Characterization of Process-related Impurity: Host Cell Proteins

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Topics Covered Today:

1. Background Information.
2. Regulatory Perspective.
3. Risk Assessment.
4. Case Studies.
5. When to develop process-specific HCP ELISA?
6. Conclusions

1. Why HCP Analysis ?

- Potential adverse effects including immunogenicity
- Process Development
- Quality Control

Heterogeneity and Impurity Analysis

Product-related Impurities /Substance

- Truncation
- Aggregation
- Deamidation
- Oxidation
- Glycosylation
- Bond mismatch
- Conformational alteration

Process-related Impurities

- Host Cell Proteins
- Residual DNA
- Endotoxin
- Protein-A
- Additives

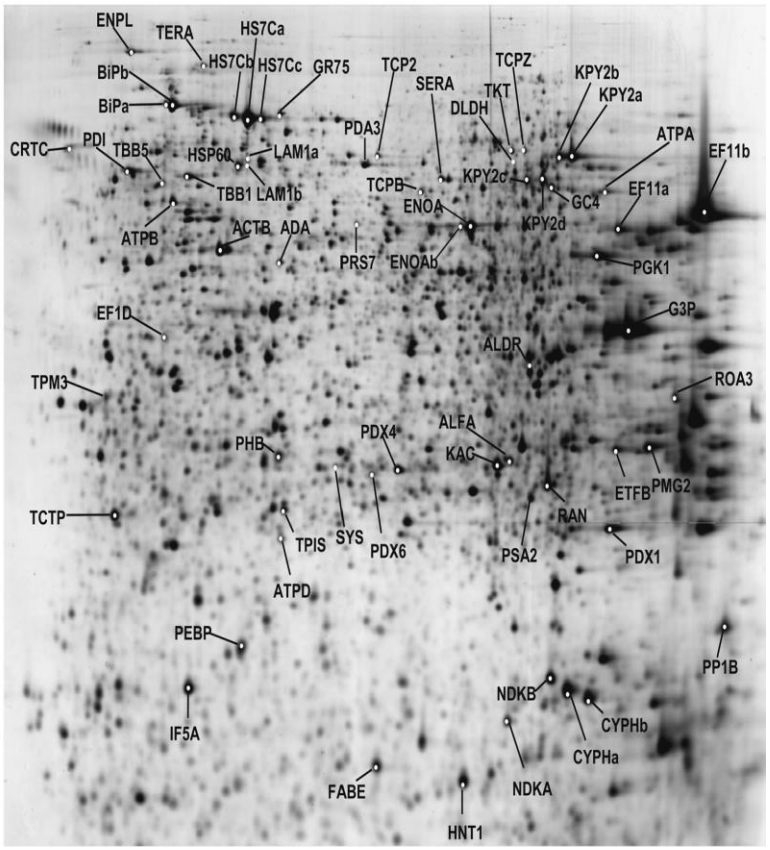
HCPs Defined

- Proteins and their modified forms from the expression system that may co-exist with product
- Content depends on many variables:
  - Type of expression system (E. coli, NS/0, CHO)
  - Genetic strain of system
  - Isolation technology
  - Purification technology
- Classified as Host-derived Process-Related Impurity
- Expressed as ng HCP per mg protein (ppm)

Complexity of HCPs

NS/0

E. coli



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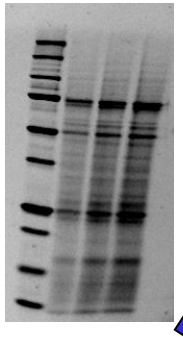
Smales, C.M et al, Biotech. Bioeng. Vol. 66, p474-489, 2004

Common Analytical Methods for HCP analysis

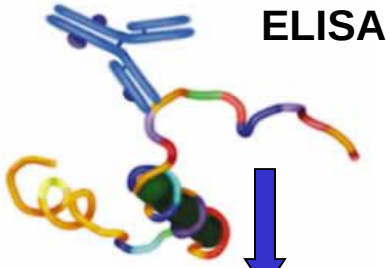
Method	Strengths	Weaknesses
HPLC	High resolution. Quantitative for Single entities	Low sensitivity
SDS-PAGE /Silver stain	Reasonable Sensitivity 2 ng/band (200 ppm) Resolves multiple proteins	Interpretation Subjective Product Interference Qualitative. Complex
Western Blot (1-D/2-D)	Immunological identity Sensitivity 0.1-1 ng/band (10-100 ppm) orthogonal Resolution	Qualitative. Complex May fail to detect some contaminants
Immunoassay	High sensitivity < 1 ng/mL Semiquantitative. Objective endpoint (1 ppm)	No resolution of Individual components. Antibody may fail to detect some contaminants

HCP Analytical Technologies

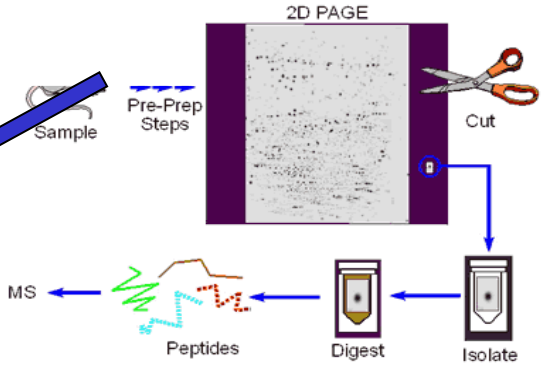
SDS-PAGE

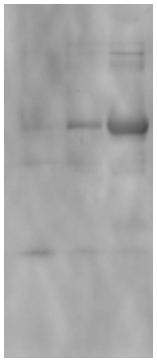


ELISA



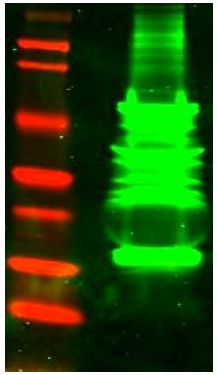
Proteomics



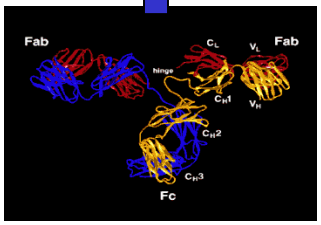


Color-based Western

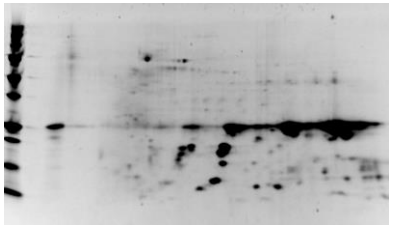
HCPs



Odyssey Infrared Image System



Bioinformatics



CCD-based High Sensitivity Imaging

Potential Issues of Current Sandwich ELISA HCP Analysis

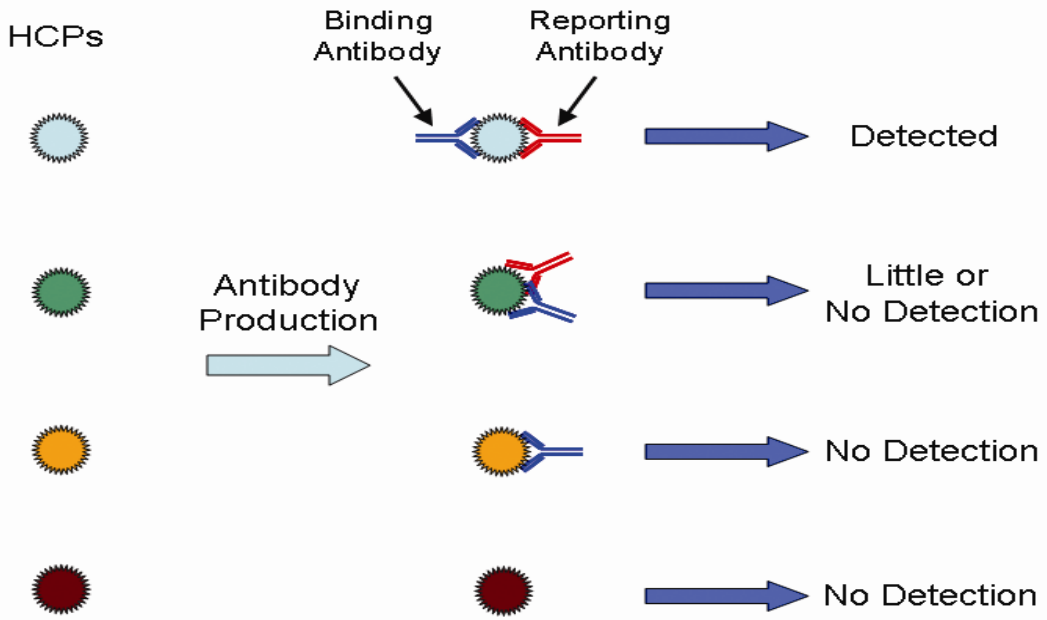
1. Not every HCP will generate good antibodies.

2. For the HCPs that do generate antibodies, not everyone will generate two good antibodies.

3. It is difficult to establish an HCP standard that will match the profile of HCPs in the final product.

4. Change of antibody population over time from different hosts.
5. Custom-specific antibody generation and ELISA method takes about 1 year to develop with estimate cost > $40,000

Current HCP Detection is a Measure of "Protein Immunogenicity"



(Xing Wang, Alan K. Hunter, Ned M. Mozier. Biotech. Bioeng. Vol. 103, p446, 2009)

Reasons to Test

- Patterns and numbers indicative of process performance

- Data required for process development
- Increasing demand as product developed

QC release testing

Comparability

Characterization for cause (e.g. process deviation investigations)

Process validation

- Regulatory Agencies expect that a program is in place and sound scientific basis for approach
- FDA Experience: 1-100 ppm

2. Guidance for Industry Q6B: Specifications

2.3.1

Adequate design of a process and knowledge of its capability are part of the strategy used to develop a manufacturing process that is controlled and reproducible, yielding a drug substance or drug product that meets specifications. In this respect, limits are justified based on critical information gained from the entire process spanning the period from early development through commercial scale production.

4.1.3

Process-related impurities (section II.A.4) 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 by the use of appropriate, well controlled manufacturing processes.

2. Guidance for Industry Q6B: Specifications

6.2.1 Process-related impurities and contaminants

These are derived from the manufacturing process(section 2.1.4) and are classified into three major categories: cell substrate-derived, cell culture derived and downstream-derived.

a) Cell substrate-derived impurities include, but are not limited to, proteins derived from the host organism, nucleic acid (host cell genomic, vector, or total DNA). For host cell proteins, a sensitive assay e.g., immunoassay, capable of detecting a wide range of protein impurities is generally utilized. In the case of an immunoassay, a polyclonal antibody used in the test is generated by immunization with a preparation of a production cell minus the product-coding gene, fusion partners, or other appropriate cell lines. The level of DNA from the host cells can be detected by direct analysis on the product (such as hybridization techniques). Clearance studies, which could include spiking experiments at the laboratory scale, to demonstrate the removal of cell substrate-derived impurities such as nucleic acids and host cell proteins may sometimes be used to eliminate the need for establishing acceptance criteria for these impurities.

3. Risk Assessments for HCPs ?

Current high confident detection (Coomassie Blue) is about 800 ppm in SDS-PAGE
(assuming Detection Limit of 8 ng/protein, and standard loading of 10 µg/ lane).

Assume an injection of 600 mg mAb/person (10 mpk, 60 kg average, then proteins with levels below 500 µg/injection will not be detected consistently (600 x 0.8 µg = 480 µg).

At what minimum level might a protein generate an immunological response?
Mouse: < 1 µg.
Rabbit: < 10 µg.
Human ? < 100 µg ?
(From: *Antibodies. A Laboratory Manual.* pp100. By Ed Harlow & David Lane.)

Proposed Risk Assessment for HCPs

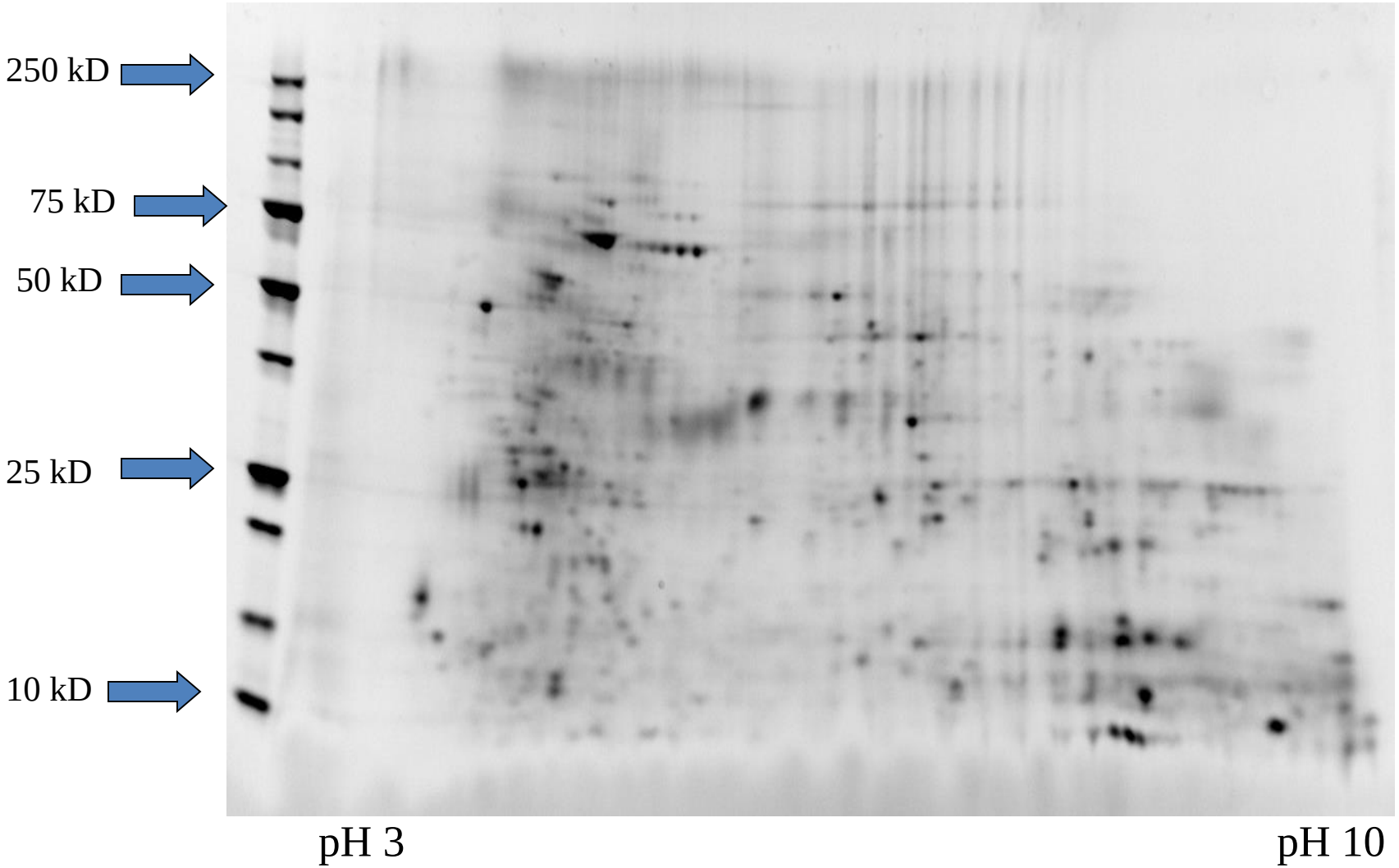
		isolation technique	HCP assay	S	O	D	
Microbial	*E. coli*	homogenate	commercial	3	5	3	45
		periplasm	commercial	3	4	3	36
		homogenate	custom	3	5	2	30
		inclusion bodies	commercial	3	3	3	27
		periplasm	custom	3	4	2	24
		inclusion bodies	custom	3	3	2	18
	Yeast	secreted	commercial	3	2	3	18
		secreted	custom	3	2	2	12
Mammalian	CHO, NS0, etc	secreted (non-mAb)	commercial	3	2	3	18
		secreted (non-mAb)	custom	3	2	2	12
	CHO, NS0, etc	secreted (mAb), +protein A/G	commercial	3	1	3	9
		secreted (mAb), +protein A/G	custom	3	1	2	6

(Xing Wang, Alan K. Hunter, Ned M. Mozier. Biotech. Bioeng. Vol. 103, p446-458, 2009)

4. Case Study

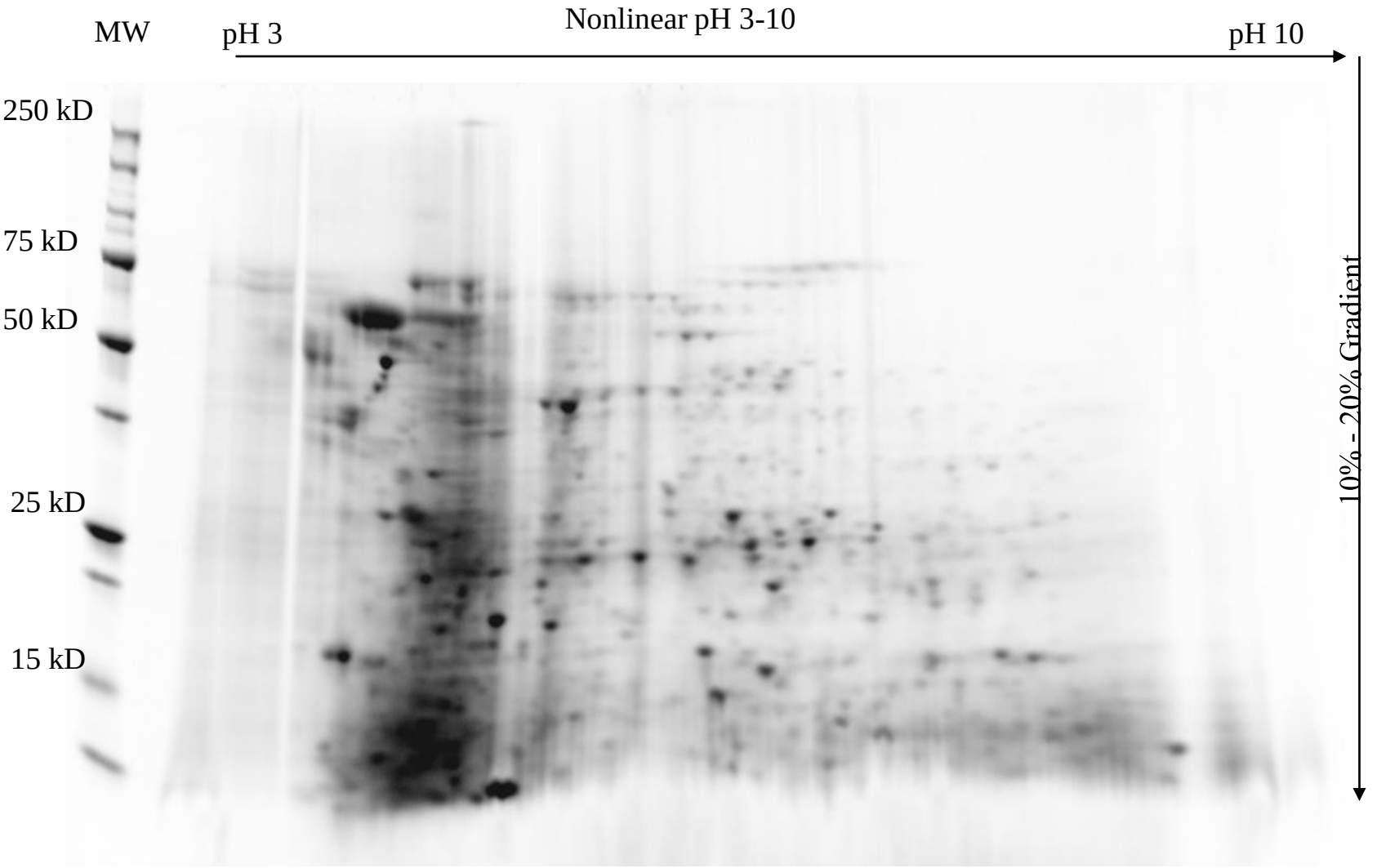
1. Comparison between Commercial HCP Kit and Process-specific Antibodies

2-D Analysis of Process-specific Null Cell Immunogen

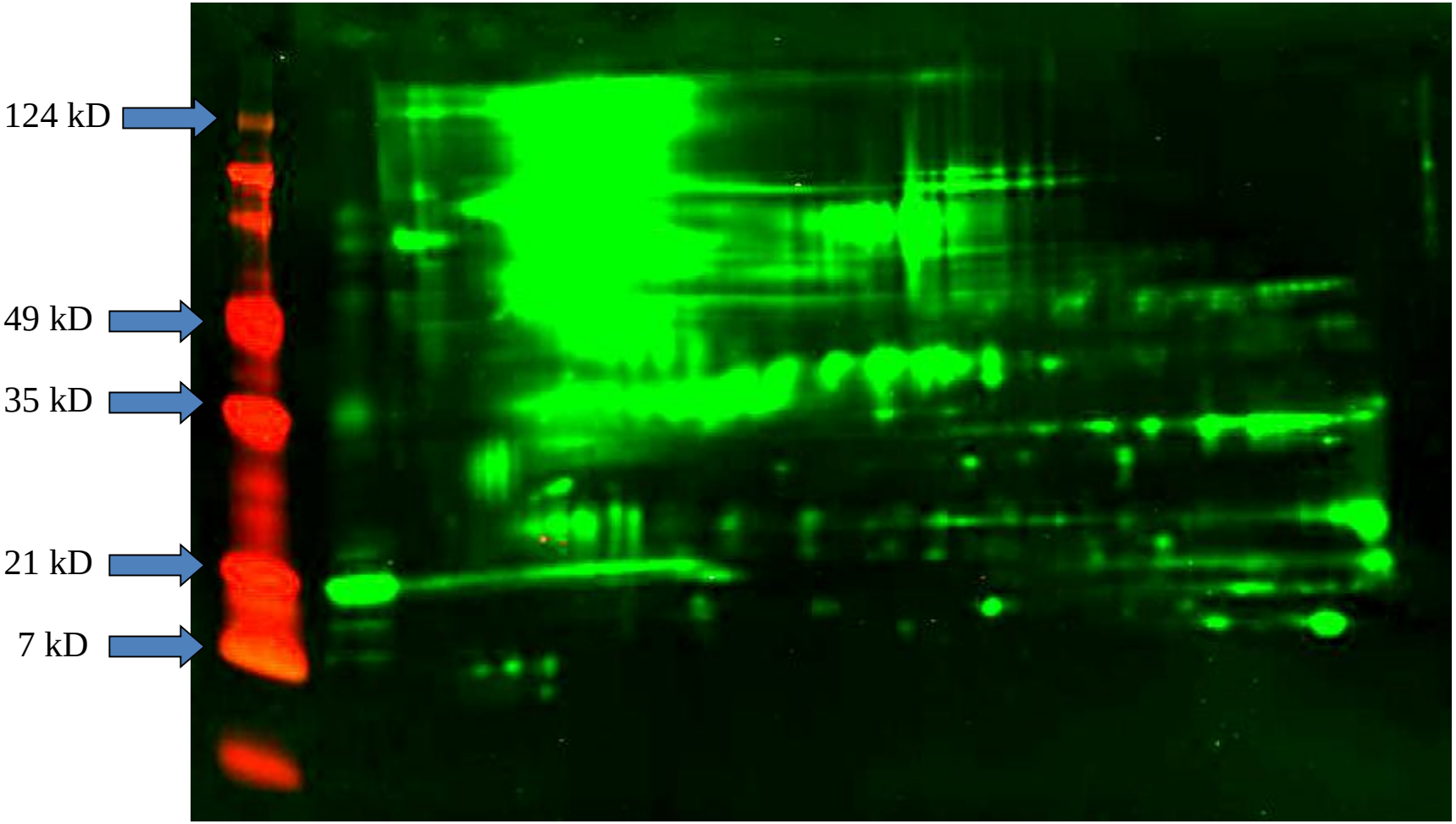


HCPs from the broth loaded at100 µg/gel

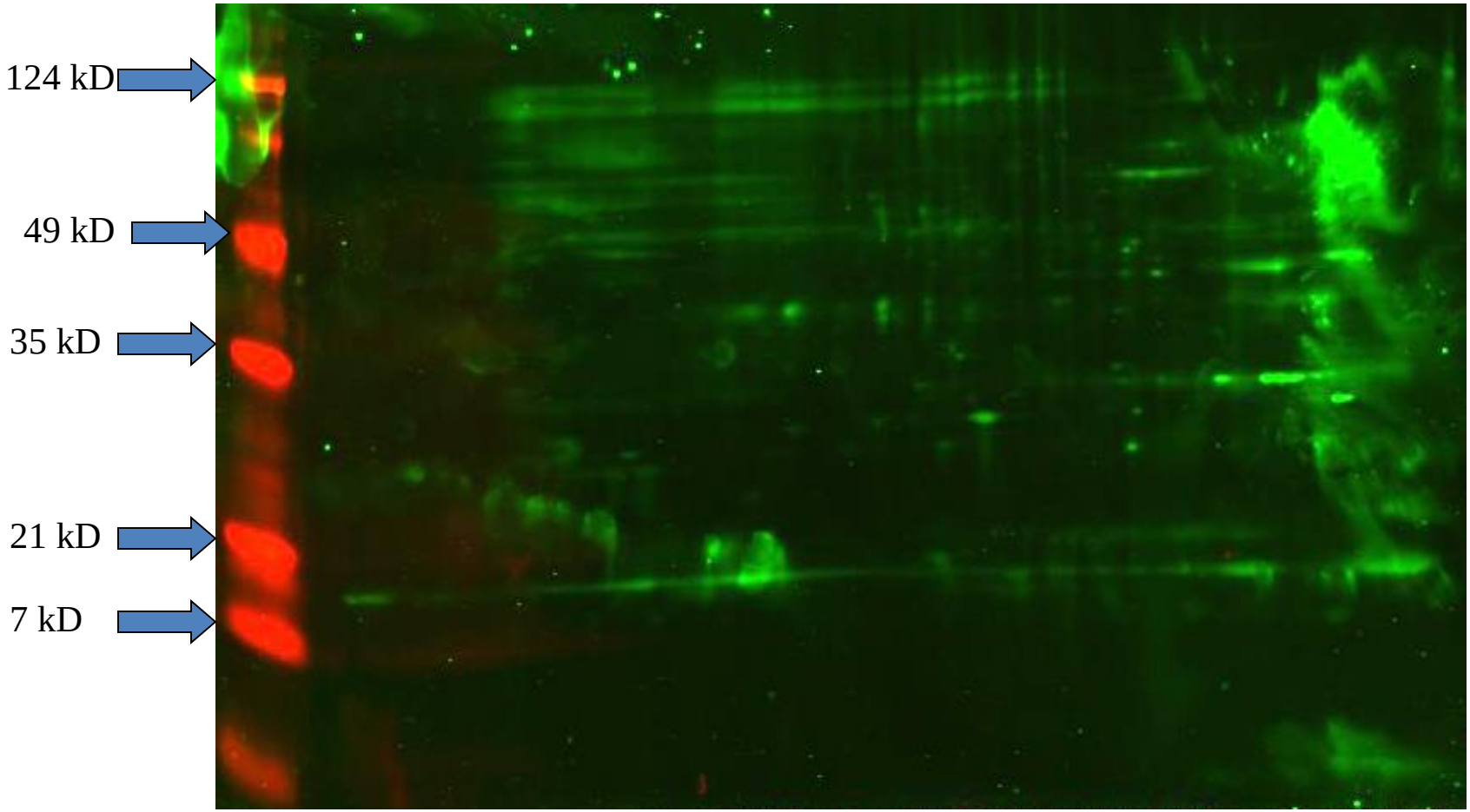
2-D Gel Analysis of Commercial HCP Immunogen



2-D Western Analysis of Null Cell Immunogen Probed with Process-specific Antibodies (typically >70% antibody coverage)



2-D Western Analysis of Null Cell Immunogen Probed with Commercial anti-HCP Antibodies (typically 25%-50% antibody coverage)



Comparison of Commercial and In-house HCP ELISA

HCP ELISA	Run 1,Commercial Kit (ng/mg)	Run 1 In-house (ng/mg)
Upstream	145	1072
Downstream	5	24
Final Drug Substance	<1	<1

HCP ELISA	Run 2, Commercial Kit (ng/mg)	Run 2 In-house (ng/mg)
Upstream	114	990
Downstream	4	48
Final Drug Substance	<1	<1

HCP ELISA	Run 3,Commercial Kit (ng/mg)	Run 3 In-house (ng/mg)
Upstream	130	1222
Downstream	4	42
Final Drug Substance	<1	<1

4. Case Study

2. Cross-reactivity between Monoclonal Antibodies and Anti-HCP Antibodies

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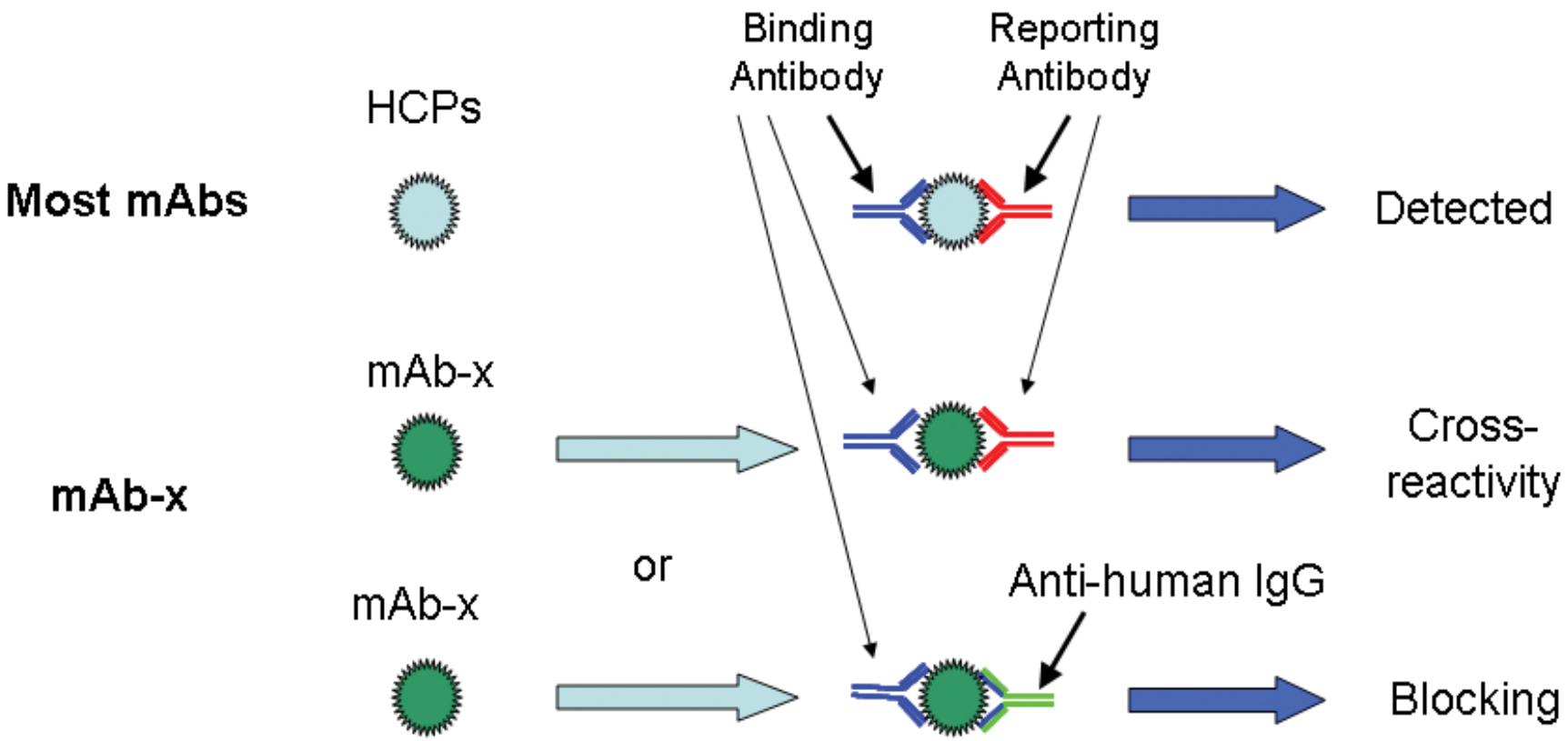
Table 1. Effect of anti-human IgG antibody blocking on HCP ELISA quantitation

Sample ID	CHO HCP ELISA without anti-human IgG antibody incubation HCP (ppm)	CHO HCP ELISA with anti-human IgG antibody incubation HCP (ppm)
mAb-1	3±0.09	4±0.18
mAb-3	3±0.14	4±0.14
mAb-4	7±0.06	7±0.12
mAb-5	6±0.01	5±0.31
mAb-6	10±0.15	7*±0.42
mAb-7	3±0.08	2±0.06
mAb-8	39±0.78	19*±0.38
mAb-9	77±3.85	17*±5.10

*Reduction in reported HCP reading by 30% or more. MAb-9 was used for most of the studies in this report. mAb-2 was analyzed in Western Blot but not in HCP ELISA due to the availability of material.

Use Anti-human Polyclonal Antibody to Block the Nonspecific Interaction

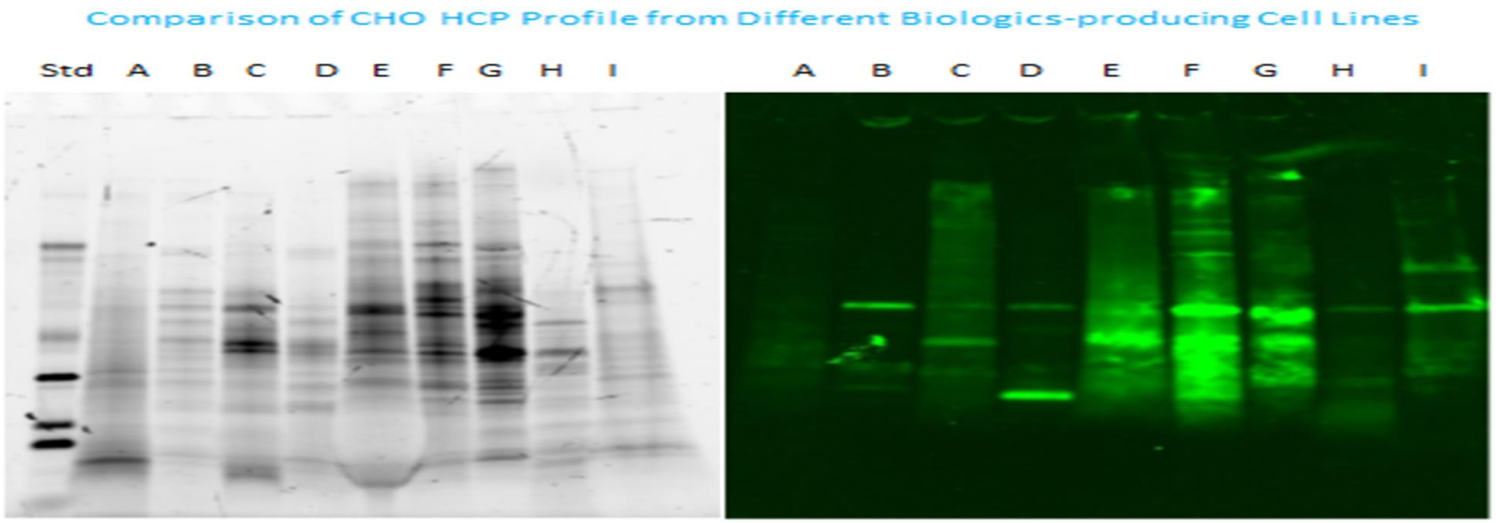


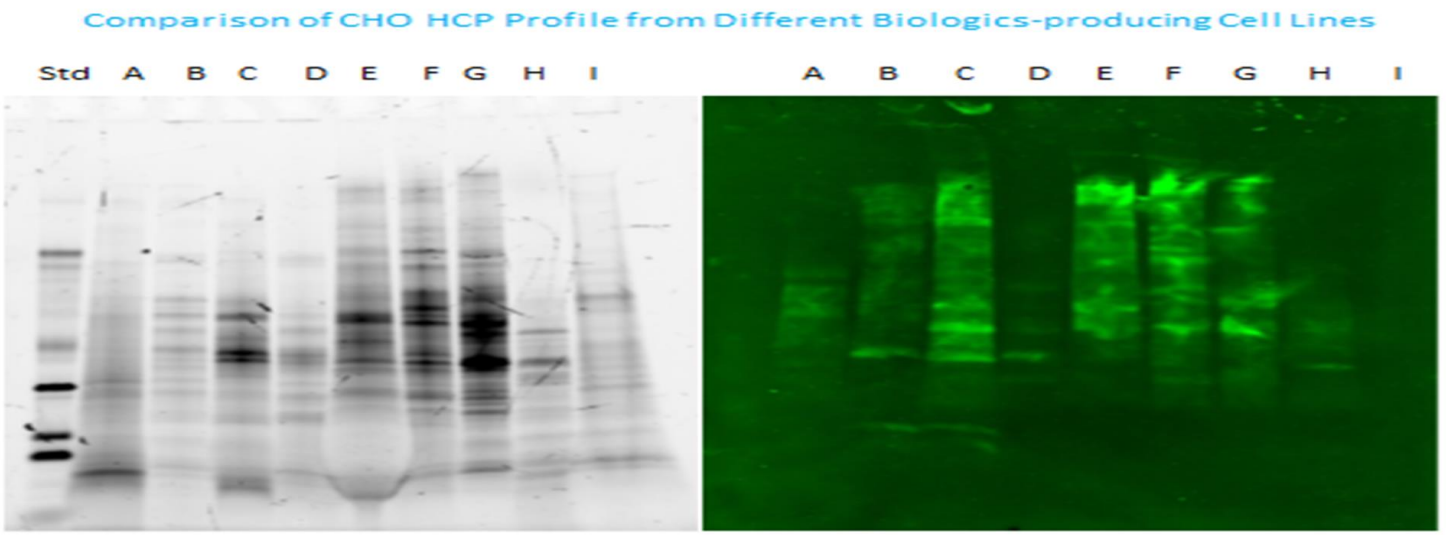
(Xing Wang, Thomas Schomogy, Kristine Wells, Ned M. Mozier. **BioProcess Int.** P18—24, 2010)

5. Generic or Process-specific HCP ELISA?

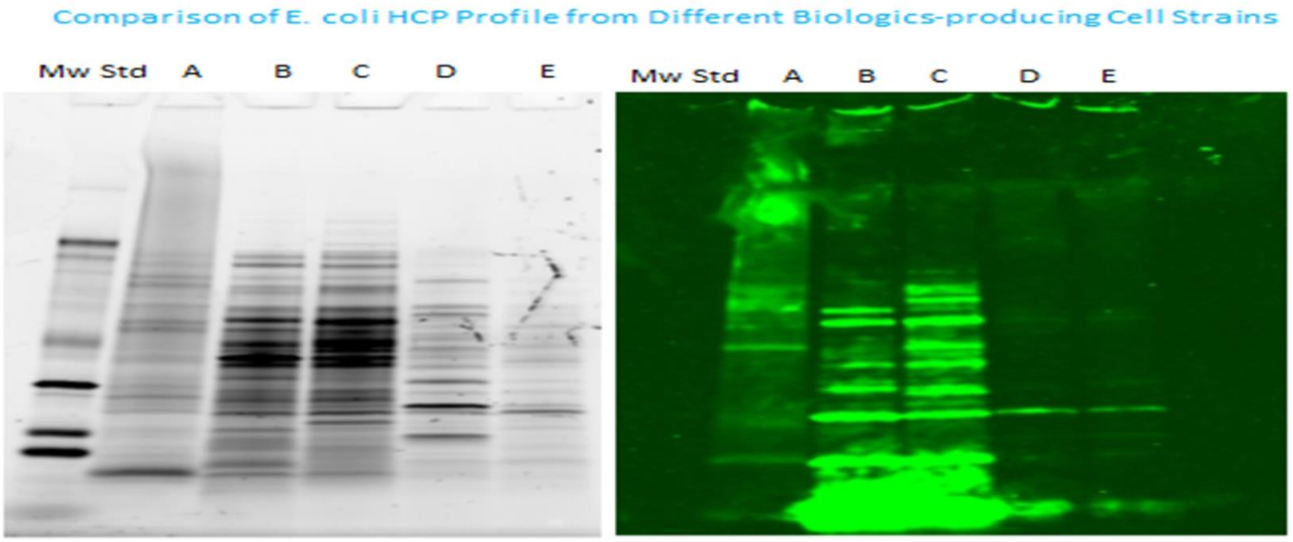
When to make the switch?

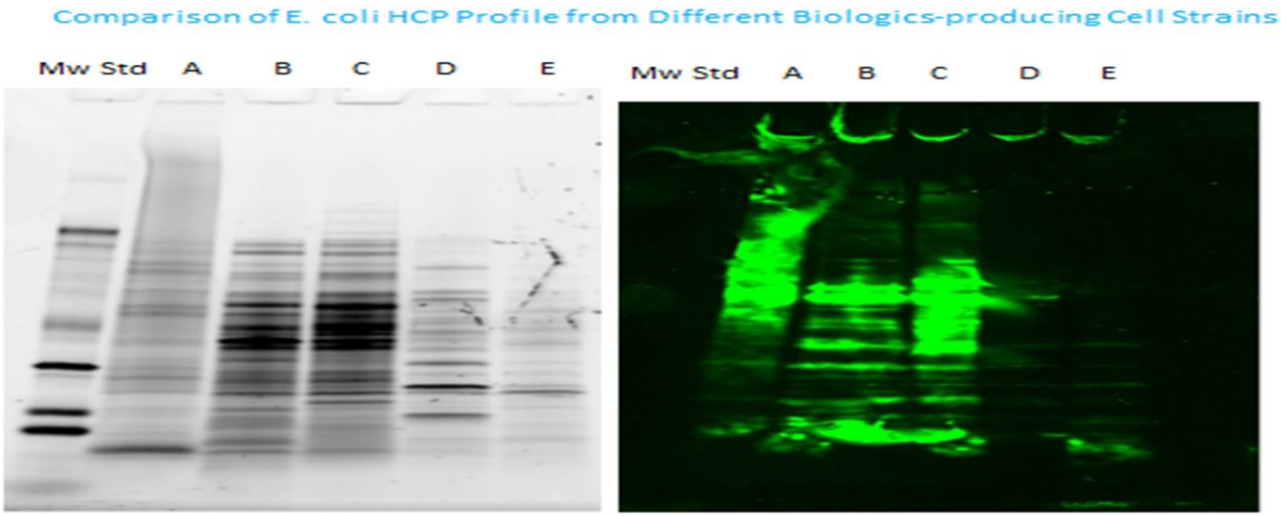
HCPs from 9 Different CHO Cell Lines, Upper Panel Western Blot with Antibodies Produced from cell line-F and Lower Panel from cell line-C.



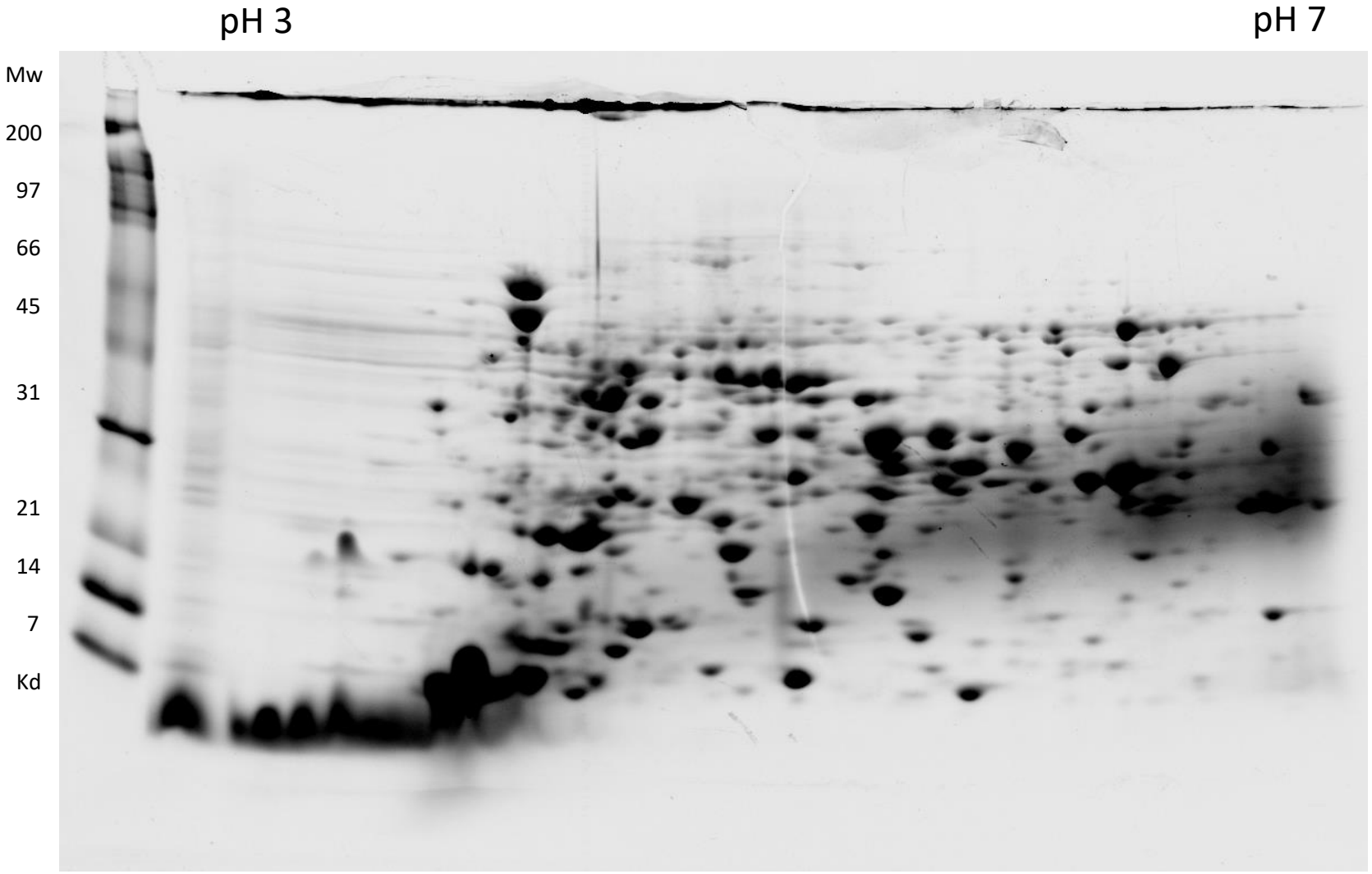


HCPs from 5 Strains of E. coli, Western Blot with Antibodies Produced from Strain C and Strain A Respectively.

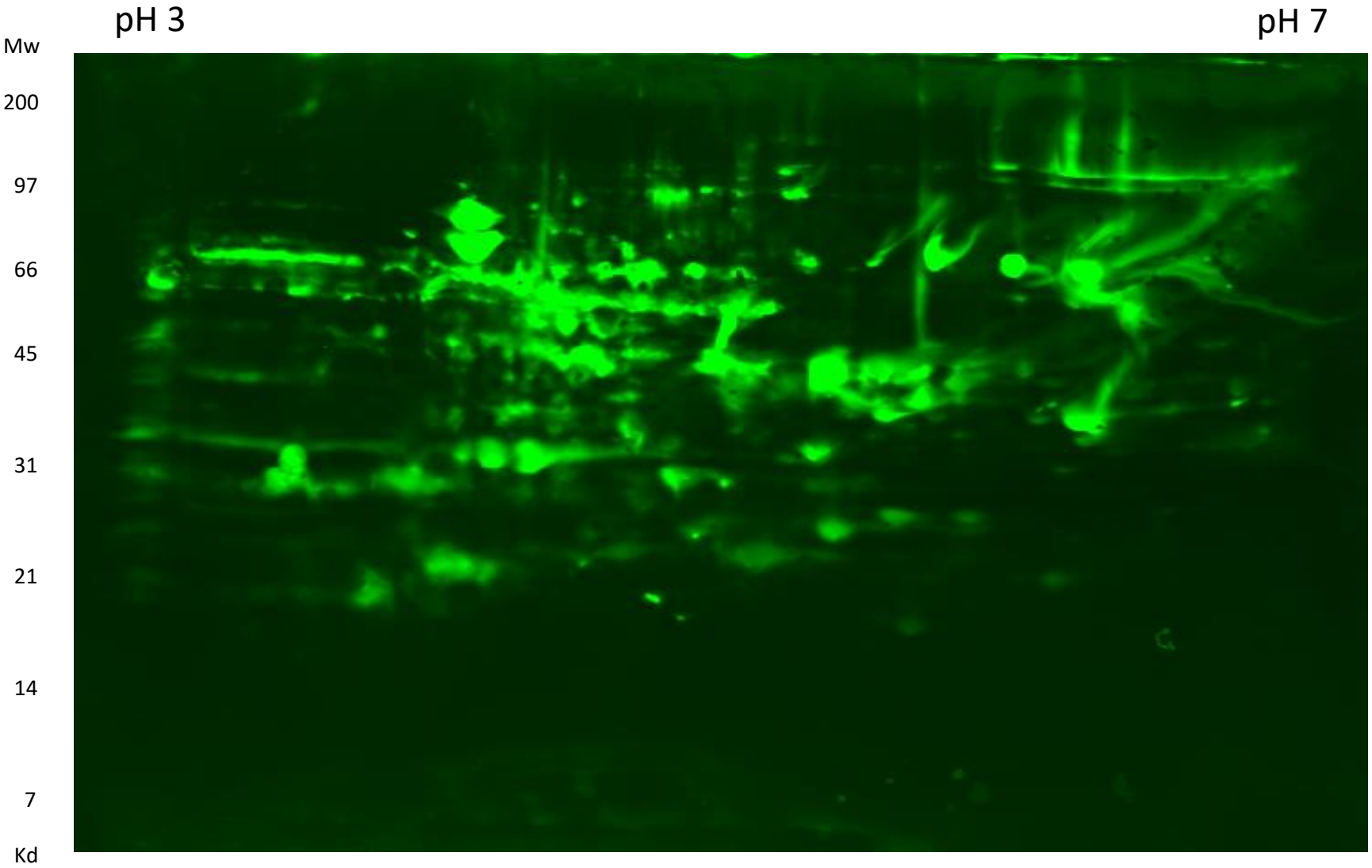




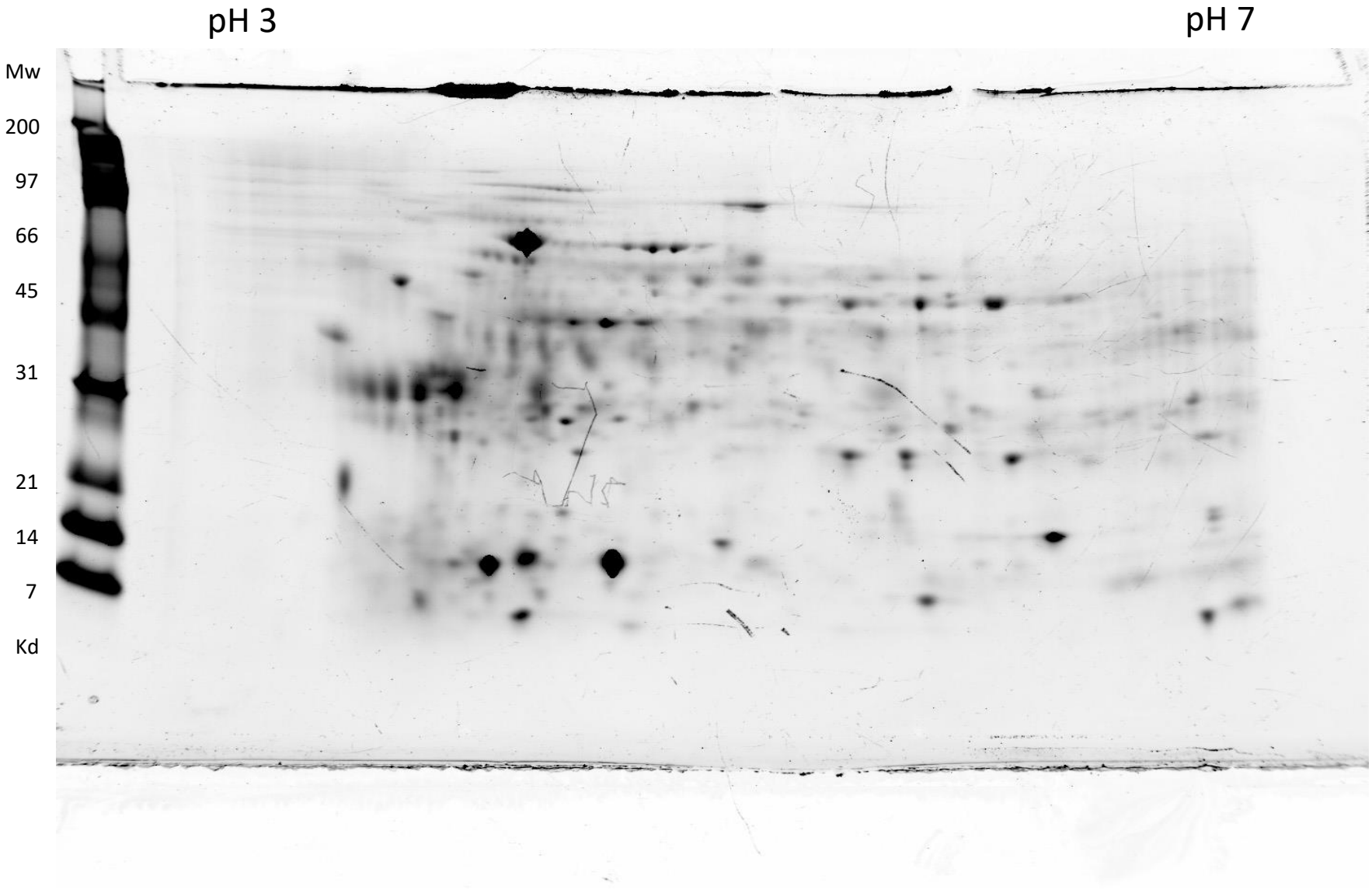
E. coli HCP Stained with Sypro Ruby after 2-D Gel Separation Acidic to Neutral Proteins, pI 3 to pI 7



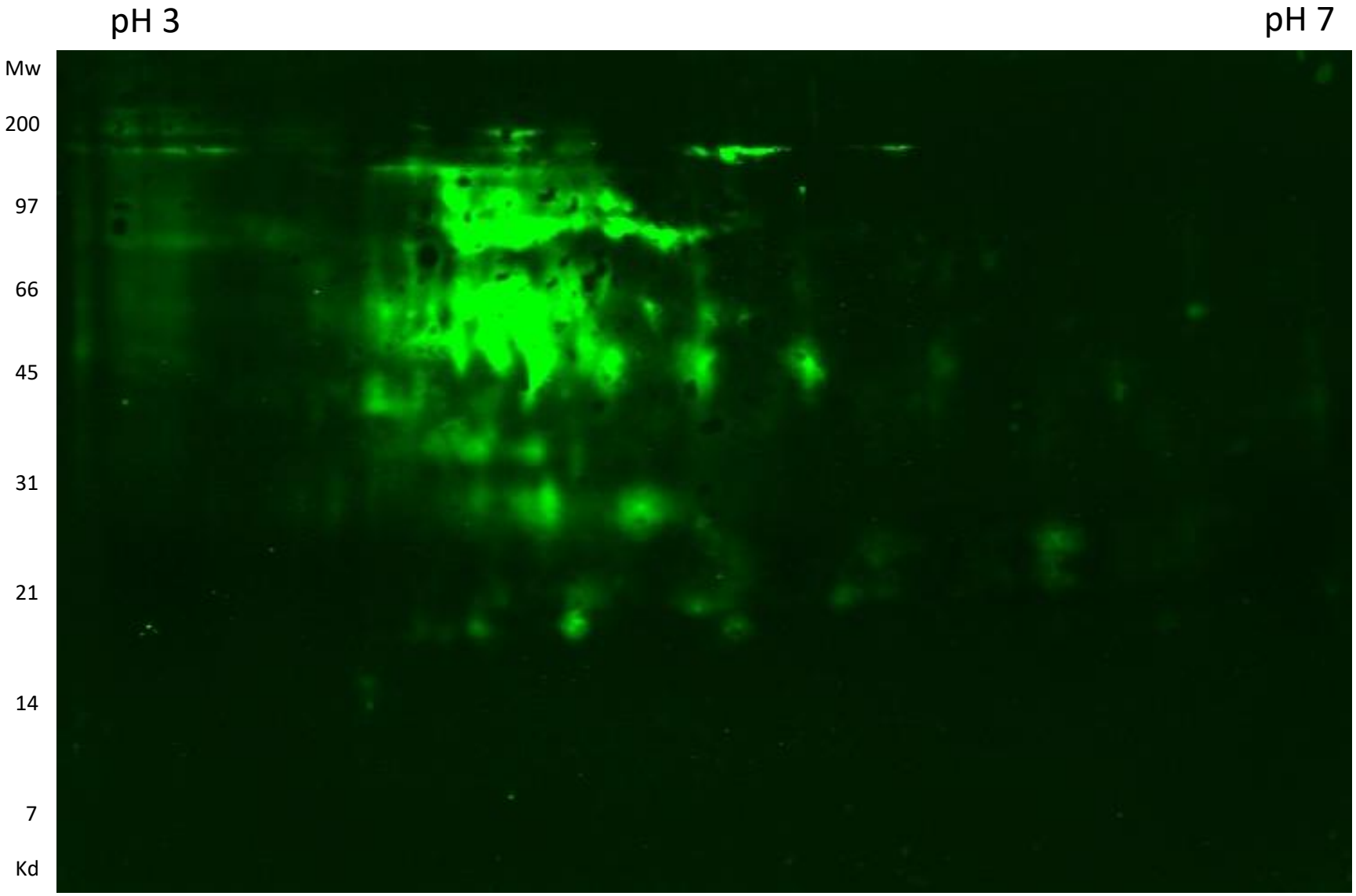
E. coli 2-D Western Blot Analysis of Antibody Coverage For Acidic to Neutral Proteins. Antibody Coverage >80%



CHO Total Protein Stained with Sypro Ruby after 2-D Gel Separation Acidic to Neutral Proteins



CHO 2-D Western Blot Analysis of Antibody Coverage For Acidic to Neutral Proteins. Antibody Coverage >70%.



Pros and Cons for Generic and Process-specific HCP ELISA

Generic HCP ELISA	Process-specific HCP ELISA
Easy to use, QC in place	Take significant up-front investment, needs internal QC.
Cost is high in long-term.	Cost is low in long-term.
No control of a Critical Reagent.	Control of Critical Reagent.
Pay per kit use.	>10,000 ELISA kits of antibody produced/project.
Antibody coverage typically low.	Antibody coverage typically high.

What Factors Determine When to Use Generic or Process-specific HCP ELISA?

- In many biotech companies, a large portion of pipeline is licensed from outside (as high as 75%).
- Only a portion of the pipeline candidates will pass Phase-II (except Biosimilars).
- It does not make financial sense to develop process-specific HCP for every early stage projects.
- Generic HCP ELISA can be used up to Phase-II.
- Going into Phase-III, a ICH-validated HCP ELISA needs to be in place for Process Validation and Drug Substance Release.
- Generic HCP ELISA can be used in Phase-III if the antibody coverage is >70% (unlikely based on numerous case studies). If generic HCP ELISA kit can't provide good coverage, a process-specific HCP ELISA kit needs to be developed, which takes from 6-8 months to more than a year to complete.

6. Conclusions

- The HCP issue is complex and needs to be understood.
- Sandwich ELISA is the gold standard for HCP quantitation in the industry.
- Orthogonal methods should be used to further characterize HCPs (1-D and 2-D Western Blot, Mass Spec., Proteomics etc.).
- Biologics at Clinical Development Phase-I and II can use commercial HCP ELISA kits.
- Biologics going into Phase-III, most likely a process-specific HCP ELISA kit needs to be developed, this process will take 6-8 months to more than a year.