Robust HCP coverage analysis with dedicated Melanie software

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Robust HCP Coverage Analysis with Dedicated Melanie Software

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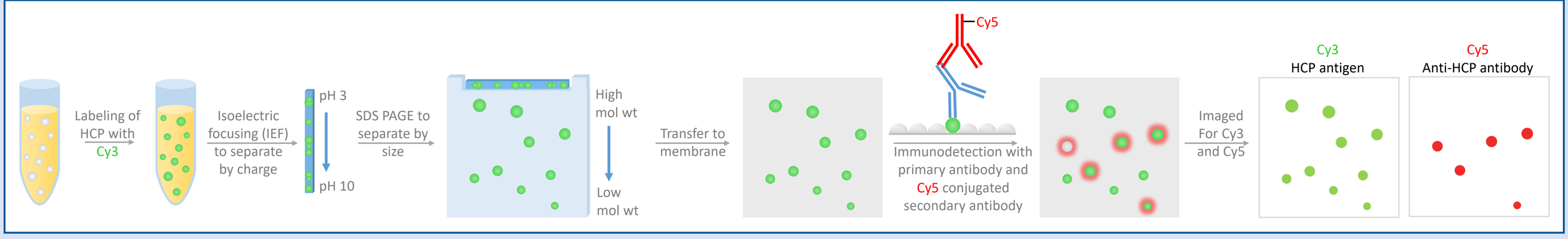
Introduction

Host Cell Protein (HCP) impurities must be carefully identified, minimized and monitored throughout development and manufacturing of biopharmaceutical products to guarantee patient safety and drug efficacy. While HCP ELISA is a critical component of HCP contaminant detection, regulatory agencies require demonstration that the polyclonal antibody mixture used in the ELISA is broadly reactive against a wide range of potential HCPs.

During the development and validation of antibodies, 2D gel electrophoresis followed by Western blotting is a standard approach to determine coverage of the HCP specific antibody, i.e. the percentage of immunodetection the antibody offers for the total population of HCP. Yet, vastly different spot patterns are often seen between independent gels and blots, as well as subjective and time-consuming image analysis with unsuitable tools have presented serious challenges to coverage assessment.

Here we show how the application of 2D Differential in Blot Electrophoresis (2D-DIBE) for the characterization of an anti-CHO cell antibody removes the need for subjective and laborious blot-to-gel matching. Image analysis with the dedicated Melanie™ Coverage software further reduces subjectivity and analysis time.

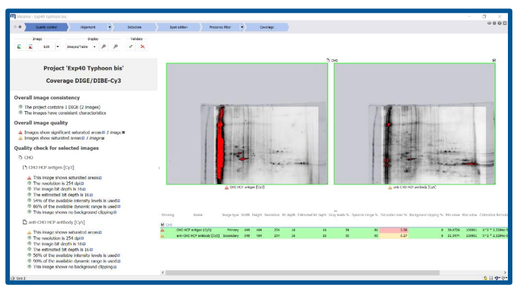
2D Differential in Blot Electrophoresis (2D-DIBE)



Methods

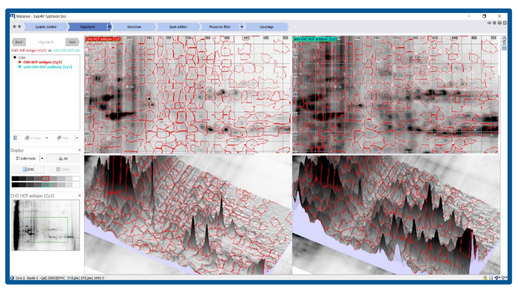
CHO-HCP sample was pre-labeled with Cy3™ and separated on a 2D gel. Protein was transferred from the gel to a LF-PVDF membrane. Blot was incubated with a generic anti-CHO-HCP polyclonal antibody (Rockland Inc.) and a Cy5™ pre-labeled secondary antibody. Blot images were acquired with Amersham™ Typhoon™ at 100 µm resolution, saved as 16 bit images and analyzed with Melanie Coverage software, using the dedicated workflow and settings below.

Image Analysis Workflow



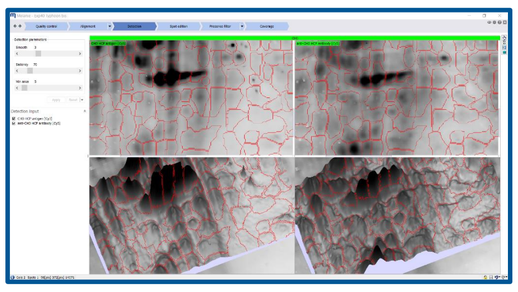
Quality control

A coverage DIGE/DIBE project was created and images imported. Cy3 was indicated as dye for the primary image (useful when analyzing replicate DIBE pairs). Automatic image quality checks were applied. Images were cropped and the contrast adjusted.



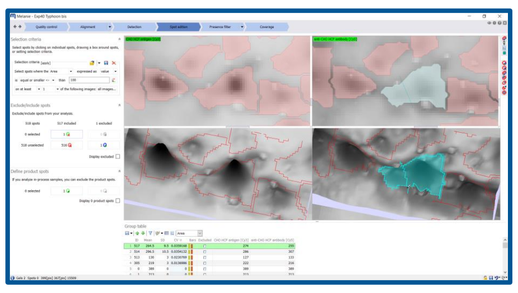
Alignment

As the 2D-DIBE images are from the same blot, no alignment was necessary. For alignment of replicate DIBE pairs, dedicated tools allow systematic review of alignment pairs (only primary images need be aligned). Where necessary, automatic or user matches can be edited in 2D and 3D views.



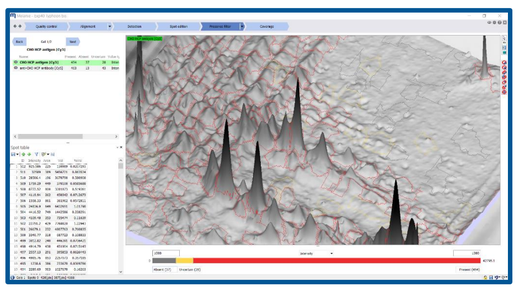
Detection

Spot detection parameters were fine-tuned (Smooth=3, Saliency=70, Min Area=5) and both images were selected to determine the single spot map. 513 spots were automatically detected.



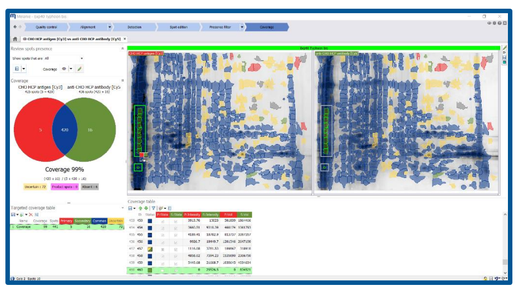
Spot edition

No spots were deleted or excluded. A few spots were added or edited (split, grow, shrink) in the 2D or 3D views while having both images visualized to reduce bias. A total of 520 spots were selected for further review.



Presence filter

For each image, a three level spot filter was applied to categorize spots as absent, uncertain, or present. Intensity was selected for filtering, and the two thresholds applied were 1000/1300 for CHO-HCP antigen and 2000/3500 for anti-CHO-HCP antibody.

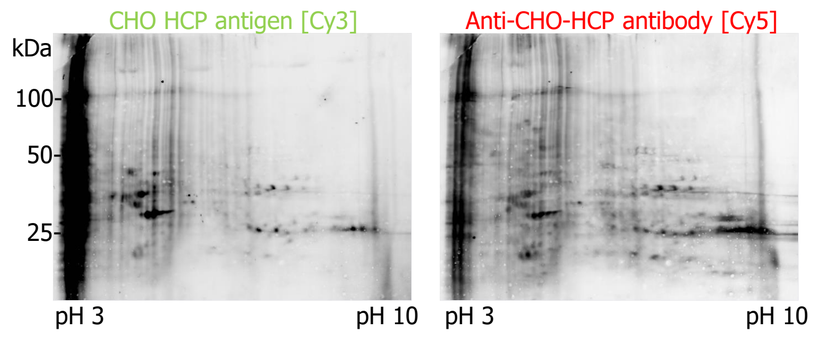


Coverage

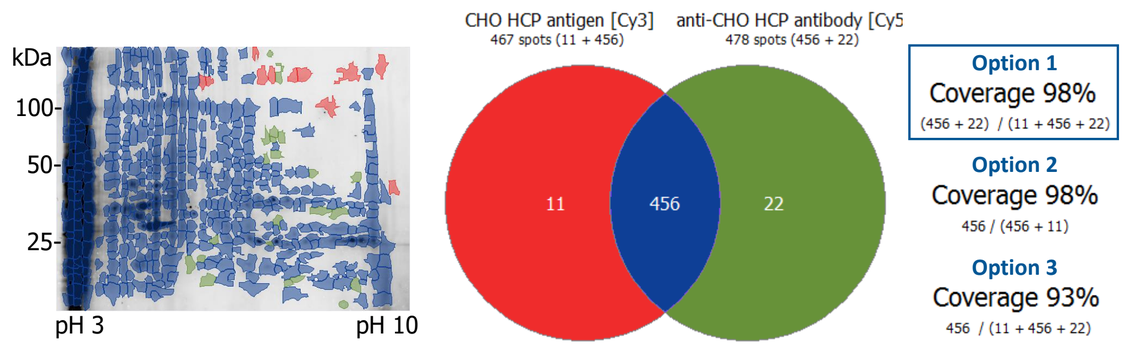
The 74 uncertain spots were quickly reviewed using the 3D view and table to confirm appropriate coverage status (presence or absence on primary image, secondary image, or both). % coverage and Venn diagram were exported after completed review. Total analysis took less than an hour.

Results and Conclusions

Raw images



Coverage results

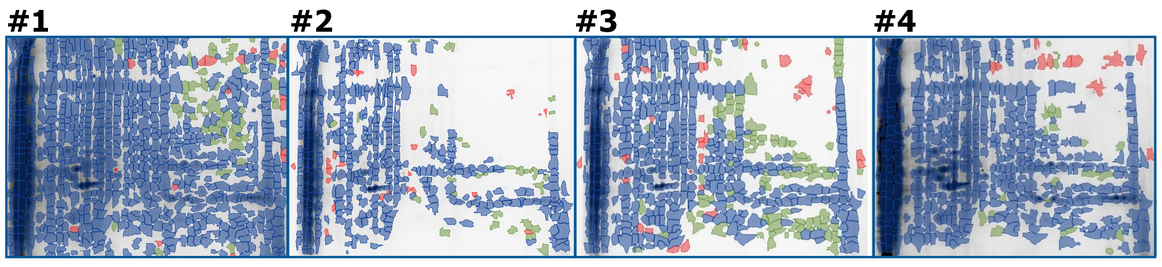


The anti-CHO-HCP antibody presented here shows high immunoreactivity with a coverage of 98% as calculated with the formula in Option 1 (setting in the software). Coverage is even 100% for spots with mol wt < 20 kDa (targeted coverage results not presented here).

Reproducibility

Images were analyzed by 4 different operators from 3 different affiliations, with varying experience with the Melanie Coverage software. All users reported that the analysis took less than an hour. Mean coverage was 96%, with a CV of 2.1%.

Analysis of 2D-DIBE data with Melanie therefore provides reliable, reproducible, unbiased, and fast coverage results to validate antibody reagents.



User	Coverage (%)	Spots	Primary	Secondary	Common
#1	98	764	18	43	703
#2	95	481	23	32	426
#3	94	572	34	101	437
#4	98	489	11	22	456

References

- Amersham Typhoon (www.gelifesciences.com/typhoon), GE Healthcare Life Sciences, Uppsala, Sweden
- Melanie Coverage software (www.2d-gel-analysis.com), developed by SIB Swiss Institute of Bioinformatics, Geneva, Switzerland and available from GE Healthcare Life Sciences, Uppsala, Sweden and GeneBio, Geneva, Switzerland.
- HCP-antibodies (http://www.rockland-inc.com/Host-Cell-Protein.aspx) developed by Rockland Immunochemicals Inc. Limerick, PA 19646, USA