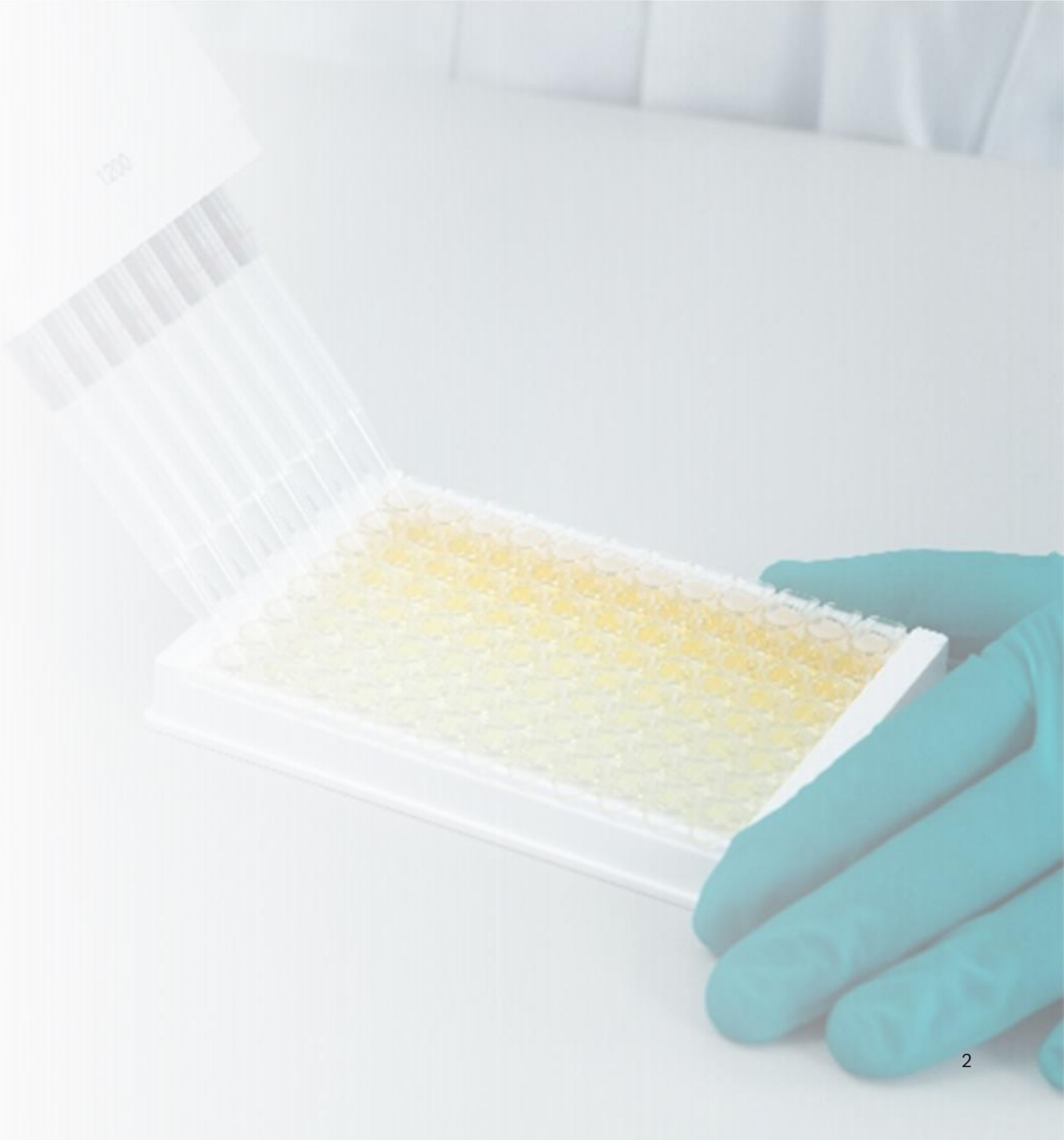
Challenging host cell protein assays for improved risk mitigation

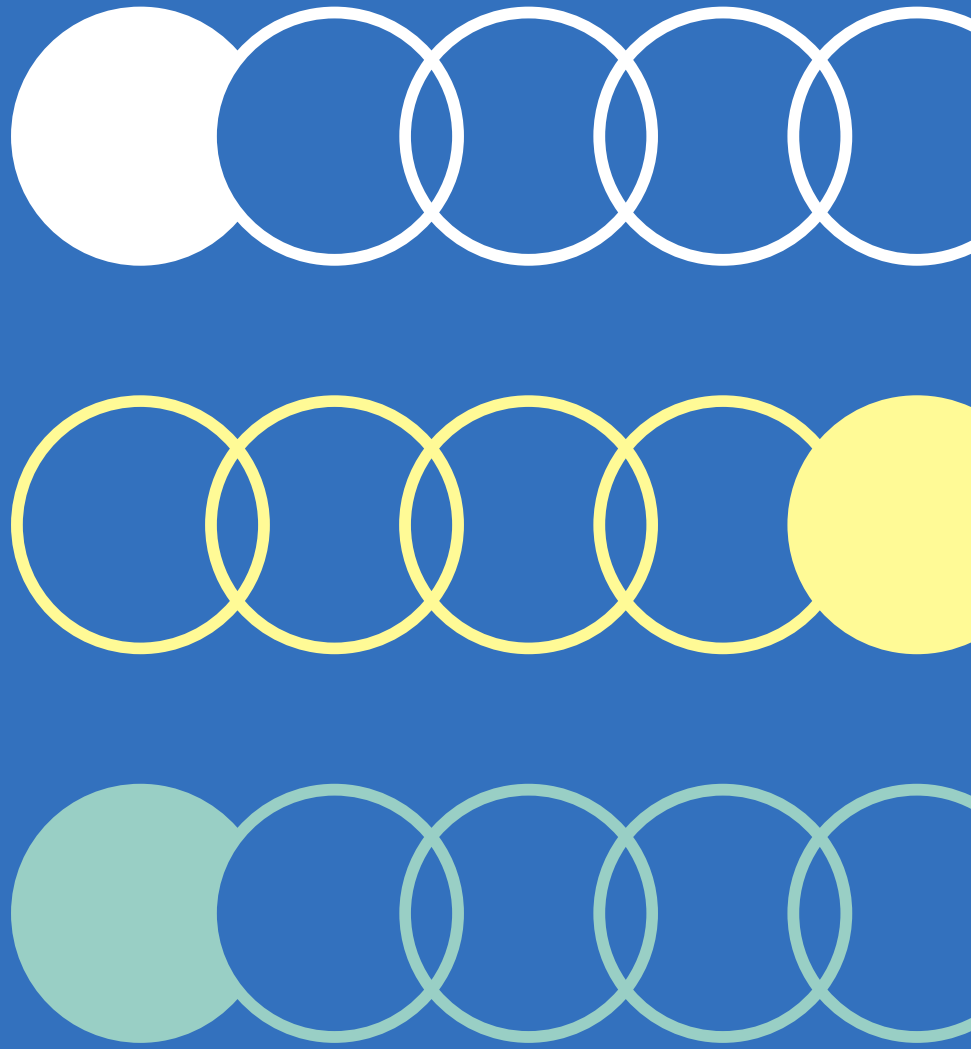
Andrew Hamilton
Scientist
Cytiva

Agenda

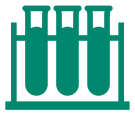
- Challenges of accurate HCP analysis
- Generic and process-specific HCP antibodies
- Considerations for selecting an HCP ELISA
- Criteria for selecting an HCP ELISA
- Considerations for an HCP coverage assay
- Outsourcing HCP risk management



Challenges of accurate HCP analysis



Challenges of accurate HCP analysis

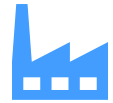


Assay

- Sample compatibility
- Antibody specificity
- Development time
- Coverage assay
- Orthogonal assays

Project

- Issues avoidance in P3
- Assay transfer to quality control
- Assay development time
- Time to market
- Acquisition of molecule

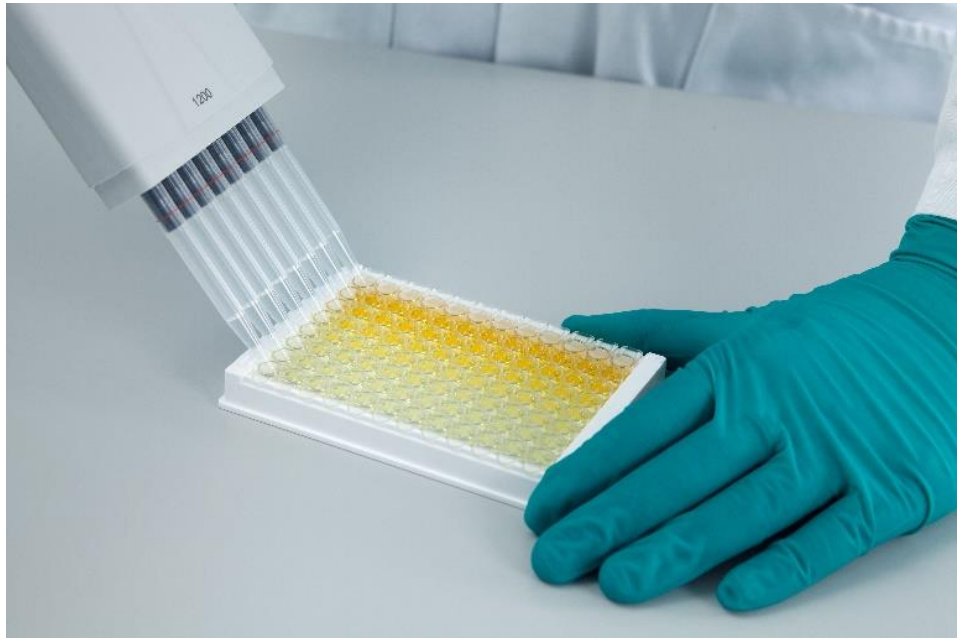


Supplier

- Security of supply
- Antibody quality
- Customer support
- Manufacturing certification
- In-house vs outsourced

ELISA: Industry standard for HCP detection

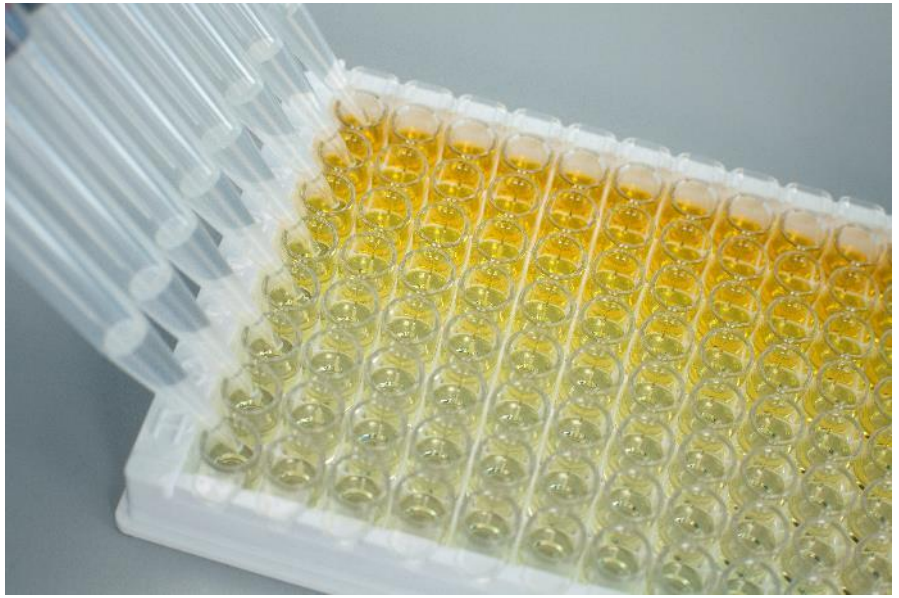
- **Most commonly used assay for HCP quantification**
- **Unique ELISA** detects thousands of proteins simultaneously with **polyclonal antibodies**
- **Required** for data submission to regulatory authorities
- Total HCP sensitivity **below one part per million**
- Easy to use assay with rapid analysis
- Approved validation available using coverage assays
- Potential for automation
- Available with **generic off-the-shelf antibodies**
- Can be customized with process-specific antibodies



Why choose **generic** antibody-based assays?

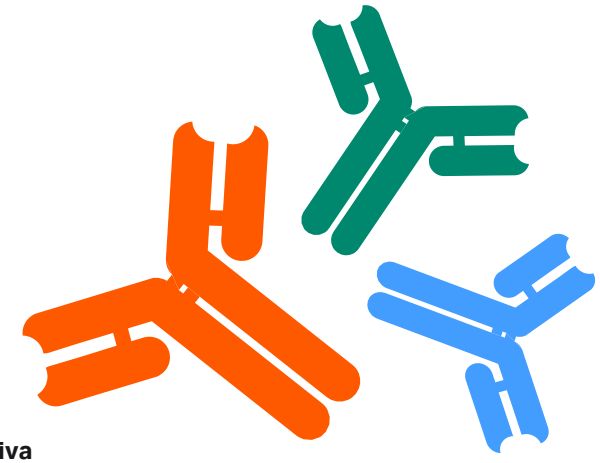
- **Inexpensive** compared to specific (custom) antibodies (USD 100K – 200K)
- Off-the-shelf: **Saves time** with antibody generation and kit development (~ 12 months)
- Can be used for data submission (Phase 3) to authorities (molecule- and country-dependent)
- Can be used **prior to Phase 2** in most scenarios
- **Approved validation** available with coverage
- Maximum flexibility

It is important to test different generic antibodies to find the most suitable kit



Why choose **process-specific** antibody-based assays?

- **Higher probability** of good sample compatibility
- Controlled **security of supply** of reagents
- Can be used for data submission (Phase 3) and **product batch release**
- **Approved validation** available with coverage
- Possibility for custom **platform assays**



Consider the most suitable antigen for generating process-specific antibodies

Why should you challenge your current HCP risk management strategy?

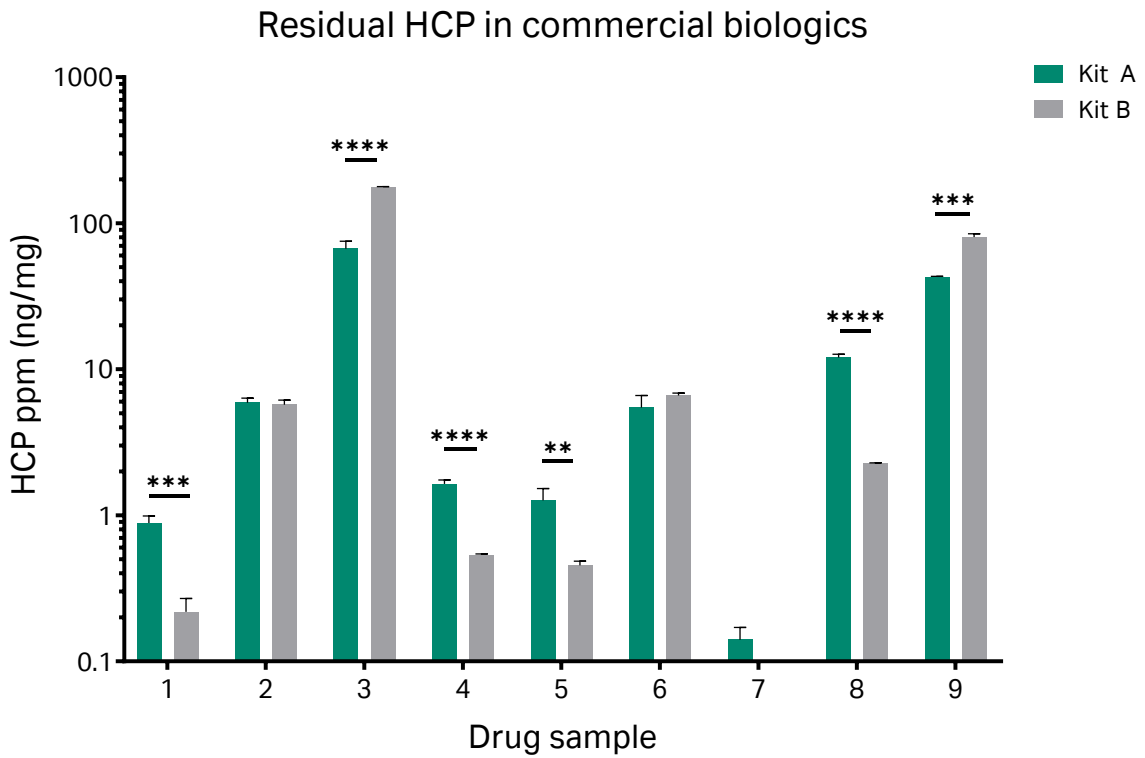
- Generic kits **do not guarantee** good sample compatibility
- HCP levels can **vary significantly** between different kits
- **Early testing** with different generic kits mitigates the risk of unexpected HCP levels later on
- **Cell culture conditions** can affect compatibility with generic kits
- Purification can inadvertently **enrich the HCPs** that generic antibodies do not cover
- **High product concentrations** can interfere with the assay
- The chosen assay must be available for the **lifetime of the molecule**

Different HCP ELISA kits report different HCP levels

We tested nine commercial biologics for residual HCP using two different kits

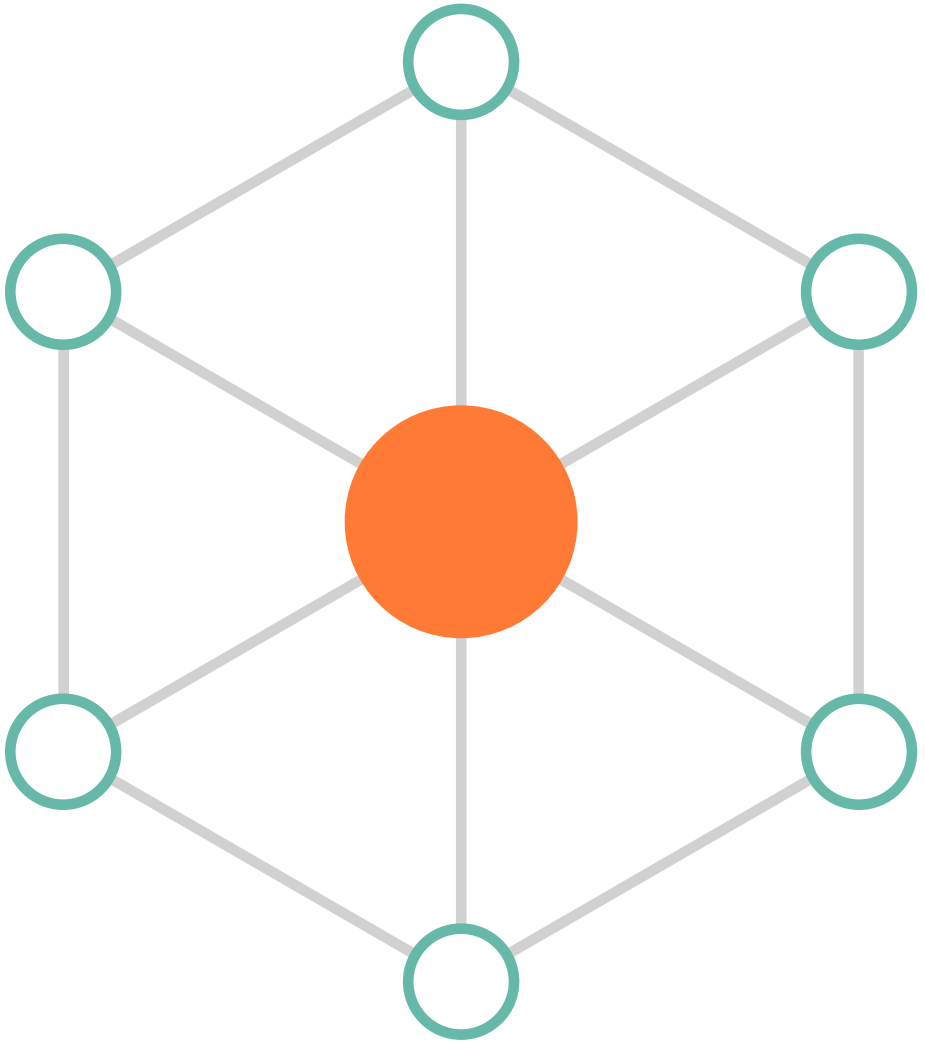
- Kit A reported higher HCP in four samples
- Kit B reported higher HCP in two samples
- Three samples had approximately equivalent HCP levels with both

It is critical to select the most suitable kit for each molecule



Residual HCP in commercial drug substances expressed as ppm (nanograms HCP per milligram of drug substance). Asterisks denote statistical significance (t-test). Error bars represent standard deviation.
** = $p < 0.01$
*** = $p < 0.001$
**** = $p < 0.0001$

Criteria for selecting an HCP ELISA



Criteria for selecting an HCP ELISA

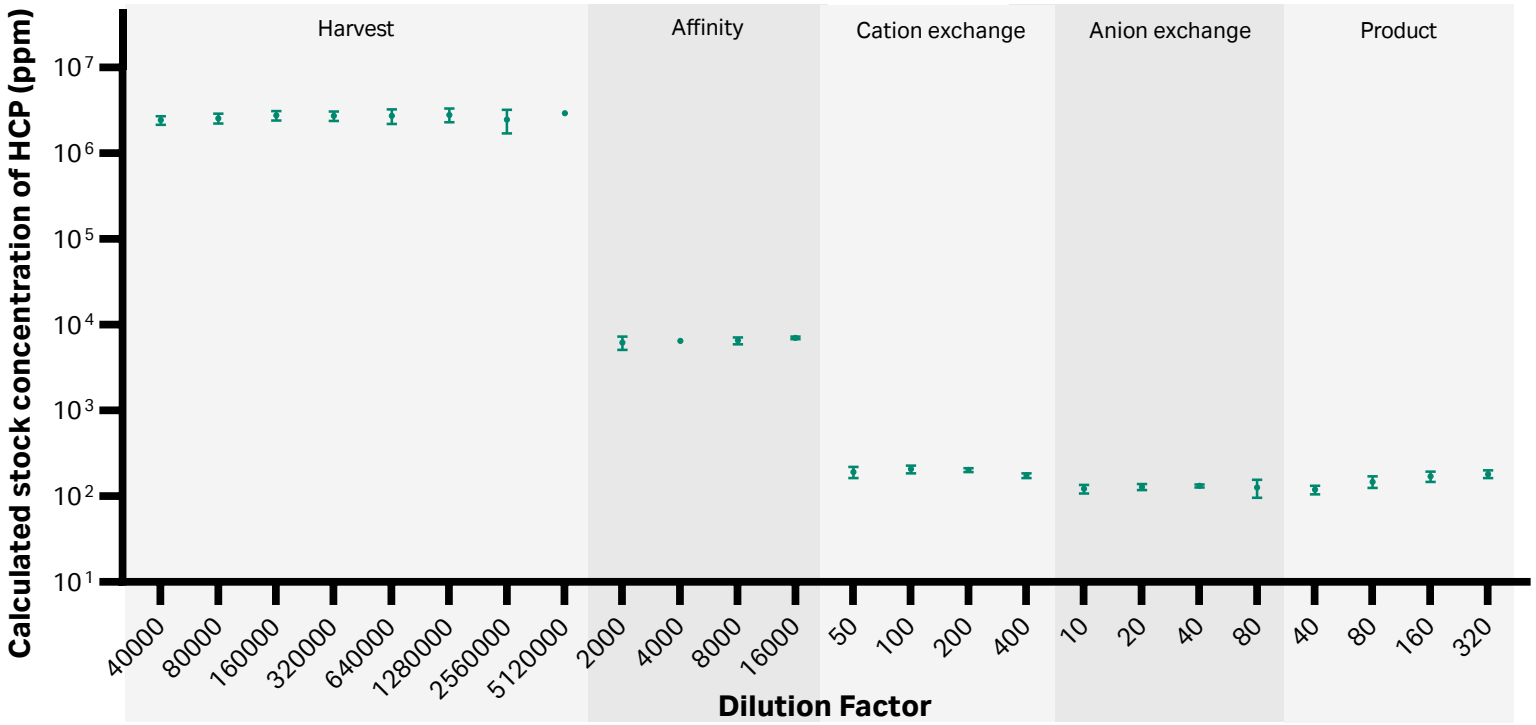
- Sample compatibility: Does the ELISA perform well with the sample?
- Dilutional linearity: Are results proportional when the test sample is diluted?
- Matrix effects: Do the product or process-specific buffers interfere with the assay?
- Accuracy: Does the ELISA accurately quantify a known, control amount of HCP?
- Sensitivity: Does the ELISA detect sufficiently low amounts of HCP?
- Antibody coverage: Do the HCP antibodies recognize a sufficient proportion of HCP?

Sample compatibility – dilutional linearity

Dilutional linearity – or parallelism – indicates how well the result scales when the sample is diluted

Good parallelism is critical across the entire purification process

This helps avoid problems later in development



Sample compatibility – spike recovery

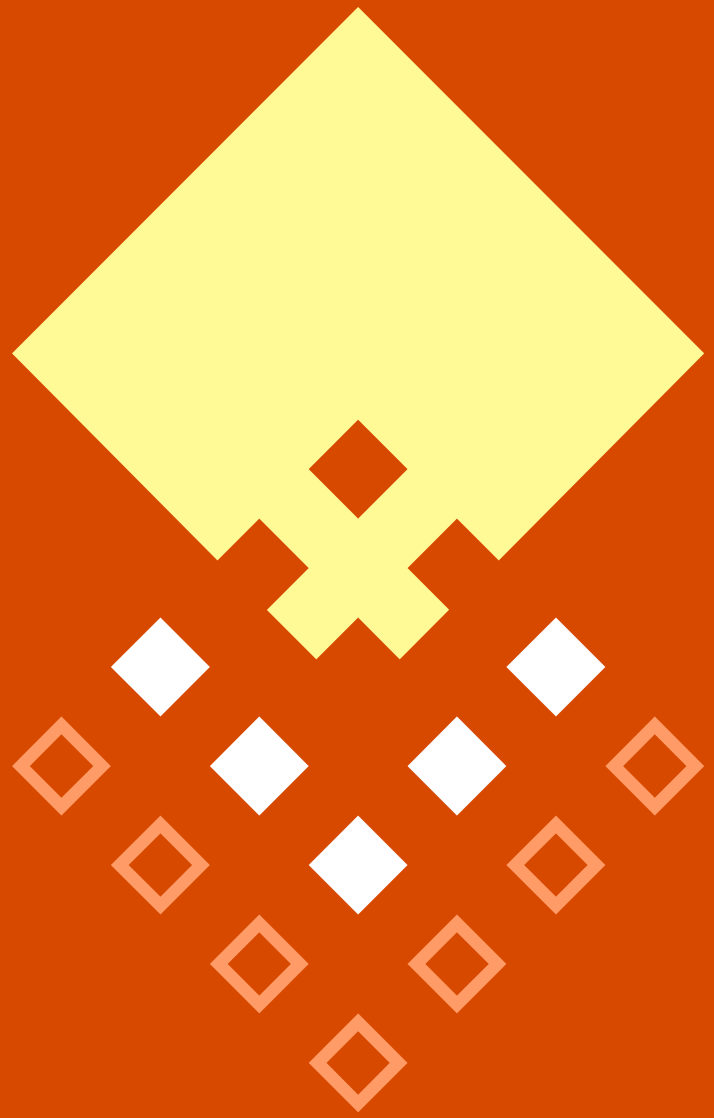
It is important to identify any detrimental effects from the sample matrix

To measure matrix effect, spike a known concentration of HCP standard into process-specific buffer

Recovery between 80% – 120% is considered valid

Matrix	Sample buffer	50mM Na Acetate pH 3.5		50mM Na Acetate pH 5.5, 100mM NaCl		25mM Phosphate pH 7.5		MAb		
Dilution	0	1:1	1:10	1:1	1:10	1:1	1:10	5 mg/mL	2.5 mg/mL	0.5 mg/mL
Mean recovery	96.60	61.22	107.54	110.25	98.87	109.00	105.99	101.60	99.19	94.38
Mean CV (%)	5.75	3.43	3.91	2.33	6.11	3.08	7.57	6.35	5.09	8.48

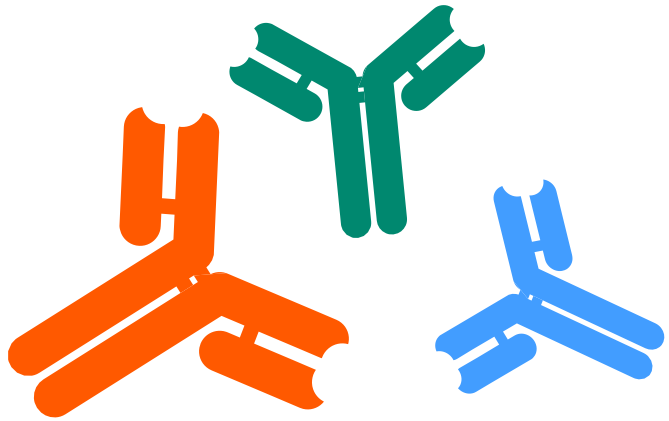
Coverage



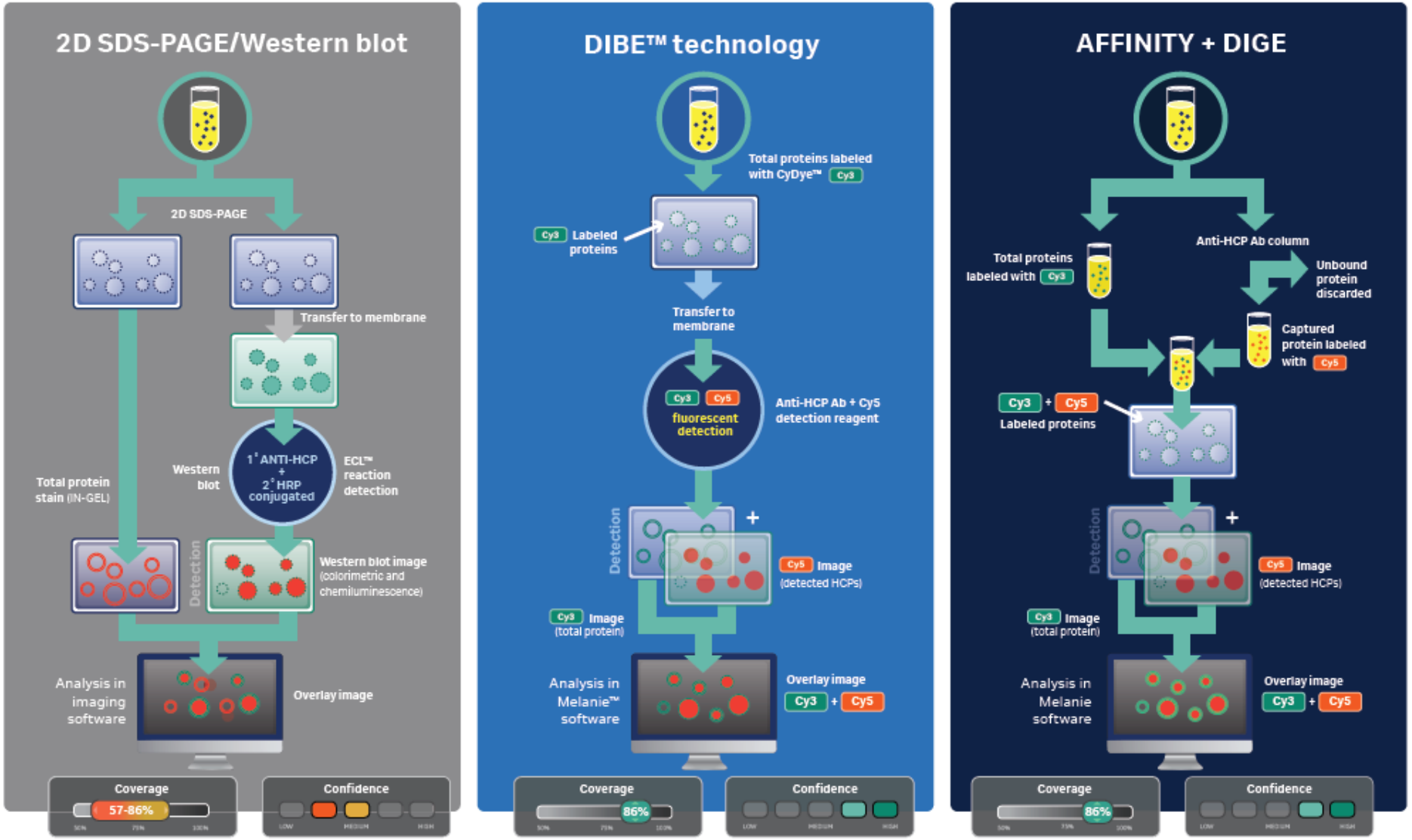
Antibody coverage

- Coverage analysis calculates the proportion of HCP detected by ELISA antibody
- Performed on upstream HCP sample to account for all possible HCP
- Can be used as a criteria for antibody screening in ELISA development
- Required to measure risk of undetected HCP in ELISA
- More than ~60% coverage is considered acceptable

Reporting coverage percentage is a requirement for regulatory filing in many regions

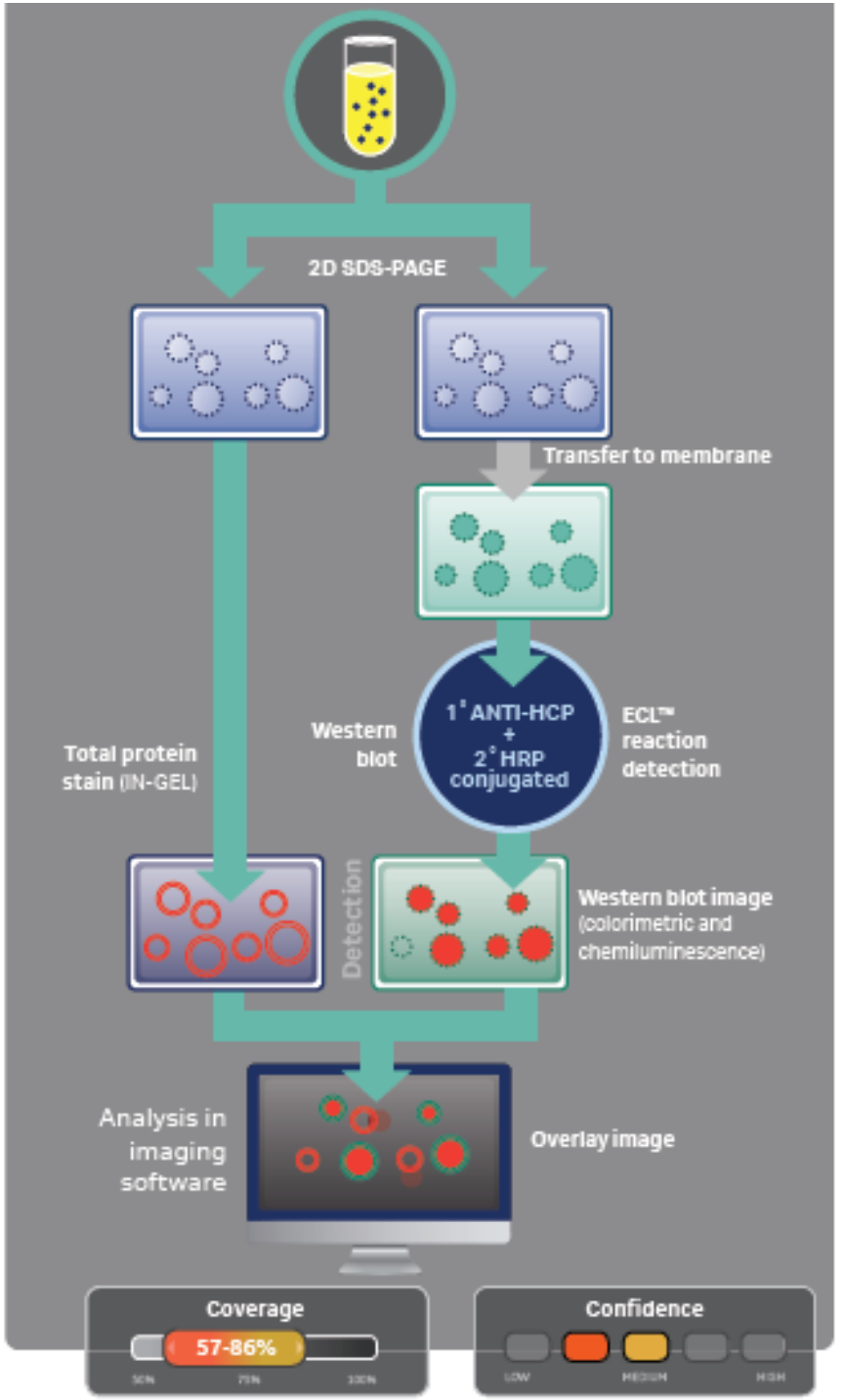


Which coverage analysis method is the most suitable?



2D SDS-PAGE/Western blot

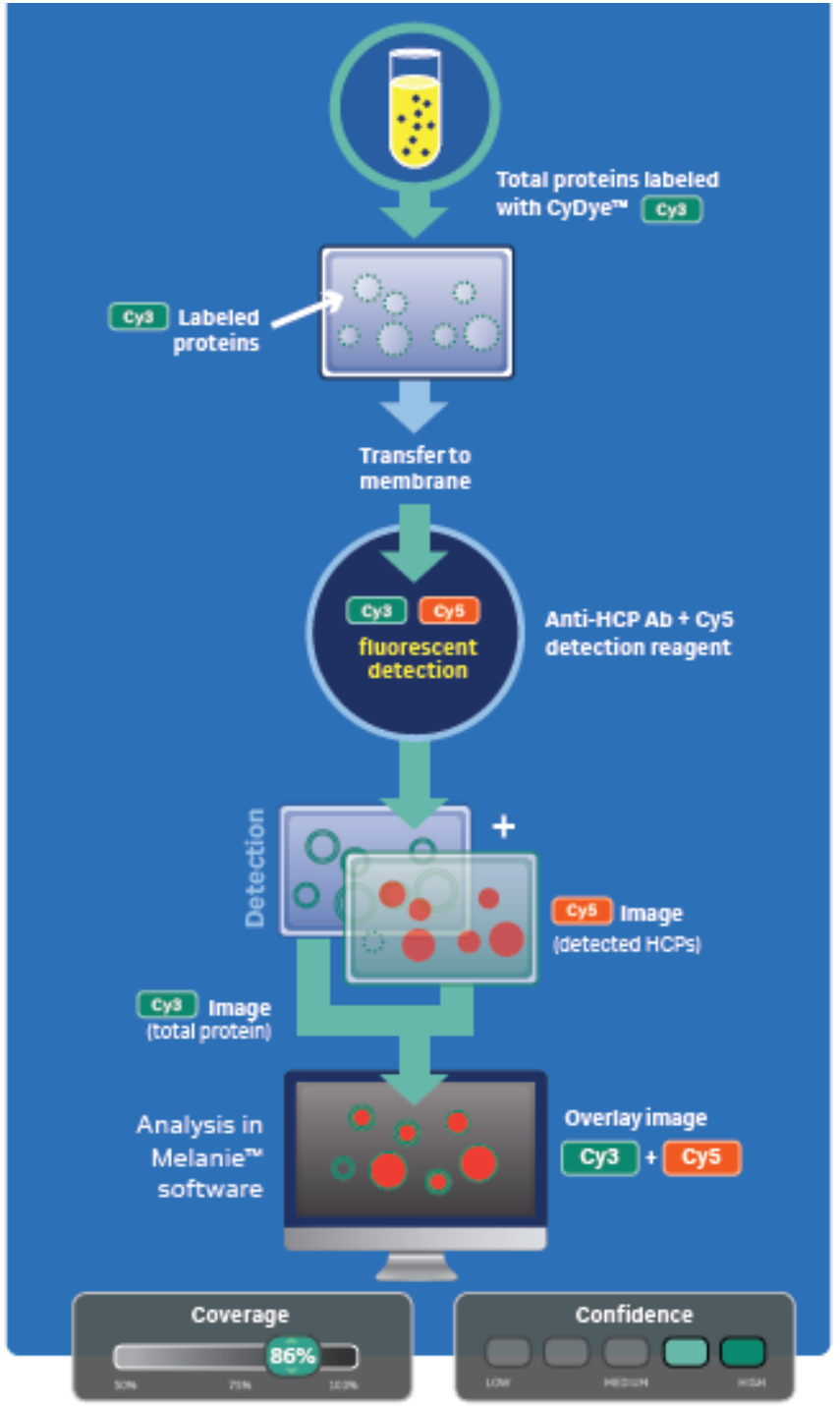
- Provides good separation of HCPs
- Using two gels doubles the time and effort needed
- 2D spot comparison is challenging and not standardized
- Coverage values can vary within and between labs
- Immunodetection in denatured conditions



DIBE technology

- Using only one gel reduces time and effort needed
- No need to use an affinity column
- Immunodetection can be more sensitive than affinity and DIGE approach
- High-resolution = accurate identification of all signals

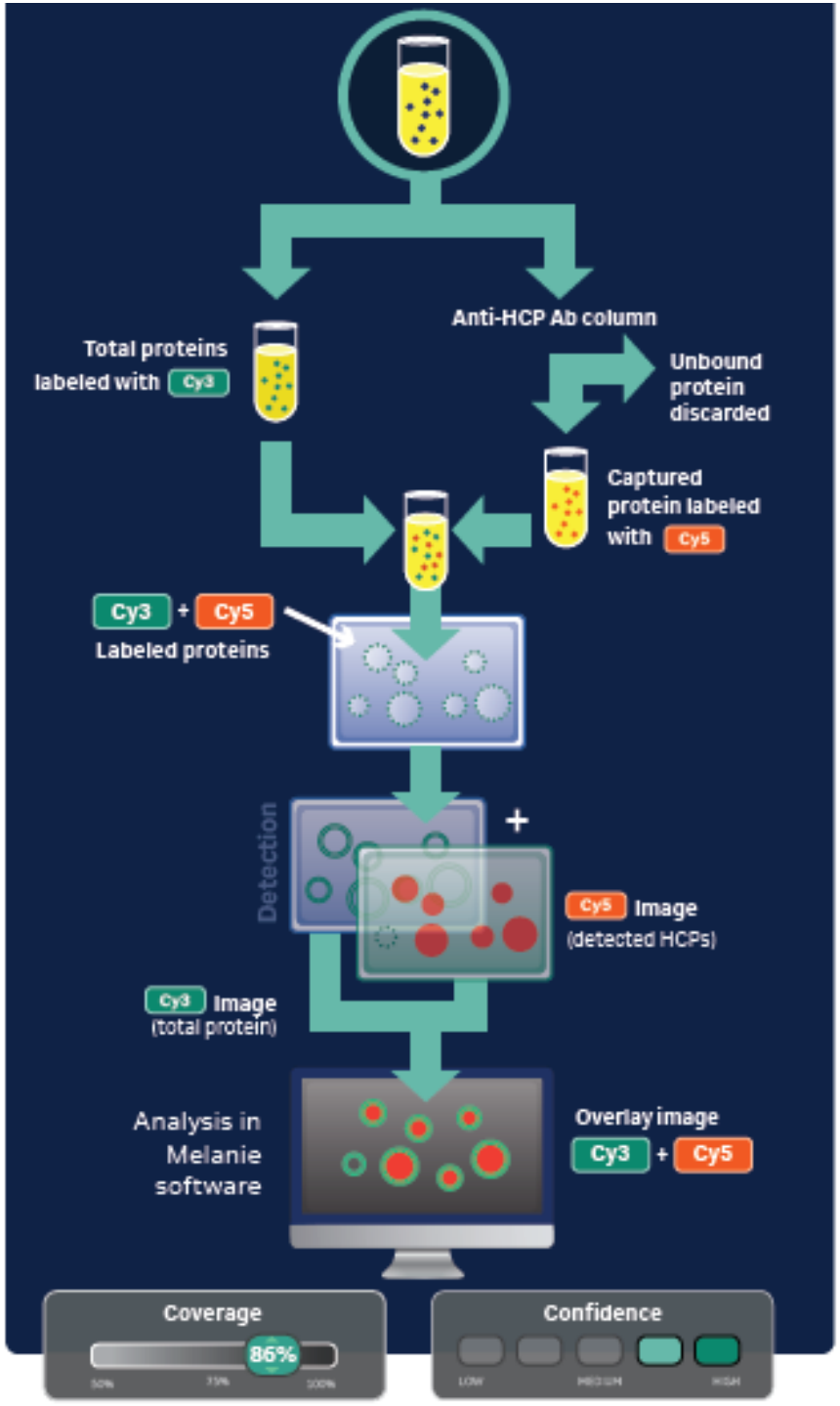
⚠ Immunodetection in denatured conditions



Affinity + DIGE

- HCP capture in native conditions
- Avoids challenges of immunoblot transfer

- Might underrepresent tightly bound HCPs
- Requires careful preparation of anti-HCP resin, which can be difficult to reproduce
- Might not be as sensitive as a Western blot



Best practices to ensure robust coverage results

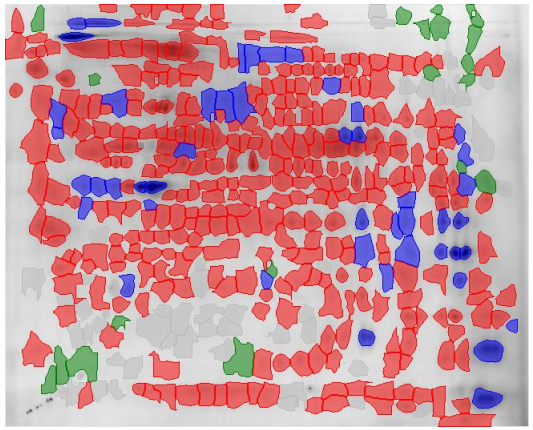
- Mock cell line using similar culture methods
- Triplicate experiments to ensure reproducibility and increase confidence
- Use the same antibody as ELISA
- Use DIBE™ technology to avoid alignment of antigen and antibody signals
- Perform blinded analysis to avoid subjectivity with spot detection
- Calculate coverage based on all proteins, not just the most abundant

Antigen choice can affect antibody coverage

- CHO-K1 null cell line – supernatant fraction
- DIBE™ coverage analysis performed with anti-CHO antibodies raised against **lysate** and **supernatant**
- CHO supernatant antibody has much higher coverage
- Higher risk for missed HCP in ELISA with lysate antibody

Selecting the most suitable antibody is critical for accurate results

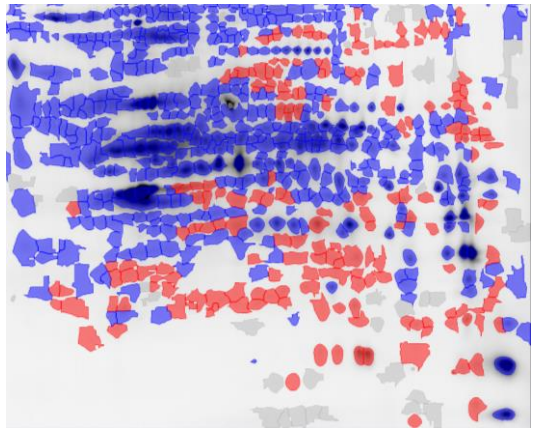
CHO-K1 supernatant sample



CHO lysate antibody

17% coverage

CHO-K1 supernatant sample



CHO supernatant antibody

74% coverage

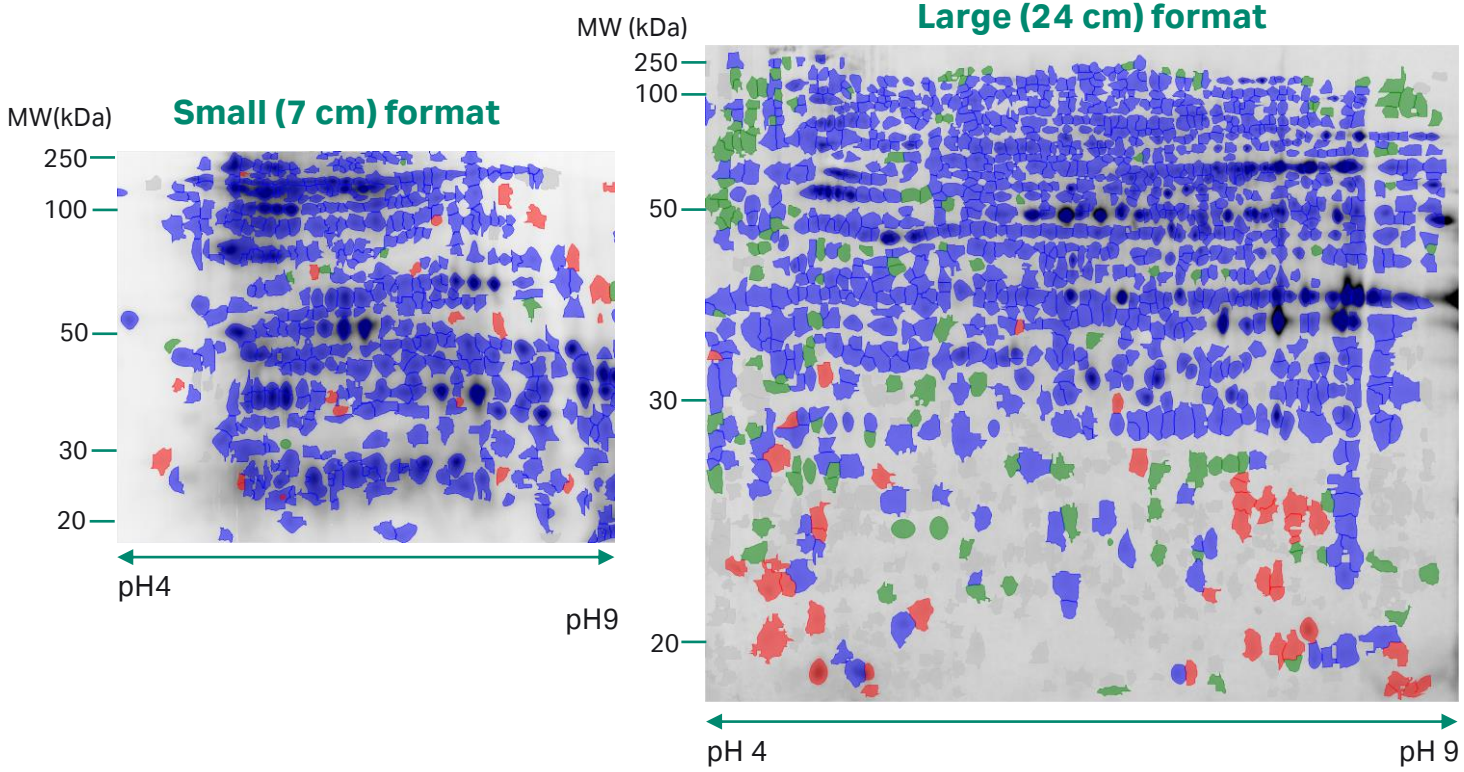
Increased robustness of coverage data using large format workflow

- Using large format gels increases the total number of spots detected
- Coverage should be similar
- Small gels suitable for routine analysis and optimization
- Large gels more suitable for data submission to authorities

More spots

More robust data

Increased confidence



Gel size	Total number of spots	Antibody coverage (%)
Small gel (7 cm)	478	92%
Large gel (24 cm)	1140	96%

Choose the most appropriate workflow for the application

7-cm small format coverage analysis

- Perfect for optimization and routine testing
- Uses less reagent and material
- Quicker protocol — faster imaging
- Easier handling
- Ideal size for CCD imaging

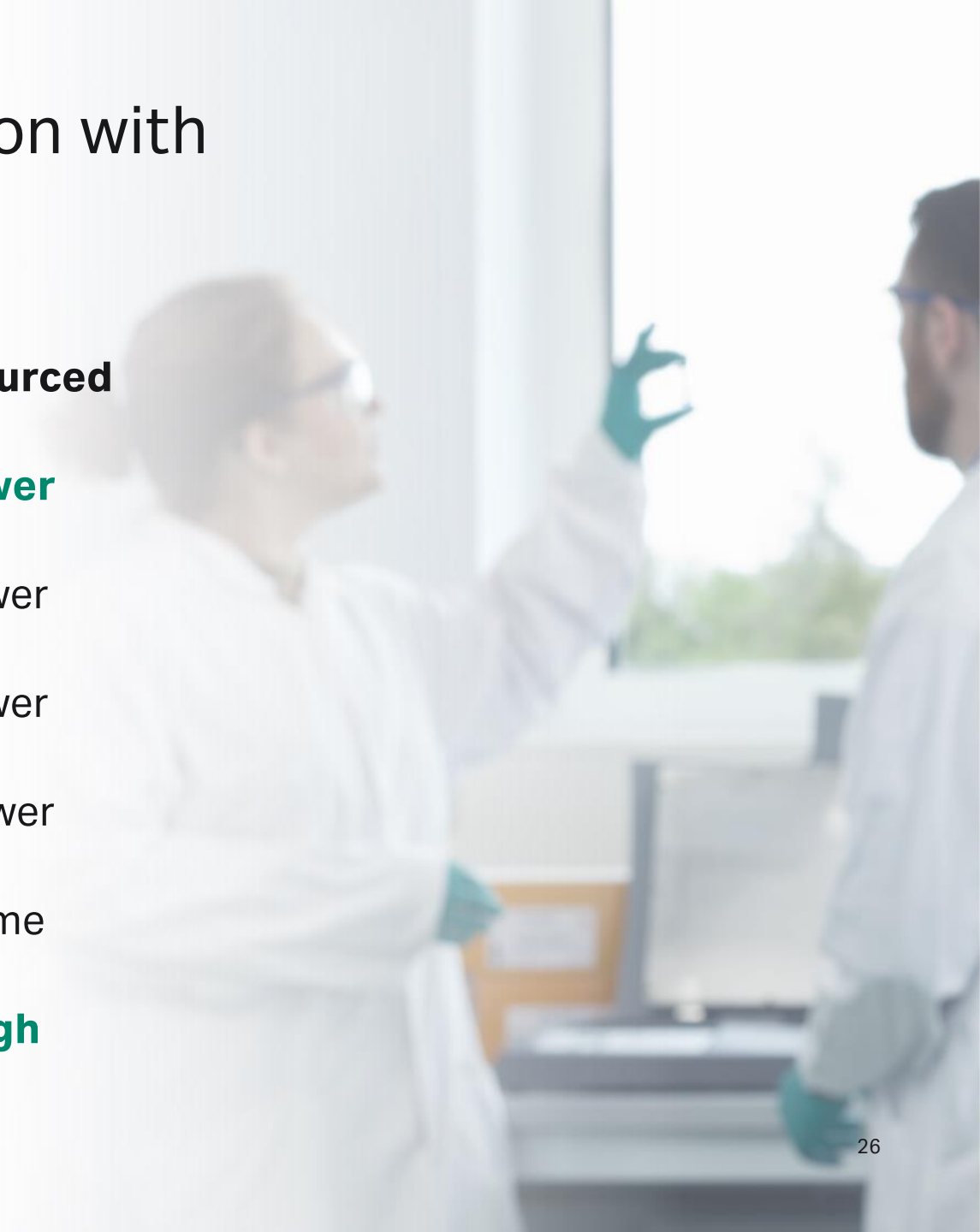
24-cm large format coverage analysis

- Ideal for regulatory filing
- Greater resolving power results in more spots
- Higher protein load increases sensitivity
- Higher spot count gives increased robustness
- Best results obtained with laser biomolecular imaging

Should I outsource HCP risk mitigation?

Maximize control of HCP risk mitigation with in-house management

Consideration	In-house	Outsourced
Costs	High	**Lower**
Data control	**High**	Lower
Process control	**High**	Lower
Reaction time	**Fast**	Slower
Transfer issues	**None**	Some
Expertise	Requires training	**High**



A multifaceted, comprehensive approach is key to success

- Considering your HCP analysis strategy early is essential to reducing risk
- You should test multiple assays to ensure optimal compatibility
- ELISA is the industry standard for HCP quantification
- Your chosen assay should be available for the lifetime of the molecule
- Coverage analysis for antibody-based assays is a regulatory requirement
- Large format coverage workflow is ideal for regulatory filing
- Outsourcing HCP management can be a useful tool

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