Evaluation of Host cell proteins in Lenti Viral Vectors

Shilpa Suravajhala, Scientist - bluebird bio

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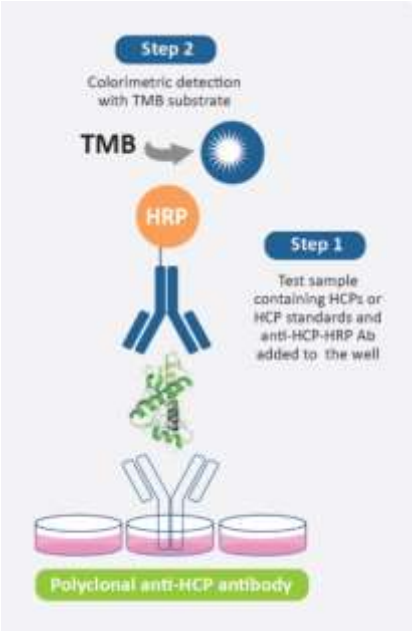
Host cell proteins in Lenti Viral Vectors

- Host cell proteins (HCPs) are proteins produced/encoded by host organisms during the production of recombinant therapeutic proteins.
- HCPs in LVV are process related impurities generated during the manufacturing process from the transformed human embryonic kidney cells (HEK293T).
- Since residual HCPs have the potential to affect product quality, safety, and efficacy understanding the clearance of HCPs during the various stages of the product purification process is crucial.

- <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals
  - Immuno Assays
  - Electrophoretic Methods
  - Western Blot Methods
  - Chromatographic and Proteomic Methods

Evaluation of Host cell proteins – Gold standard method

- Sandwich ELISA - most commonly used method for monitoring HCPs
  - Assess the total amount of HCPs
  - Understand/track changes throughout the manufacturing process
    - Benefits: relatively easy to train/run, low cost, high throughput
    - Limitations: dependent on coverage of the Ab, ID of the proteins is not possible



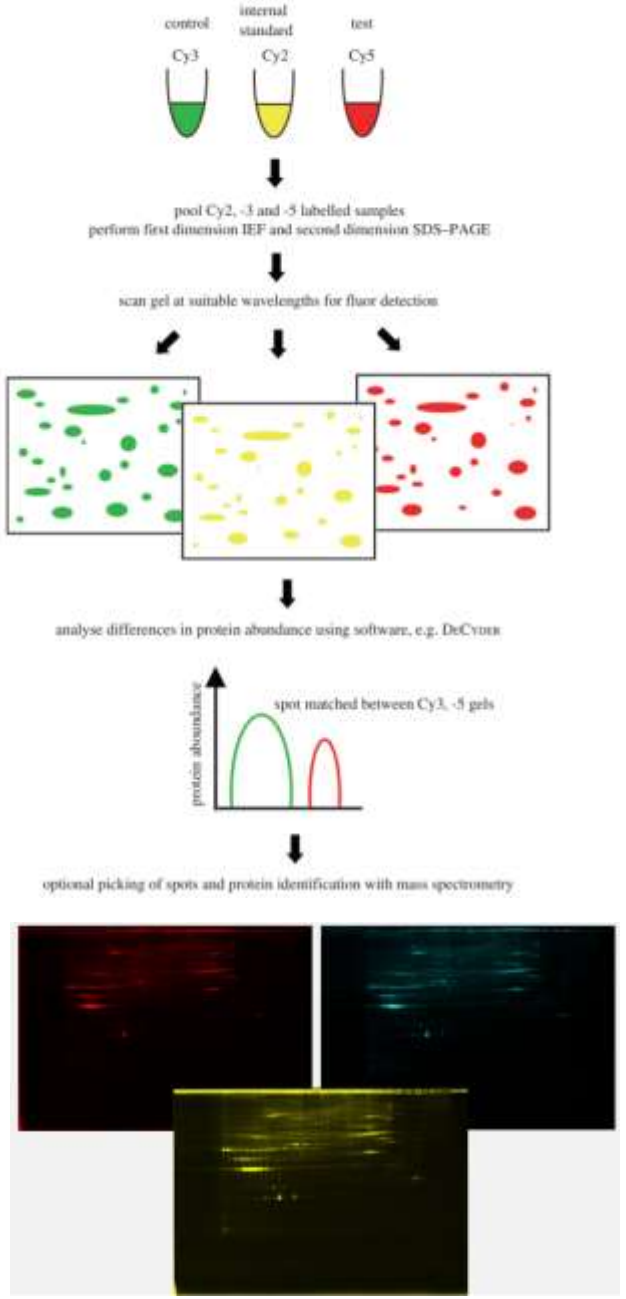
HCP (ng/mL)						
Sample	Cell culture fluid	Nuclease Treatment	Harvested cell culture fluid	Eluate	Intermediate Bulk	Final Product (LVV)
Test Article-1	5,586	23,193	8,101	2,285	58,818	36,874

- Regulatory agencies prefer additional complementary information
  - Information on the antibody coverage
    - Coverage: % of HCP species an anti-HCP antibody detects out of the total HCP species present in a sample
  - Clearance study using orthogonal technologies
    - Surrogate host cell proteins can be spiked during DP Manufacturing to monitor clearance throughout the process

Figure: https://www.cygnustechnologies.com/hcp-immunoassays

Evaluation of Host cell proteins - Orthogonal Techniques

- 2-D fluorescence differential gel electrophoresis (2D DIGE)
  - isoelectric focusing (IEF) to separate proteins according to their isoelectric point (pI) followed by SDS-PAGE to separate proteins according to molecular weight
  - Applications:
    - Process comparability (aLVV vs sLVV),
    - Clearance: comparing upstream to purified downstream samples
    - Evaluation of antibody coverage
  - Limitations:
    - Laborious, analyst variability during spot identification, low throughput
- LC-MS
  - powerful tool for analysis of HCPs during process development due to its sensitivity, selectivity and adaptability.
  - Useful for evaluation of antibody coverage information.
  - Enrichment of HCP's via immunoaffinity is typically performed
  - Limitations:
    - detection of low abundance proteins
    - Requires subject matter experts (SME's) for analysis
    - Expensive for routine testing (unless the analysis is performed in-house)



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Evaluation of Host cell proteins -Capillary Electrophoresis

- Analysis:
  - Sample, separation matrix, stacking matrix, antibodies and reagents are loaded
  - Voltage is applied to enable separation by molecular weight
  - UV light immobilizes the proteins to the capillary wall
  - Immunoprobed with primary, followed by secondary HRP conjugate and detected by Chemiluminescence

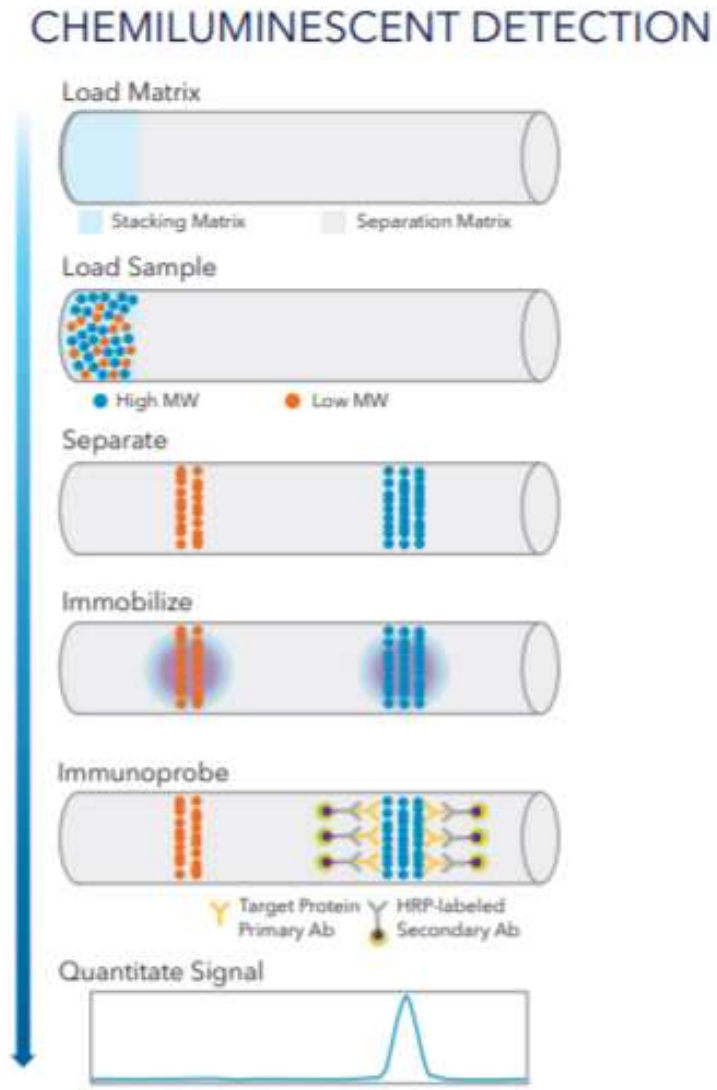
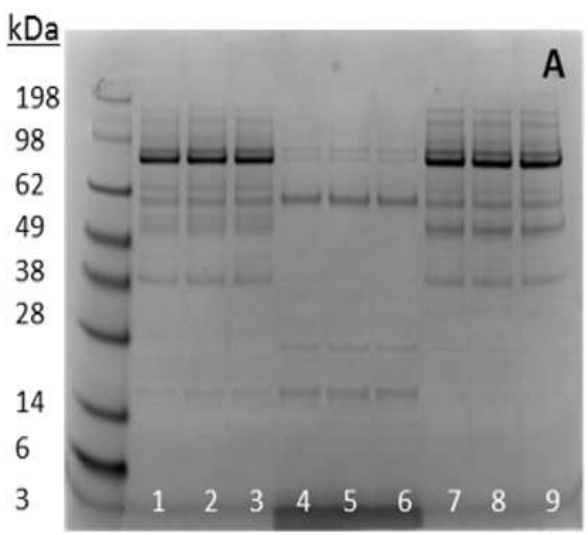


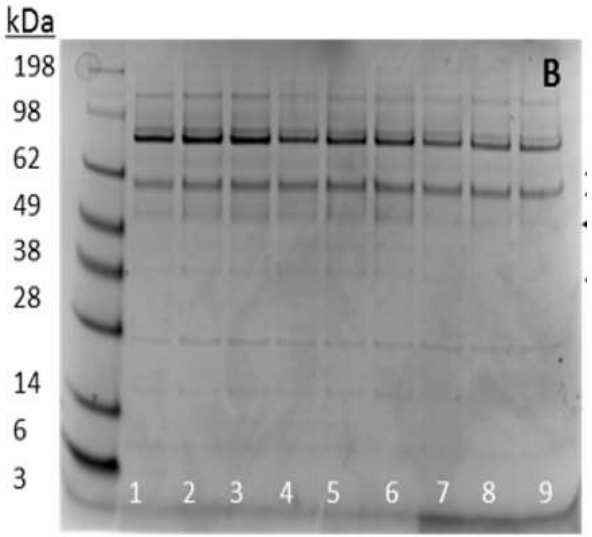
Figure downloaded from :https://www.proteinsimple.com/documents/Jess_Product_Brochure_RevC.pdf

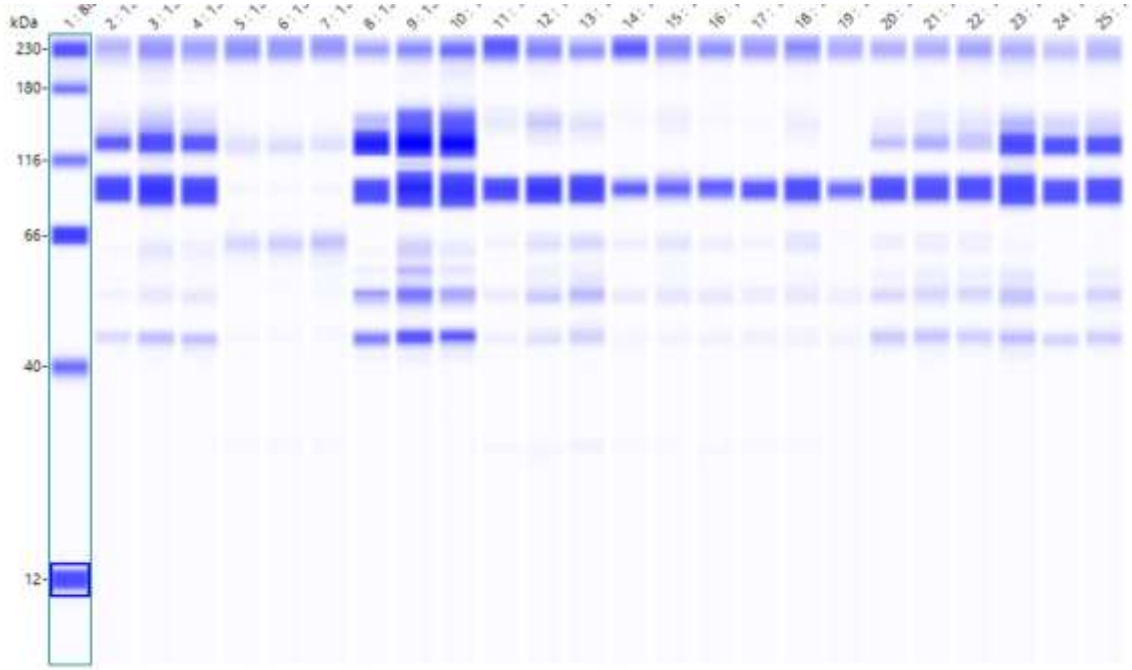
Evaluation of Host cell proteins -Capillary Electrophoresis

Utilizes sub-millimeter glass capillaries and applied electric potential to separate proteins according to size

- Applications:
  - Characterization tool to evaluate
    - Total protein profile, Specific protein profiles
    - Qualitative and Quantitative
    - clearance of proteins

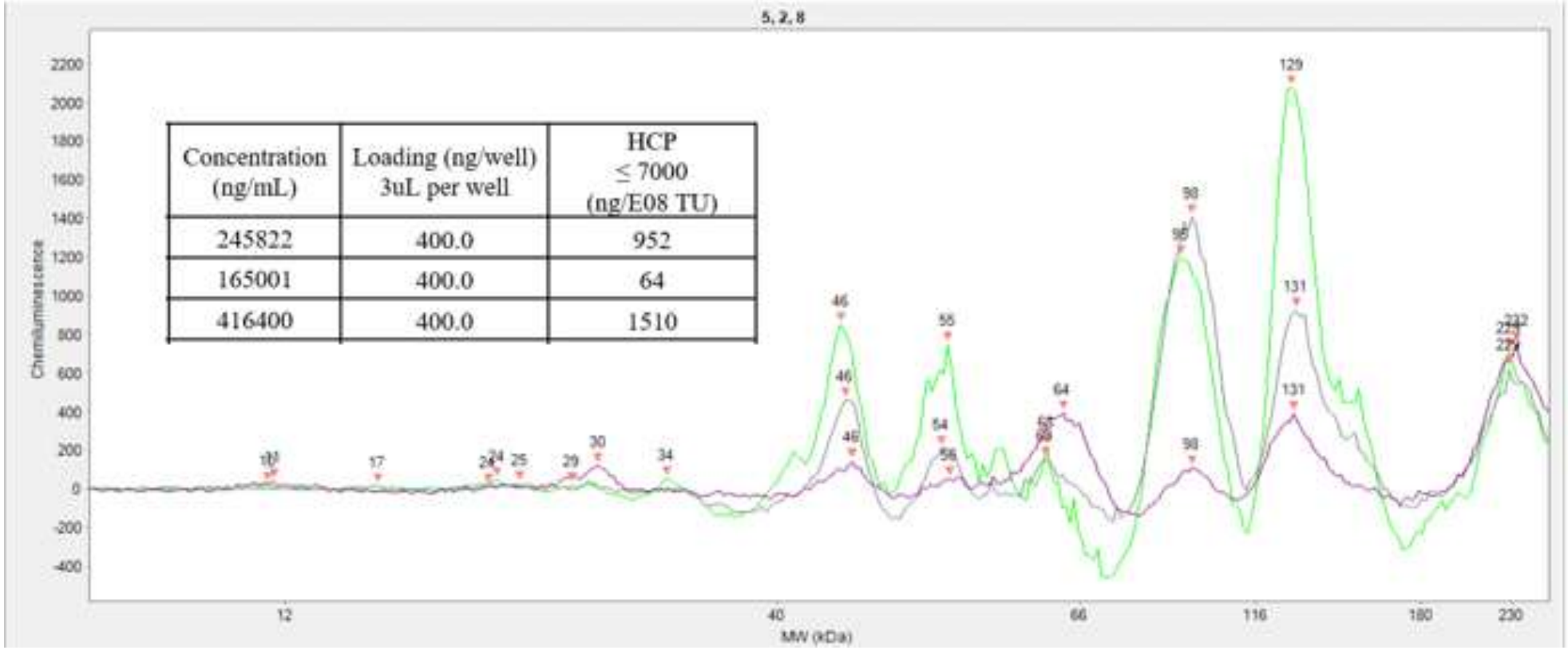






Evaluation of Host cell proteins -Capillary Electrophoresis

Concentration (ng/mL)	Loading (ng/well) 3uL per well	HCP ≤ 7000 (ng/E08 TU)
245822	400.0	952
165001	400.0	64
416400	400.0	1510

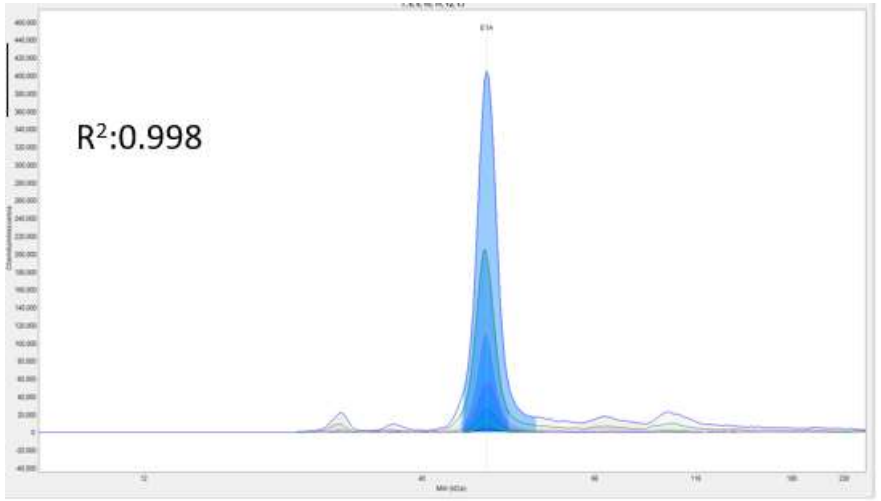


Clearance of E1A

- E1A and SV40 (surrogate protein markers) known to be HEK293T-specific were tracked during spiking experiments in small scale transductions to evaluate clearance across the various wash steps

E1A Tested Range: 1.5625 – 250 ng/mL

E1A Working Range: 1.5625 – 25 ng/mL

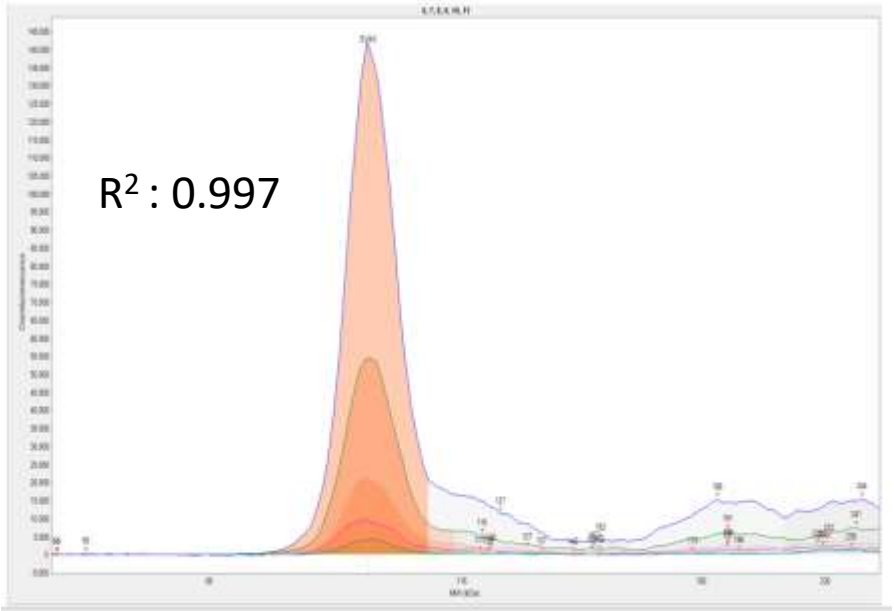


Sample	Measured Concentration (ng/mL)	log Clearance
High Spike Mock Sample	32.20	N/A
High Spike Wash 1	3.553	0.0278
High Spike Wash 2	<LOQ (1.5625)	>0.3846
High Spike Wash 3		
High Spike Wash 4		
Low Spike Mock Sample	1.742	N/A
Low Spike Wash 1	<LOQ (1.5625)	
Low Spike Wash 2		
Low Spike Wash 3		
Low Spike Wash 4		
sLVV No Spike Mock Sample	<LOQ (1.5625)	N/A
sLVV No Spike Wash 1		
sLVV No Spike Wash 2		
sLVV No Spike Wash 3		

Clearance of SV40

Tested range: 9.375 – 1500 ng/mL

SV40 Working Range: 9.375 -150 ng/mL



Sample	Measured Concentration (ng/mL)	log Clearance
High Spike Mock Sample	165.7	N/A
High Spike Wash 1	12.32	0.1994
High Spike Wash 2	<LOQ (9.375)	>0.3178
High Spike Wash 3		
High Spike Wash 4		
Low Spike Mock Sample	46.11	N/A
Low Spike Wash 1	<LOQ (9.375)	
Low Spike Wash 2		
Low Spike Wash 3		
Low Spike Wash 4		
No Spike Mock Sample	16.62	N/A
No Spike Wash 1	<LOQ (9.375)	
No Spike Wash 2		
No Spike Wash 3		

Questions

- Thank you!