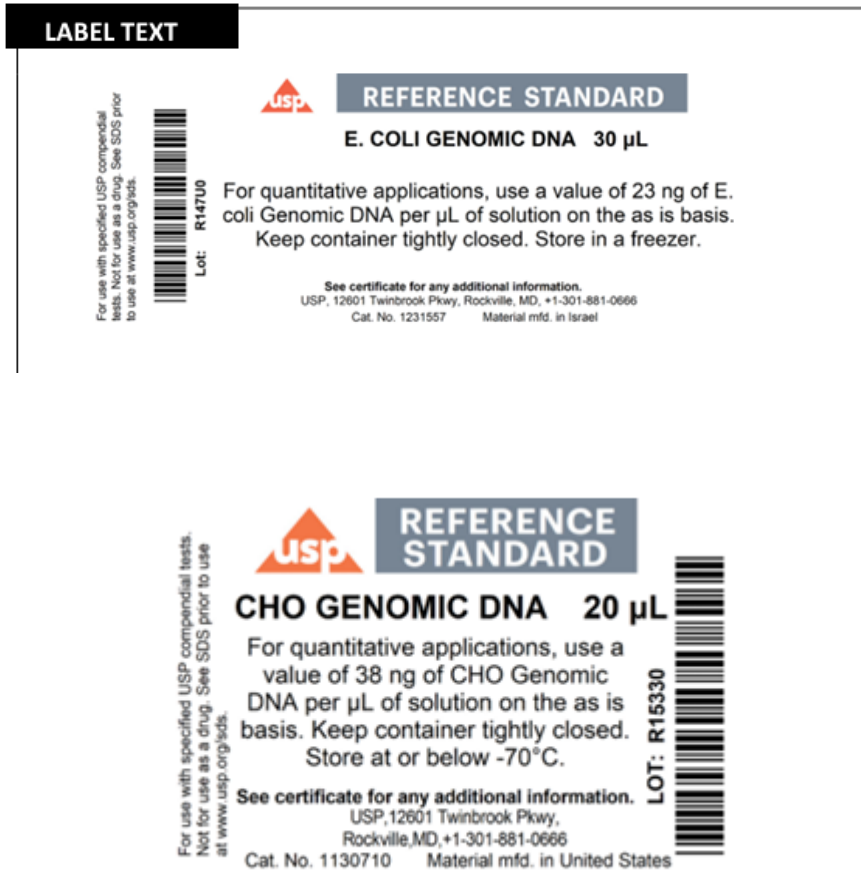
USP *E. coli* and CHO Genomic DNA Reference Standards

- Highly-characterized quantitative reference standards by multiple USP labs and collaborator labs
- Concentration determined using the qPCR analysis per USP <509> and verified by low volume DNA quantification method (A260)
- Applications
  - Use as a standard to measure residual DNA in the products
  - Assess the system suitability of analytical procedures
  - Serve as a positive control for extractions



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Upcoming Webinar

ATCC-USP JOINT WEBINAR:

RESIDUAL DNA TESTING

FEBRUARY 8
11:00 AM EST

Leka Papazisi, DVM, Ph.D.
Principal Scientist, Microbiology R&D,
Product Life Cycle, ATCC

Ying Han, Ph.D.
Senior Scientist II, Global Biologics
USP

Register Now

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3 RESIDUAL HOST CELL PROTEIN STANDARDS

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Regulatory Guidance on Host Cell Proteins

- No specific limits exist in regulatory guidance
  - ICH Q6B Specifications
    - *Process-related impurities in the drug substance may include cell culture media, **host cell proteins**, DNA, monoclonal antibodies or chromatographic media used in purification, solvents, and buffer components. **These impurities should be minimized** using appropriate, well controlled manufacturing processes.*
- HCP levels should be measured in:
  - preclinical lots used in toxicology assessment
  - all lots during clinical development
  - process validation samples from the final manufacturing process
- After approval, HCP monitoring may be required as an element of the control system.

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Impact of Host Cell Proteins

- **Residual HCPs have the potential to affect product quality, safety, and efficacy, including:**
  - Unwanted immune responses to HCPs
  - HCPs can have bioactivity
    - homology to endogenous human proteins
  - HCPs may be enzymes that cleave the drug substance or excipients that can affect stability, potency
    - e.g., lipases, proteases
- **Risks can vary based on many factors, including:**
  - Dose (mg biologics/kg body weight)
  - Route of administration
  - Frequency of dosing (acute or chronic indications)
  - Patient receives more than one biotherapeutic
  - Patient population (immune-compromised, etc.)

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USP Public Standards

- **Monographs**
  - Specifications for pharmaceutical articles in commerce (from release through product shelf life)
  - Tests, assays, and acceptance criteria needed to demonstrate the article meets required quality standards

- **General Chapters**
  - Procedural chapters numbered below <1000>: Typically contain requirements and validated methods and may have associated reference standards
  - Informational chapters numbered <1000> to <1999>: Best practices and considerations

- **Physical Reference Materials**
  - **Reference Standard:** ensure the identity, strength, quality and purity of pharmaceutical articles and relate directly to procedures in the USP compendia
  - **Analytical Reference Materials (ARM)**: non-compendial, well characterized materials

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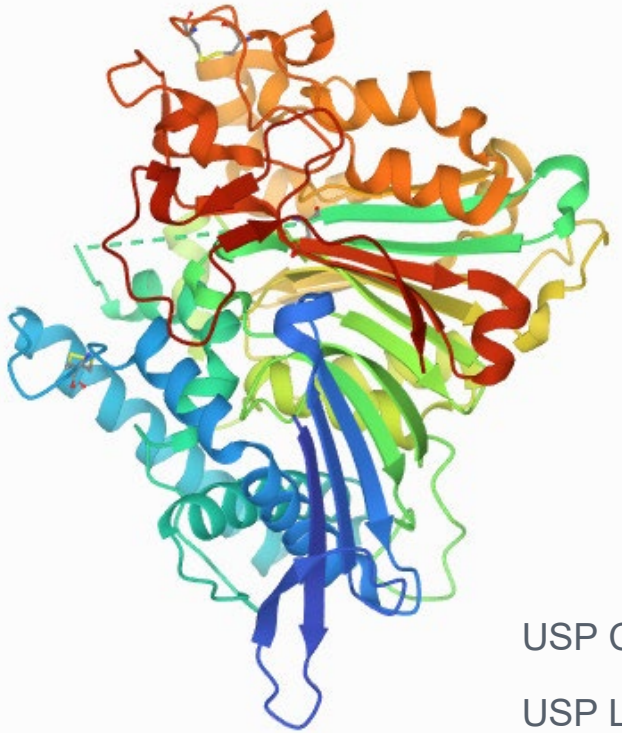
USP PLBL2 Analytical Reference Material

- ELISA
  - Confirm if a total HCP ELISA can detect PLBL2
  - Compare performance of anti-PLBL2 ELISAs
  - Include as control for anti-PLBL2 ELISA
- Western Blotting
  - Confirm total HCP antibodies can detect PLBL2
  - Compare anti-PLBL2 antibodies
- Control for development of MRM for PLBL2
  - Identification and quantitation of PLBL2
- Could be included in LC-MS/MS System Suitability testing



USP Catalog No.: 1582716

USP Lot No.: F174K0

Protein Sequence provided

- UniProtKB accession: G3I6T1

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

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HCP Peptide Analytical Reference Materials Development

Peptides

- Peptide specifications
  - stable isotope labeled Lys or Arg (SIL)
  - high purity (>95%)
  - sequences selected based on publications and feedback from stakeholders
  - Form Factor
    - dissolved peptide (liquid)
    - stored frozen ≤-65°C
  - *Projected release Spring 2024*
    - *Clusterin, Lipoprotein Lipase (3 peptides each)*
- Tests
  - Appearance
  - Identification
    - MS/MS
    - NMR
  - Impurities by HPLC
  - Quantitation by qNMR
  - Quantitation by AAA
  - Solubility assessment

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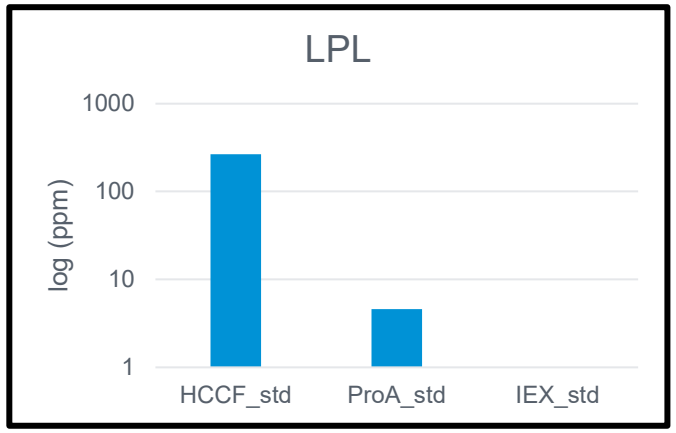
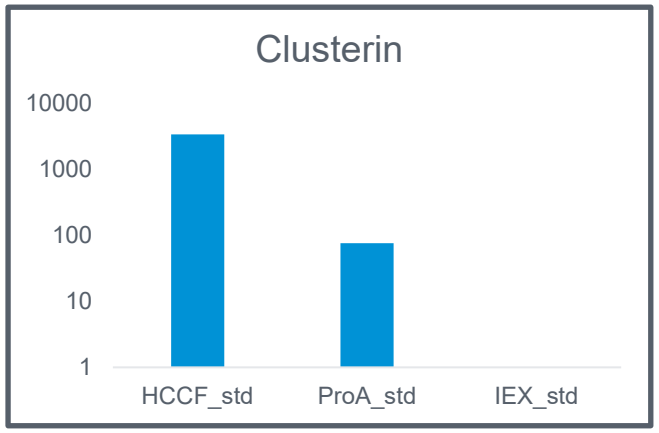
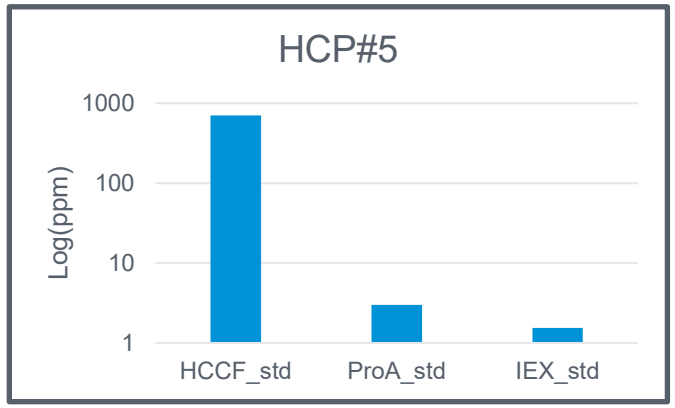
Quantitation using SIL Peptides

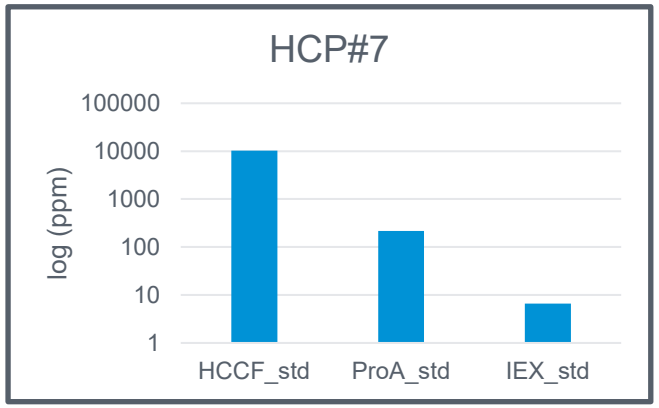
SIL peptides facilitated robust assessment of HCP clearance

- Producing cell line
  - HCCF, Protein A Pool, IEX Pool
  - Standard (denaturing)
  - DDA workflow, triplicate measurements
- SIL peptides solubilized and combined into one standard solution and spiked into digested sample
- Peptide amounts are average of triplicate measurements
- Protein amounts were averaged from multiple peptides when available









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Host Cell Protein in USP-NF

- USP-NF in <1132> *Residual Host Cell Protein Measurement in Biopharmaceuticals*
  - Official in USP-NF
  - 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 developed by Host Cell Protein Expert Panel
  - *<1132.1> Residual Host Cell Protein Measurement in Biopharmaceuticals by Mass Spectrometry*
  - Contains general guidance and best practices
  - *Published in PF 49(3)*
    - Comments under review!

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GC <1132.1> Highlights

- Approaches
  - Relative to Product Protein
  - Relative to Spiked-in Proteins
  - Relative to Spiked-in Peptides
- Results Reporting
  - List of identified proteins
  - List of identified proteins with levels (concentrations)
- Comparison to ELISA based methods
  - Multi-analyte ELISA yields ng/mL total HCP
    - Results expressed as ng HCP per mg product (ng/mg)
  - Individual HCP ELISA yields ng/mL single HCP
  - ***Note that ELISA and LC-MS/MS are complementary methods!***

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Proposed New GC <1132.1> Overview

- The chapter was developed by Host Cell Protein Expert Panel
  - Panel made up of expert volunteers from industry and academia with regulatory representation (FDA)
  - Contains general guidance and best practices
  - Published in PF 49(3)
    - Comment period closed
    - **Over 150 comments** received from industry, consultants, industrial organizations and regulators
- Next Steps
  - Review and reconciliation of comments by USP and Expert Panel
  - Review and Approval by Expert Committee (BIO2)
  - Public comments and responses summarized and published on USP's website
  - Publish in USP-NF

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MICROBIOLOGY SOLUTIONS

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Rapid Microbial Methods SC: 2020-2025 Cycle

The USP Microbiology EC is working to draft a series of official rapid microbial contamination tests based on the validation data from the peer-reviewed literature, submission from sponsor and, if necessary, collaborative proof of concept and validation studies

General Chapters		Update
<72> Respiration-Based Microbiological Methods for the Detection of Contamination in Short Life Products	PF 46(6)	• Revising <72> & <73> based on comments from PF, EC members and GLs • Revised and Published in PF50(1)
<73> ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short Life Products		
<1071> Rapid Microbiological Methods for the Detection of Contamination in Short-Life Products - A Risk-Based Approach	Official	• Ongoing major revision • Published in PF50(1)
<74> Solid Phase Cytometry-Based Microbial Method for the Detection of Contamination in Short Life Products	PF 48(5)	• Ongoing major review by the new MEC • <77> will be republished in PF soon
<77> Mycoplasma Nucleic Acid Amplification Tests		

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USP Microbial Methods for Detection of contamination <72> & <73>

- Culture media and incubation temperature: Growth Promotion Test of Aerobes, Anaerobes, and Fungi
- Method suitability test: demonstrate that the signs of growth in the presence of the product is comparable to the growth in the absence of the product
- Test for microbial detection in the product to be examined: consideration of volume of articles
- Monitoring and interpretation of results: Growth in the microbiological media is monitored at frequent, regular intervals, and the signal is captured by the instrument. Once positive threshold is met, a visual and/or audible alarm identifies the sample as positive

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USP <72> Respiration Based Microbiological Methods for the Detection of Contamination in Short-Life Products

- A growth-based method with direct inoculation
- Monitoring growth by detecting carbon dioxide production
  - Colorimetric or Fluorometric detection or
  - Detect pressure changes in the headspace of the culture bottle due to gas consumption and/or production
- Advantage of the method
  - Broad equivalency to compendial methods
  - Automation and periodic monitoring of the media for early detection of microbial growth
  - Small sample size and direct inoculation of cultures without interference from product-related turbidity
  - Automated data analysis, acquisition, reporting, and archiving
- Disadvantage of the method: inability to detect microorganisms that do not grow under the culture conditions utilized

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Mycoplasma testing General Chapters

- <63> Mycoplasma Tests Official
  - Method (A): agar and broth media procedure
  - Method (B): indicator cell culture procedure
  - Provision to use a validated nucleic acid amplification test as an alternative method
- <77> Mycoplasma Nucleic Acid Amplification Tests Proposed PF 48(5)
  - Method(s): nucleic acid amplification test procedure(s)
  - General chapter
    - Not an alternative method to <63>
    - <63> is not the referee method

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Proposed USP <77> Mycoplasma Nucleic Acid Amplification Tests

- A new nucleic acid-based mycoplasma general test is comparable to Method A and B of <63> Mycoplasma Tests
  - Mature method with validation data
  - Specificity – greater than 100 species of mollicutes, six species are most problematic
  - Limit of detection equivalent to <63>
- The tests described in the chapter will be suitable for near real time in-process and finished product ATMP cutting the time to result from 28 days to the same day.
- Considerations for sample treatment, QC standards, suitability testing, interpretation of results and investigation of invalid results

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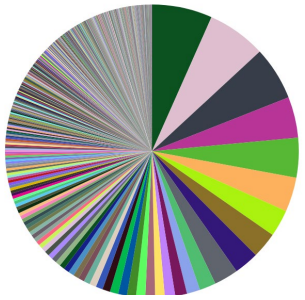
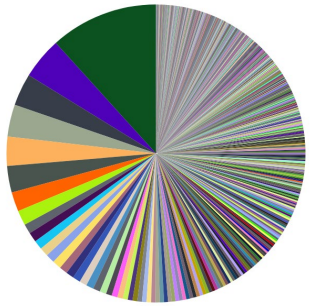
ATCC®'s comprehensive collection of microbes

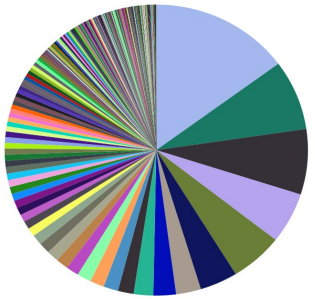
- Comprehensive microbial collection with enhanced authentication
  - 70,000+ bacteria, fungi, viruses, and protozoa
  - Over 1,300 microbial type strains
  - Over 4,000 cell lines
- Brand recognition
  - Organizations and regulatory agencies specify ATCC® cultures in their standards and guidelines
  - USP, ISO, FDA, CLSI, USDA, ASTM, AOAC, WHO
  - Over 475 reference strains recommended for use in quality control
- ATCC® has live microbes and derivatives, including inactivated materials and nucleic acids
- ATCC® uses a variety of advanced techniques to characterize and authenticate biomaterials—no single method of identification is sufficient



Bacteriology
1226 genera



Mycology
1864 genera



Virology
200 genera

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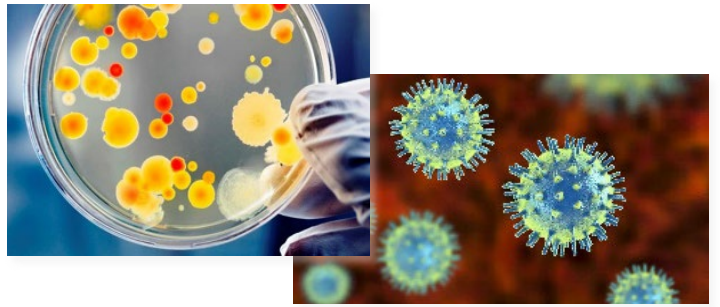
Reliable biomaterials should be used as controls

Types of materials to choose:

Reference Material	Benefit	Disadvantage
Live microbes	Sustainable source, maintains complexity of the intact microorganism, provides entire genome	Difficulty accessing materials, biosafety
Inactivated materials	Ability to access to pathogens in BSL 1 labs	Cells may no longer perform as live microbe
Genomic DNA/RNA	Ease of access, safe to use	May not mimic live microbe
Synthetic oligonucleotides	Easy to design and synthesize, allows access to non-culturable materials	May not resemble complexity of the whole genome

Other things to consider:

- Use fully authenticated materials
- Avoid contamination or misidentification



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Rapid respiration-based microbial detection methods

USP Chapter <72>

- Biologics must be tested for microbial contamination at harvest and during processing to final product
- Rapid microbial testing methods are required for cell and gene therapy products due to rapid turn around time needed to get materials to patients

Organism Type	Positive Controls	
	Organism Name	Type Strain
Aerobic Bacteria	*Staphylococcus aureus*	ATCC® 6538™
	Bacillus subtilis	ATCC® 6633™
	Pseudomonas aeruginosa	ATCC® 9027™
Anaerobic Bacteria	*Clostridium sporogenes*	ATCC® 19404™
	Cutibacterium acnes (formerly *Propionibacterium acnes*)	ATCC® 11827™
Fungi	*Candida albicans*	ATCC® 10231™
	Aspergillus brasiliensis (formerly *A. niger*)	ATCC® 16404™
	Penicillium chrysogenum (also known as *P. notatum*)	ATCC® 10106™

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Mycoplasma testing

USP Chapters <63> and <77>

Application During Bioproduction	Reference Positive Controls	
	Live Cultures	Nucleic Acid Standards (gDNA)
Human vaccines & cell culture	*M. orale* (ATCC® 23714™)	*M. orale* CH 19299 (ATCC® 23714D™)
	M. hyorhinis (ATCC® 17981™)	*M. hyorhinis* BTS-7 (ATCC® 17981D™)
	M. fermentans (ATCC® 19989™)	*M. fermentans* PG18 (ATCC® 19989™)
	M. pneumoniae (ATCC® 15531™)	*M. pneumoniae* FH (ATCC® 15531D™)
	A. laidlawii (ATCC® 23206™)	*A. laidlawii* PG8 (ATCC® 23206D™)
Bovine materials	*M. arginini* (ATCC® 25528™)	*M. arginini* G230 (ATCC® 23838D™)
	A. laidlawii (ATCC® 23206™)	*A. Laidlawii* PG8 (ATCC® 23206D™)
Avian materials	*M. synoviae* (ATCC® 25204™)	*M. Synoviae* WVU 1853 (ATCC® qCRM-25204D™)
	M. gallisepticum (ATCC® 19610™)	*M. gallisepticum* PG 31 (ATCC® 19610™)
Porcine materials	*M. hyorhinis* (ATCC® 17981™)	*M. hyorhinis* BTS-7 (ATCC® 17981D™)
Insect and plant cell lines	*Spiroplasma citri* (ATCC® 27556™)	*S. citri* Morocco-R8-A2 (ATCC® 27556D™)

- Live cultures are released with the CFU titer in their CoA
- Nucleic acids are released with the genome copy number information in their CoA

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Summary

- Quality control testing is critical for establishing reliable process development and full-scale manufacturing for safe and effective vaccines and biologics.
- The development and sustainability of a robust QC pipeline for bioproduction modalities requires reliable standards and analytical reference materials, e.g., authenticated live microorganisms and highly purified viruses, host cell DNA, host cell proteins, and endotoxin.
- Fast-paced CGT protocols and their short shelf-life products necessitate rapid microbiological QC tests to ensure timely delivery of safe and effective treatments.
- The collaboration between ATCC and USP is an alliance between trusted leaders to deliver comprehensive quality assessment tools and solutions that increase confidence in the quality of medicine—including biologics—and reduce risk from development through delivery.

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Questions?