Host Cell Protein Analytics in Viral Vector Manufacturing for Cell & Gene Therapies

Alla Zilberman, Ph.D.

Vice President, Technical Marketing

CYGNUS TECHNOLOGIES

part of Maravai LifeSciences

Cygnus Technologies Introduction

Unmatched Product and Service Portfolio

- Generic HCP ELISA kits for **24 expression platforms**
- Custom services for orthogonal assay characterization
- Custom HCP kits for late stage and marketed products
- Generic ELISA kits for purification leachates and cell culture media additives (Protein A, BSA, Insulin, etc)

Decades of Experience

- Pioneer in HCP Analytics with **over 25 years** as the market leader
- Deep understanding of all areas of process-related impurity testing: purification development, assay qualification, orthogonal testing, regulatory expectations

Credibility

- Thought leaders within HCP Analytics market
- Known as **"Gold Standard"** globally
- Develop and advise best practices to clients and regulatory bodies

Trusted by Industry and Regulators

- Established relationships with customers and regulators
- **Over 600 AAE/2D-PAGE and AAE-MS** Ab coverage projects
- **Over 200 process-specific HCP assay** supporting late stage and marketed biologics
- **Cygnus kits are filed in 29 of 29 FDA and EMA approved CAR-T cell and Gene Therapy products**

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Examples of Approved Cell and Gene Therapies Supported by Cygnus HCP Kits

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Regulatory Environment

What did we learn from mAb bioprocess?

- HCP Impurities are a real safety concern [immunogenicity, DS stability]
- Updated USP and European guideline are more harmonized for mAb and rProteins (<100 pm with a trend to <10 ppm for mAbs)
- Regulators are aware of limitations of older orthogonal methods for HCP assay “fit-for-purpose” characterization
- HCP assessment is not a “Check the box ” exercise

FDA Guidance for Industry: CMC Information for Human Gene Therapy IND Applications (Jan 2020) require testing for process impurities such as HCP, HC + “production system” DNA, and other process-related impurities (BSA, HSA, Benzonase/Endonuclease, AAV affinity resins leachates)

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Host Cell Protein, Host Cell DNA and Other Process Impurity ELISA Kits for CGTP

Gene Therapy

- HEK 293 [ELISA, Gyrolab®, Bio-Techne Ella™]
- HeLa
- PER.C6®
- Sf9
- BHK
- Vero
- *E. coli*
- CAP®
- MRC5
- Human, Vero, *E. coli* AccuRes™ Quantitative DNA Kits

Cell Therapy [genetically modified]

- HEK 293
- PG13
- Human AccuRes™ Quantitative DNA Kit

Growth Media Additives

- BSA
- HSA
- Bovine Transferrin
- Human Transferrin

Processing Enzymes & Purification Leachates

- EndonucleaseGTP®
- rTrypsin ELISA Kit
- Avitide AVIPure® • AAV2 • AAV8 • AAV9 Residual Ligand Assays
- IsoTag™ AAV Residual Reagent
- IsoTag™ LV Residual Reagent

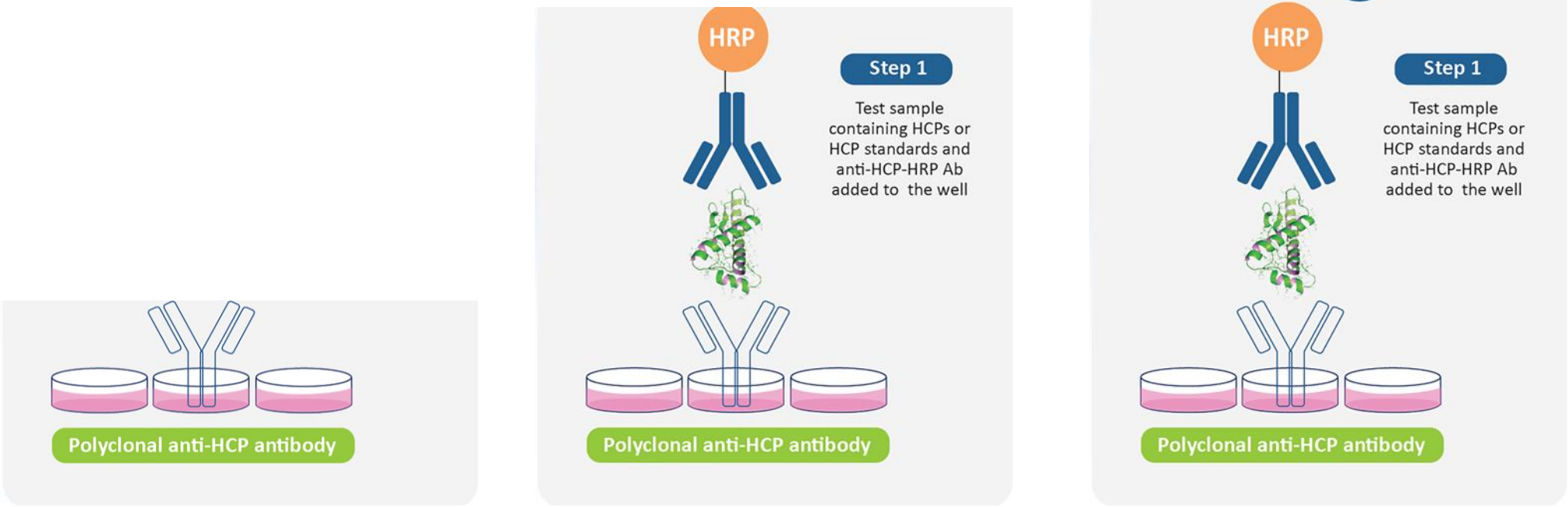
CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

HCP Immunoassays and HCP Analysis by LC-MS

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

HCP ELISA – The Gold Standard for Process Monitoring and Product Lot Release

- LOD: ~0.35 ng/ml
- LLOQ: ~2 ng/ml
- Also available on Gyrolab® and ELLA PS Platforms



Typical Cygnus HCP ELISA – Simultaneous Protocol

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

HCP Analysis by ELISA

ELISA is a gold standard for process monitoring and product lot release

- Semi-quantitative measurement of a statistical sample of HCPs in a sample
- Excellent sensitivity and selectivity
- Does not require special expertise to run
- Effective in relatively high levels of product protein
- Easily transferable, reliable, robust

+

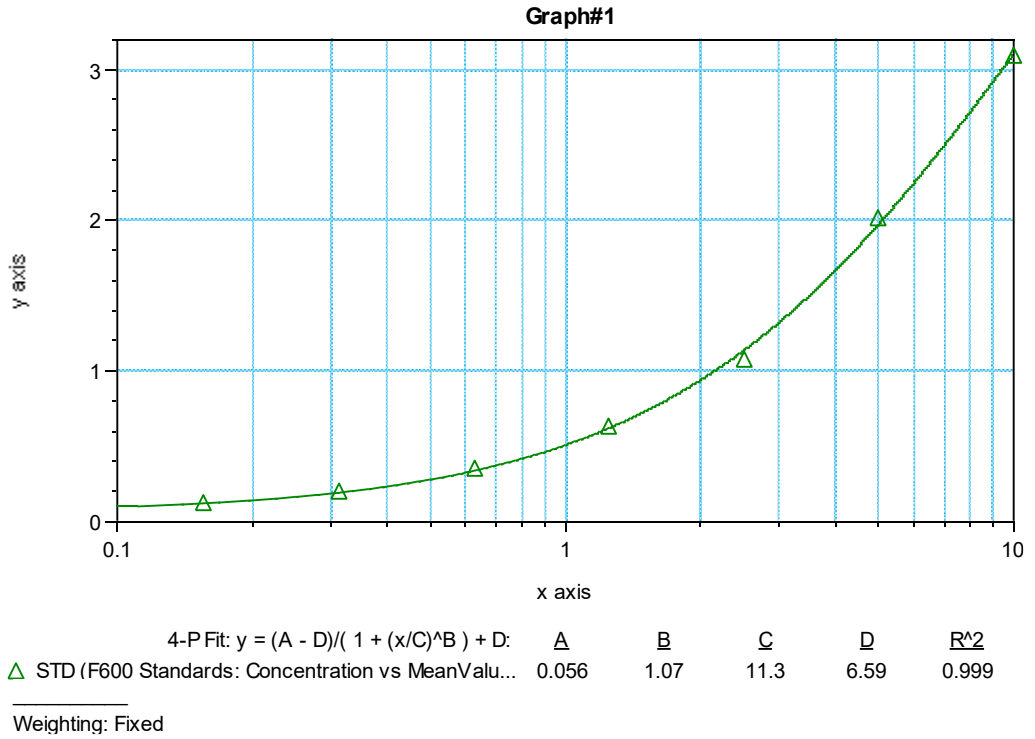
- Reports total HCP equivalents (not ng/mL)
- Results subject to change with change in kit/reagents
- Can take 12-months to generate new reagents

-

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Assay Qualification

- Dilution Linearity
- Accuracy
- Precision
- Range: LOD/LLOQ/ULOQ
- Coverage & Characterization by AAE



- ICH Q11 “Development and Manufacture of Drug Substances (Chemical Entities Biotechnological/Biological Entities)”, 2012
- ICH Q6B “Test Procedures and Acceptance Criteria for Biotechnological/Biological Products,” 1999
- USP Chapter 1132 “Residual Host Cell Protein Measurement in Biopharmaceuticals,” 2016
- USP Chapter 1132.1 ““Residual Host Cell Protein Measurement in Biopharmaceuticals by Mass Spectrometry”, 2025
- European Pharmacopoeia 2.6.34

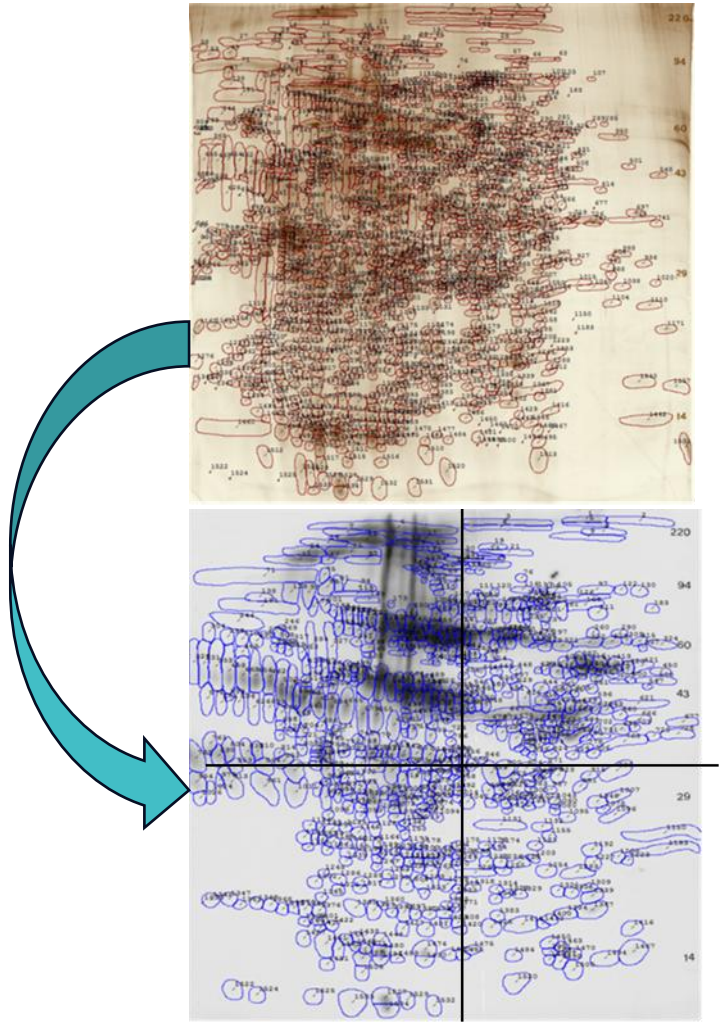
CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

A Trip Down Memory Lane (2D Western Blot, an outdated method)

Coverage Analysis by 2D Western Blot

Performed to demonstrate that the Ab used in the ELISA are broadly reactive to the HCPs in the expression platform

- Conventional Method: 2D Western blot compared to Silver Stain
- Specifications:
  - Reactive with the majority of spots found on the silver stain gel
  - Must have spots in each quadrant of the gel where spots are found on the silver stain

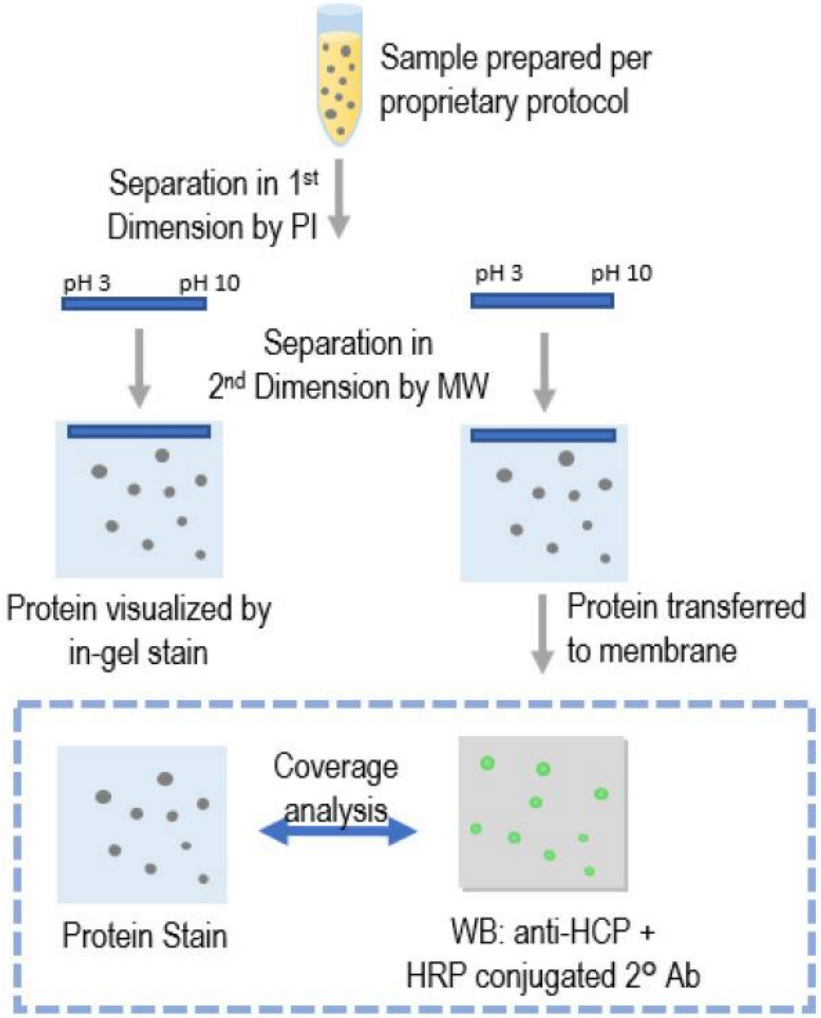


CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

A Trip Down Memory Lane (2D Western Blot, an outdated method)

Limitations of 2D Western Blot

- Sample preparation
- Running the gels
- Transfer to a membrane
- Fixing the gels
- Immunological challenges
- Non-specificity



CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Antibody Affinity Extraction (AAE™)

- Overview of the technology
- Advantages over previous methods

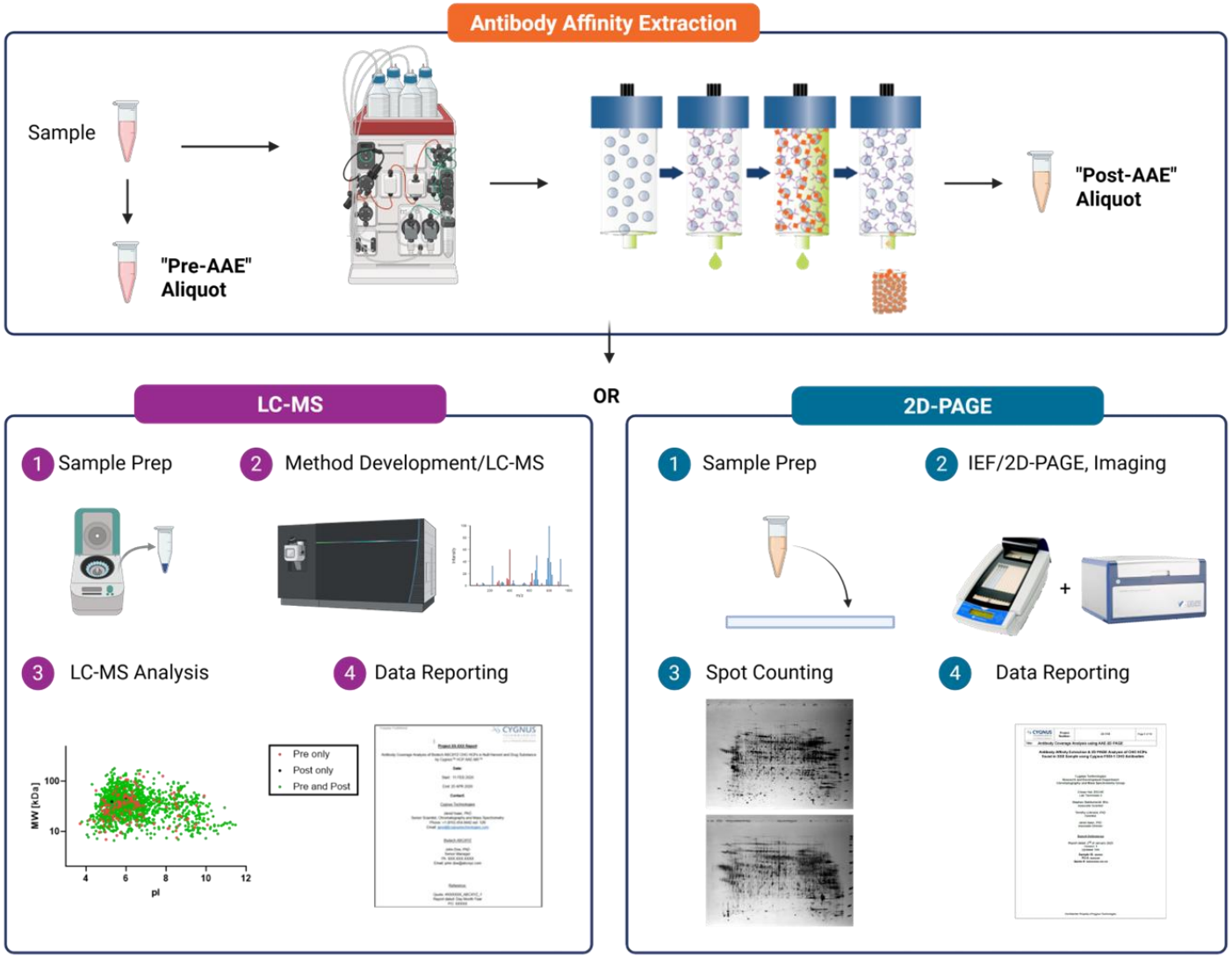
Mass Spectrometry

- Identification of HCP in mock/null harvest for coverage
- Identification of HCP in product expressing harvest for coverage
- Identification of HCP in drug substance

Antibody Affinity Extraction (AAE™)

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Antibody Affinity Extraction (AAE™)



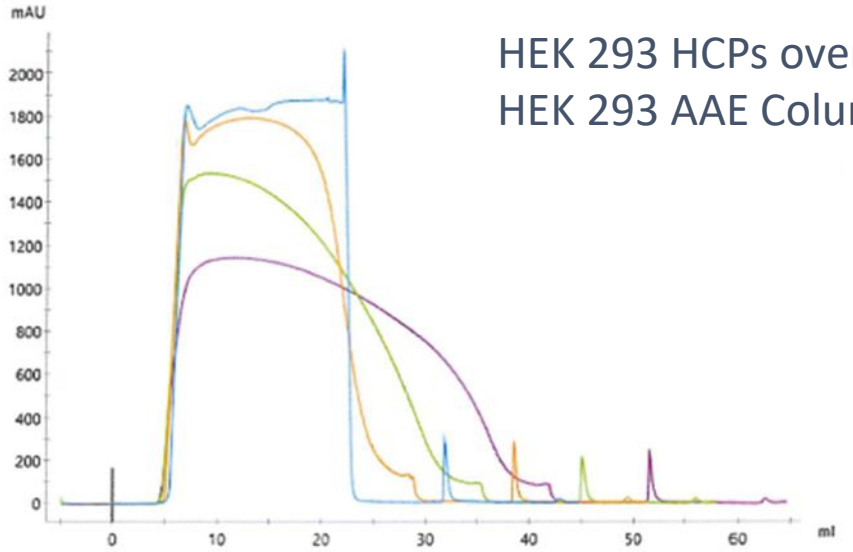
CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

AAE™ Method Specificity Assessment

Overcoming the Limitations of 2D Western Blot

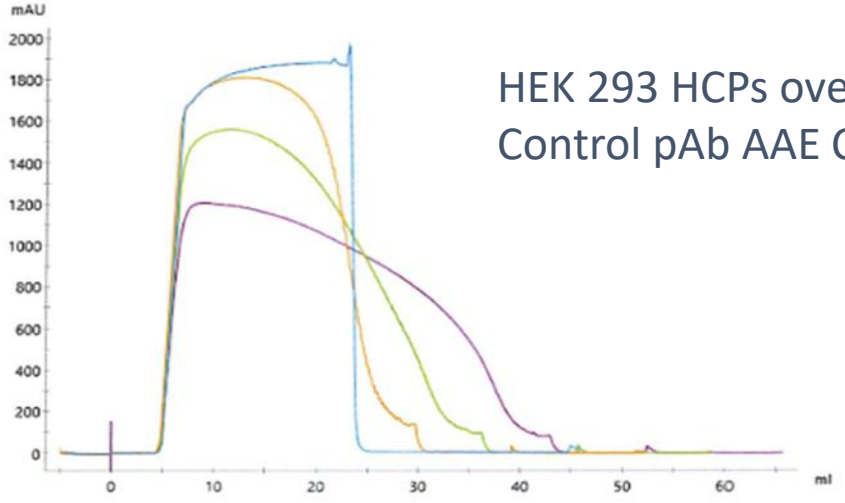
- ~~Sample Preparation~~
- Running the gels
- ~~Transfer to a Membrane~~
- Fixing the gels
- ~~Immunological challenges~~
- ~~Non-specificity~~

A



HEK 293 HCPs over
HEK 293 AAE Column

B



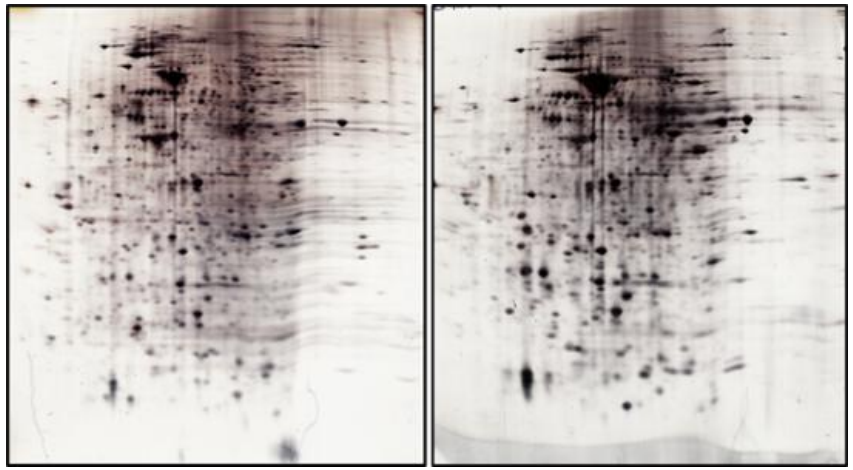
HEK 293 HCPs over
Control pAb AAE Column

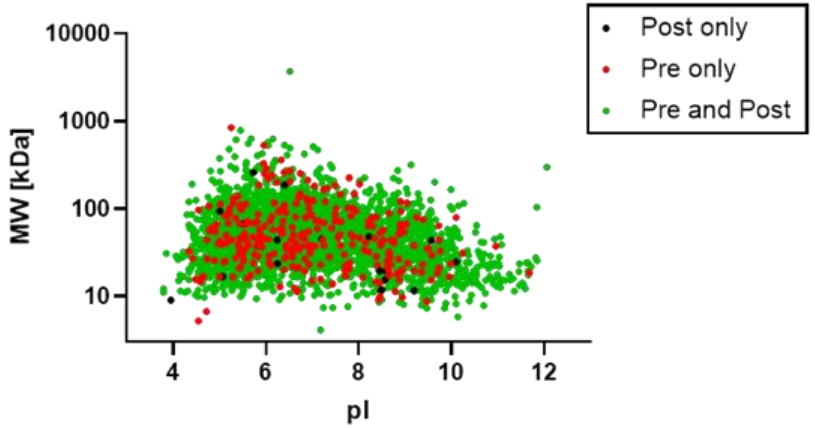
CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

HEK 293 HCP Antibody (F650S) Coverage Assessment

Silver Stain of “Pre-AAE” HEK 293 Antigen Sample

Silver Stain of “Post-AAE” HEK 293 Antigen Sample





Detection Method	PRE-AAE (all HCPs)	POST-AAE (immunoreactive HCPs)	% pAb Coverage
2D-PAGE	1687	1604	95
MS	3075	2812	91

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Individual HCP pAb Reactivity by MS

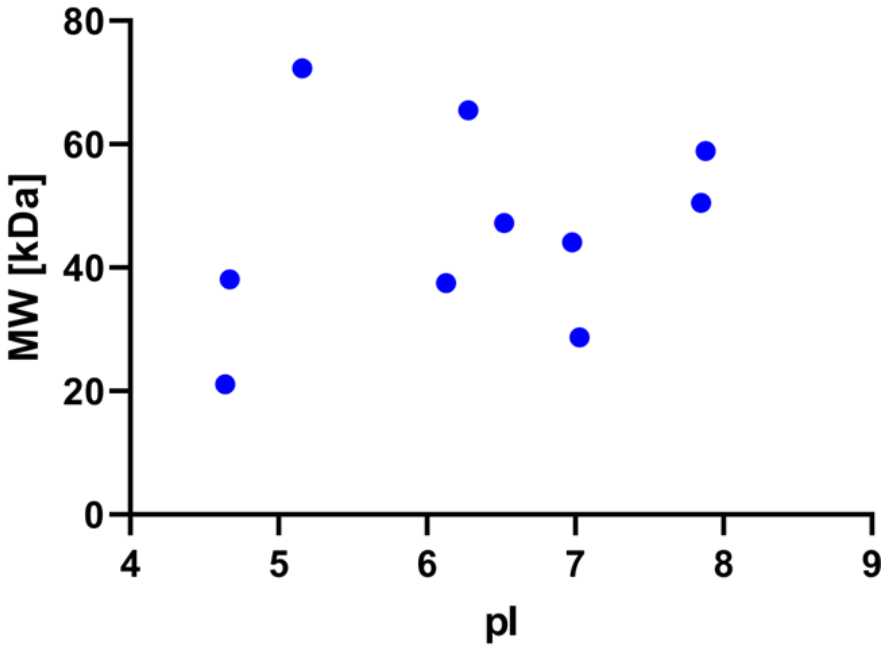
Potential High-Risk HCPs	PRE	F650S	F650S R	pI	MW
78 kDa glucose regulated protein (BiP)	Y	Y	Y	5.07	72332.96
Actin	Y	Y	Y	5.29	41736.73
Aldose reductase related protein 2 (Aldo-keto reductase)	Y	Y	Y	7.66	36019.6
Alpha enolase	Y	Y	Y	7.01	47169.0
Carboxypeptidase D	Y	Y	Y	5.68	152931.1
Cathepsin B	N	N	N	5.88	37821.6
Cathepsin D	Y	Y	Y	6.1	44552.2
Cathepsin E	N	N	N	4.69	42793.5
Chondroitin sulfate proteoglycan 4	N	N	N	5.27	250536.8
Clusterin	N	N	N	5.88	52494.6
Cofilin 1	Y	Y	Y	8.22	18502.5
Elongation factor 1a1	Y	Y	Y	9.1	50140.9
Elongation factor 2	Y	Y	Y	6.41	95338.1
Flagellin (Salmonella paratyphi A (strain ATCC 9150 / SARB42)	N	N	N	5.03	51950.2
Galectin 3 binding protein	Y	Y	Y	5.12	65331.0
Glutathione S transferase P	Y	Y	Y	5.43	23355.8
Glyceraldehyde 3 phosphate dehydrogenase	Y	Y	Y	8.57	36053.2
G-protein coupled receptor 56	N	N	N	8.79	57473.2
Heat shock cognate 71 kDa protein	Y	Y	Y	5.37	70898.1
Heat shock protein HSP 90	Y	Y	Y	4.94	84659.7
Lipoprotein Lipase	N	N	N	8.37	53162.5
Lysosomal protective protein	N	N	N	6.16	54466.1
Matrix Metalloproteinase 19	N	N	N	7.22	57357.0
Metalloproteinase inhibitor 1	N	N	N	8.46	23170.9
Monocyte chemoattractant protein 1 (C-C motif chemokine 2)	N	N	N	9.4	11025.0
Nidogen-1	N	N	N	5.12	136377.0
Peptidyl-prolyl cis-trans isomerase	Y	Y	Y	8.27	19257.2
Peroxiredoxin 1	Y	Y	Y	8.27	22110.4
Phosphoglycerate kinase 1	Y	Y	Y	8.3	44614.7
Phospholipase A2 (Group XV lysosomal)	Y	Y	Y	6.26	46657.8
Phospholipase B like 2	Y	Y	Y	6.34	65471.8
Procollagen C endopeptidase enhancer 1	N	N	N	7.41	47972.5
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	Y	Y	Y	6.46	83550.19
Procollagen-lysine (Procollagen-lysine 5-dioxygenase)	Y	Y	Y	6.54	83504.2
Protein disulfide isomerase	Y	Y	Y	4.76	57116.37
Pyruvate kinase	Y	Y	Y	7.96	57936.9
Serine protease HTRA1	N	N	N	8.09	51287.0
SPARC (Osteonectin)	N	N	N	4.73	34632.2
Sulfated glycoprotein 1 (Prosaposin)	Y	Y	Y	5.06	58112.6
T-complex protein 1 subunit alpha	Y	Y	Y	5.8	60343.6
Thioredoxin 1	Y	Y	Y	4.82	11737.5

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

HCP Identification in DS

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

HCPs and their potential mechanisms



Jones, M. et al *Biotechnol. Bioeng*. 2021
Li, X. et al *Biotechnol. Prog.* 2021
Li, X. et al *bioRxiv*. 2020
Wang, F. et al Bioprocess Intl. 2018
Vanderlaan, M. et al *Biotechnol. Prog.* 2018
Durocher, V. et al. *J. of Biotech*. 2017
Valente, K. et al *Biotechnol. Prog.* 2015
Levy, N. et al. *Biotechnol Bioeng.* 2014

Drug Degradation

Serine Protease HTRA1
Cathepsins
Matrix Metalloproteinase

Immunogenicity

Phospholipase B domain containing 2

PS20 PS80 Degradation

Lipoprotein lipase
Phospholipase A2 Group XV
Phospholipase A2 Group VII
Phospholipase A1

Drug Aggregation

Protein Disulfide Isomerase

Endoplasmic reticulum chaperone BiP
Heat shock proteins

Drug Binding

Clusterin

N-Glycan Degradation

Hexaminidase B (HEXB)

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

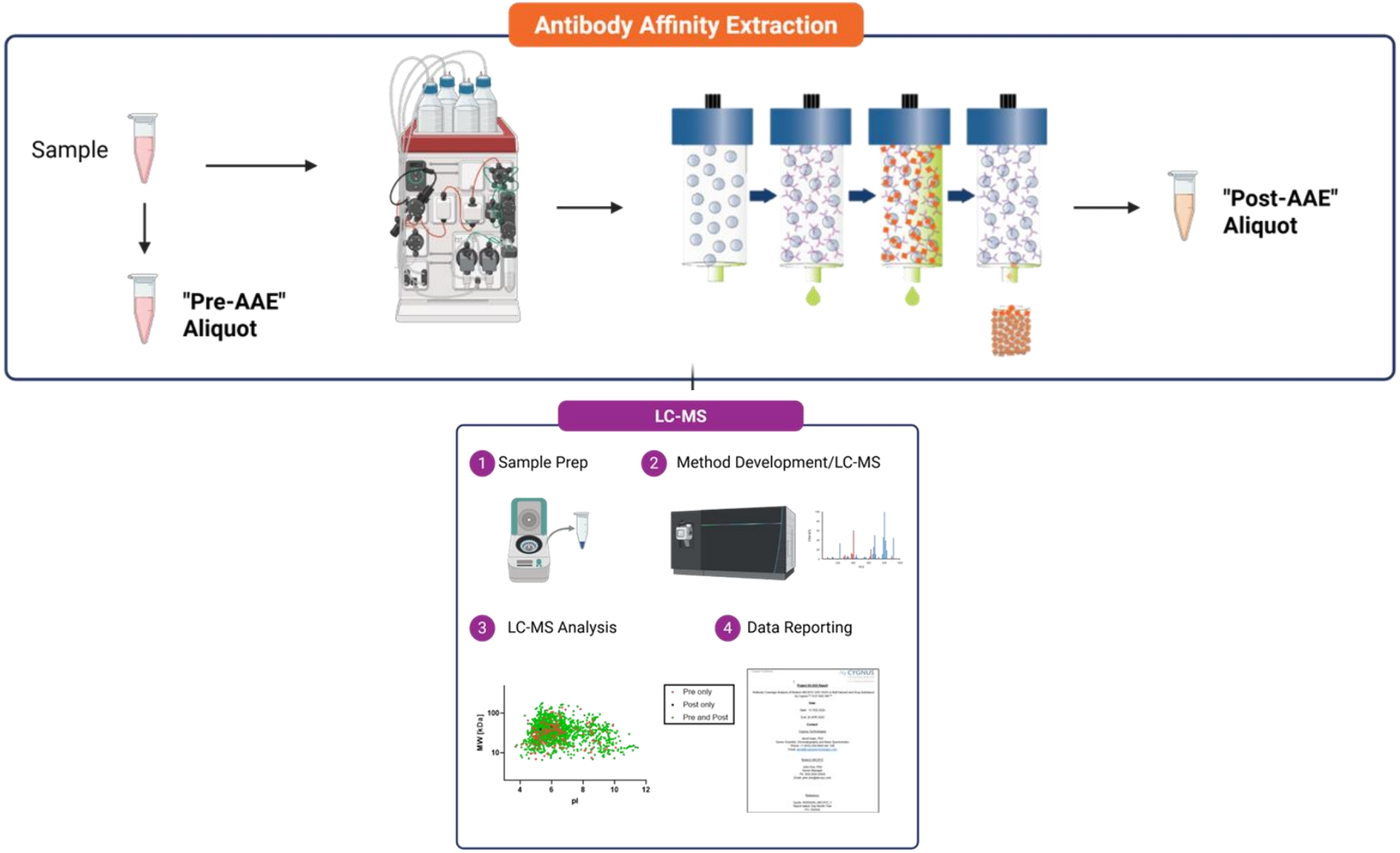
Importance of LC-MS/MS-based HCP Profiling

Host Cell Protein Clinical Safety Risk Assessment—An Updated Industry Review (Biophorum Consortium Publication; *Biotechnology and Bioengineering, Jul 2025*)

*"It is recommended to perform HCP profiling by mass spectrometry **as early as possible** to guide process optimization at each stage of clinical development leading toward commercialization to reduce "high-risk" HCPs to levels that ensure patient safety."*

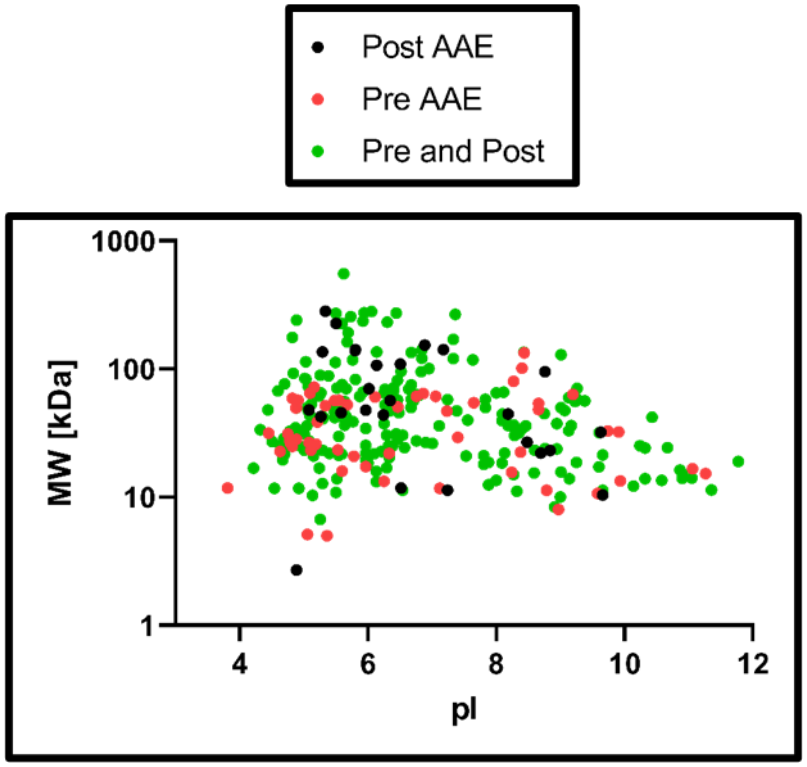
CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Antibody Affinity Extraction (AAE™)



CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Individual HCP pAb Reactivity to HCPs in the Final DS by MS



F650S: 87%

- 251 Pre AAE spots
- 218 Post AAE spots

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

USP Chapter 1132.1
“Residual Host Cell Protein Measurement in Biopharmaceuticals by Mass Spectrometry”

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

USP Chapter 1132.1 - Establishes Common Industry Terminology and Methodology

- Acknowledges lack of standardized methods (a Wild-West type situation)
- Provides an overview of the capability of MS methods for HCP identification and quantitation as well as their limitations.
- Discusses best practices for instrument selection, sample preparation, LC separation, MS data acquisition, MS data analysis and reporting.
- Emphasizes that MS and HCP-ELISA are different methods, and thus MS results may differ from those obtained by ELISA.
- Makes the LC-MS analysis comparable between different labs
- Expands our HCP analysis toolbox

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

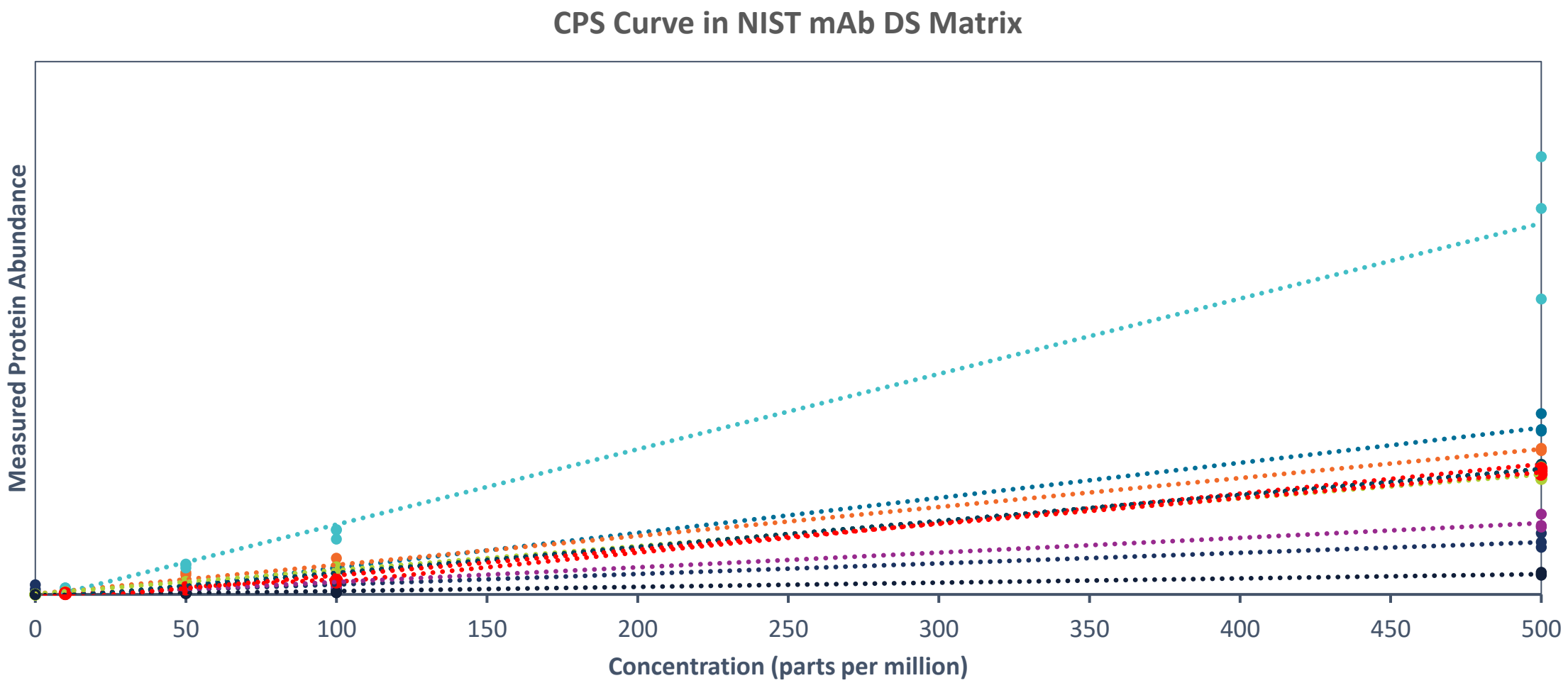
Analytical Methods in USP Chapter 1132.1

	Product Protein	Spiked-in Proteins	Spiked-in Peptides
Sensitivity	Poor	Moderate	High
Quantitative Range	Unclear	2-4 orders of magnitude	1-2 orders of magnitude
Precision	High (CV < 20%)	High (CV < 20%)	High (CV < 20%)
Accuracy	Poor	Acceptable (Order of magnitude)	Great (within 2-fold)
Ease of use	Simple; no additional reagents	Moderate; no complex method development	Difficult; thorough method development required

Global Relative Quantification of HCP by inSPECt™ MS with the Cygnus Protein Standard (CPS)

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Global Relative Quantification Based Upon the Median Response Factor of CPS



CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

inSPECt™ MS Quantification of HCP in NIST mAb is Comparable to Prior Reports

HCP	Beaumal *et al.* 2023	inSPECt™ MS
Fructose-biphosphate aldolase A	189 ppm	240 ppm
Fructose-biphosphate aldolase C	84 ppm	83 ppm
Protein-disulfide isomerase A6	83 ppm	148 ppm
Glucose-6-phosphate isomerase	32 ppm	87 ppm

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

inSPECt™ MS: Spike and Recovery for Accuracy and Precision

HCP	100 ppm		500 ppm		1000 ppm	
	%Error	%CV	%Error	%CV	%Error	%CV
Lysosomal phospholipase A and acyltransferase	26%	2%	32%	2%	34%	4%
Clusterin	6%	3%	5%	3%	11%	6%
Glutathione S-transferase P 1	23%	5%	27%	2%	31%	7%

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Cygnus Technologies Services: End-to-end, comprehensive MS solutions for HCP assessment in your bioprocess

Coverage Analysis by AAE-MS™

- Highly predictive of HCP antibody performance in HCP ELISA
- Identifies unique proteins, not spots
- The only reliable choice for coverage of HCPs in DS-containing harvest samples

Identification of immunoreactive HCPs in IPC and final DS

- AAE-MS identifies immunoreactive HCPs in the final DS
- Identifies HCPs thar persist through DSP and may contribute to loss of dilution linearity
- Enables targeted downstream process optimization

inSPECt™ MS for HCP Quantification

- DIA-based HCP quantification relative to Cygnus optimized protein standard
- Resolves and quantifies thousands of HCPs with exceptional accuracy and precision

C6XL for absolute HCP quantification

- Absolute quantification of six CHO lipases using targeted proteomic approach and SIL peptide standards (CHO 6xLipase™ MS Assay)
- Additional assays in development

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

Acknowledgments

Eric Bishop, MS

Jared Isaac, PhD

Tim Licknack, PhD

Stephen Stahlschmidt, BS

Johanna Barnhill, MS

Brinkley Thornton, MS

Thank You

Driving your success at every stage

CYGNUS TECHNOLOGIES

part of Maravai LifeSciences