2D-SDS PAGE Analysis Methods For Determining HCP Coverage Using Process Derived anti-HCP Polyclonal Antibodies

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ABSTRACT

Analysis of 2D-SDS spots was performed using two different software systems: Biorad's PD Quest spot analysis software and Total Lab's Spot Map software. Images were obtained using the Biorad Chemi Doc system. Gels were run with 100µg of protein and silver stained with a development time of 120 seconds. Protein was transferred from gels to PVDF membranes and blots were incubated with sera obtained from animals producing anti-HCP antibodies. Chemiluminescent FemtoMax™ Super Sensitive HRP Substrate was used as the 2° antibody and images were captured at three exposure times: 1.0 sec, 3.2 sec, 5.5 sec. We will demonstrate the workflow of each spot analysis software as well as variations in determining final coverage percentage of anti-HCP antibodies on the blot.

INTRODUCTION

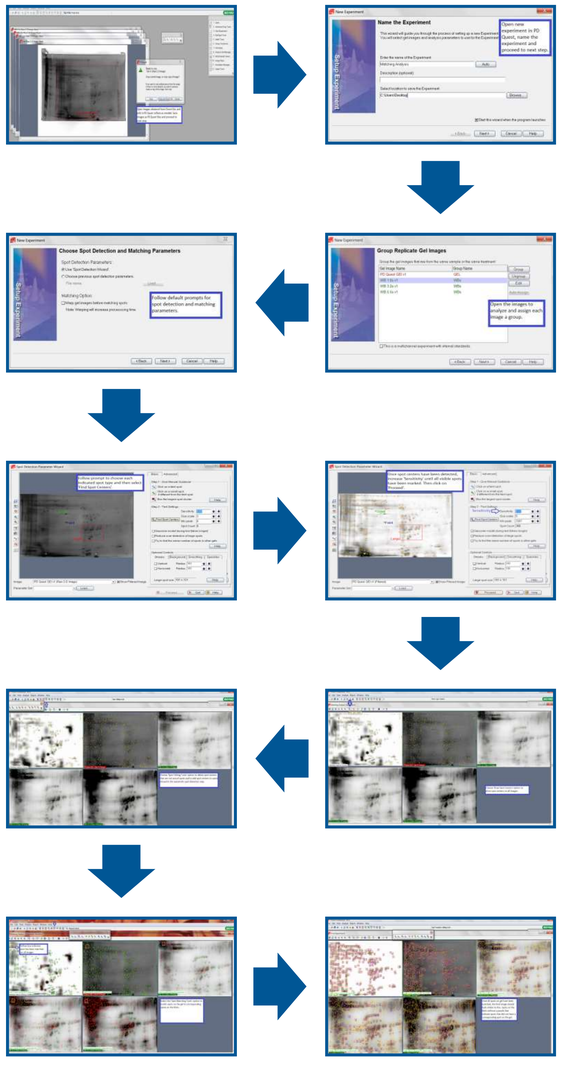
Process-related impurities from host cell proteins (HCP) can potentially contaminate large molecule biopharmaceutical products such as therapeutic monoclonal antibodies and elicit a negative immune response from patients. As such HCPs need to be identified and monitored throughout the drug development and manufacturing process to ensure efficacy of drug substance as well as to prevent any untoward health issues in patients. Clearance of HCPs through downstream purification is required to produce safe and efficacious therapeutic proteins. To investigate the presence of residual contamination with HCPs during the bioprocessing purification stream and in the final biopharmaceutical product, the development of customized polyclonal antibody reagents with maximum coverage against native HCP extracts is required.

In the development of an HCP antibody, the immunogenicity and abundance of individual HCPs can vary widely presenting a unique challenge. Validation of the HCP antibody at every step of development is key to ensure good coverage. The implementation of two-dimensional (2D) SDS-PAGE assessment, by which complex protein mixtures are separated according to isoelectric point and molecular weight followed by western blotting, can deliver additional insight into the capability of an anti-HCP polyclonal antibody. Here we present the characterization of a process specific anti-Pichia pastoris HCP antibody using 2 different analysis platforms: Biorad's PD Quest and Total Lab's Spot Map. The ultimate goal of performing 2D SDS-PAGE is to determine the coverage of the anti-HCP polyclonal antibody in a sample of HCPs. A higher percentage of coverage indicates that HCPs are being recognized by more anti-HCP polyclonal antibodies. These anti-HCP polyclonal antibodies can in turn be used to purify drug substance of a HCPs at appropriate points in the developmental process. The aim in 2D SDS-PAGE analysis is to utilize an analysis software that is straightforward and consistent in providing coverage data over a varying set of exposure times.

WORKFLOW

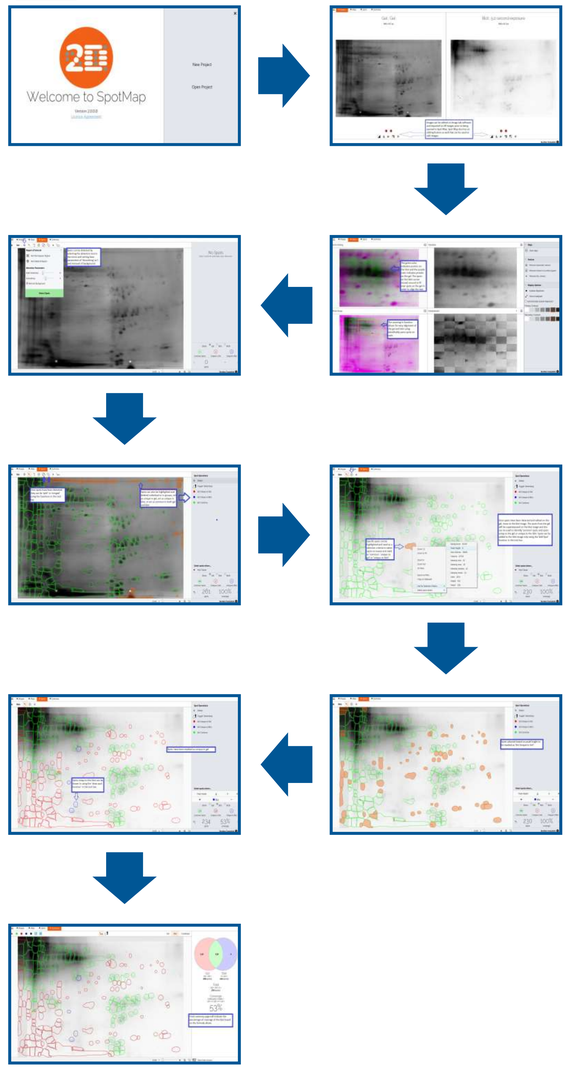
PD Quest Analysis Workflow

Figure I



Spot Map Analysis Workflow

Figure 2



RESULTS

Images Before Analysis

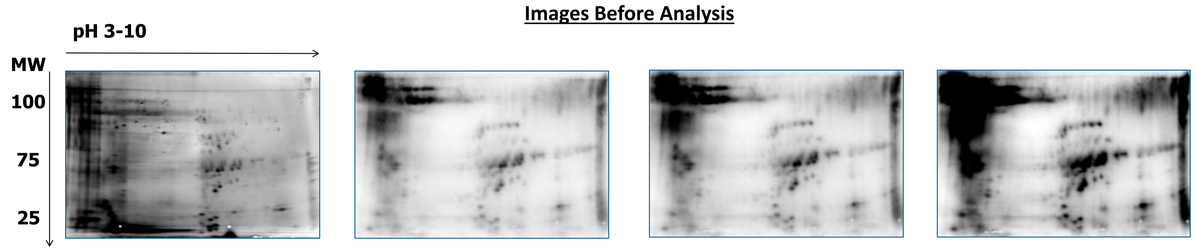


Figure 3A: Silver stained gel. 120 second development time. Imaged on a Chemi Doc Imaging system.

Figure 3B: 1.0 Second exposure time using Femtomax agent. Imaged on a Chemi Doc Imaging system.

Figure 3C: 3.2 Second exposure time using Femtomax agent. Imaged on a Chemi Doc Imaging system.

Figure 3D: 5.5 Second exposure time using Femtomax agent. Imaged on a Chemi Doc Imaging system.

Determining Coverage

PD Quest Analysis

Figure 4A: Gel Image

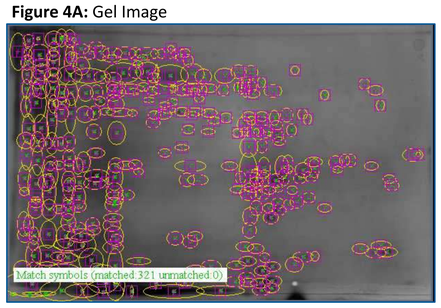
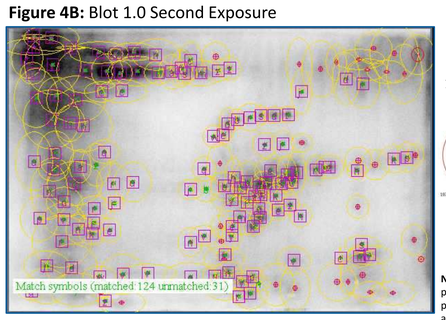
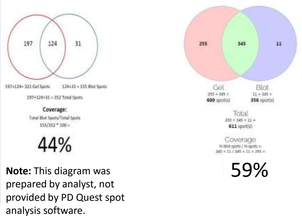


Figure 4B: Blot 1.0 Second Exposure

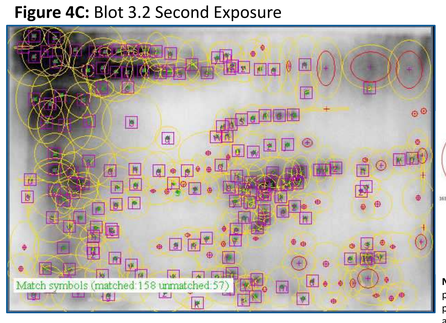


1.0 Second Exposure Coverage Comparison

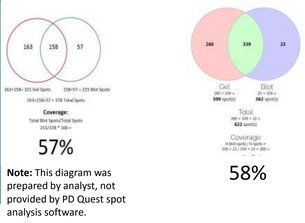


Note: This diagram was prepared by analyst, not provided by PD Quest spot analysis software.

Figure 4C: Blot 3.2 Second Exposure

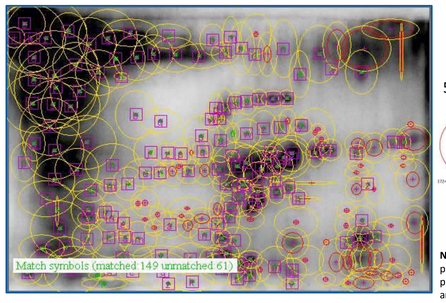


3.2 Second Exposure Coverage Comparison

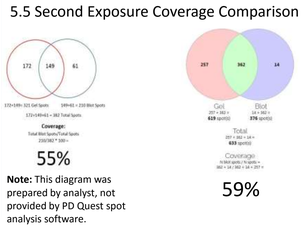


Note: This diagram was prepared by analyst, not provided by PD Quest spot analysis software.

Figure 4D: Blot 5.5 Second Exposure



5.5 Second Exposure Coverage Comparison



Note: This diagram was prepared by analyst, not provided by PD Quest spot analysis software.

Spot Map Analysis

Figure 5A: Gel Image

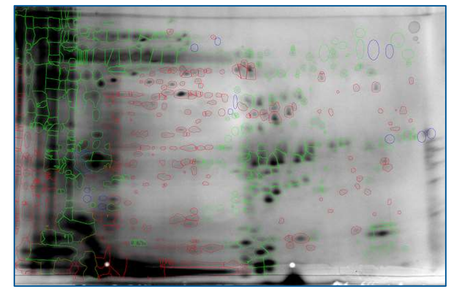


Figure 5B: Blot 1.0 Second Exposure

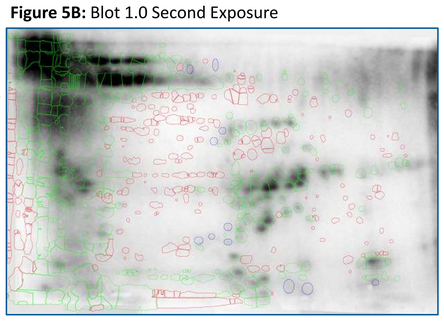


Figure 5C: Blot 3.2 Second Exposure

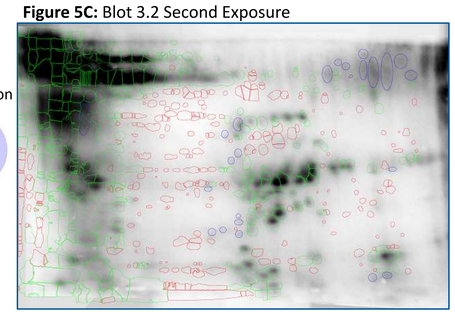
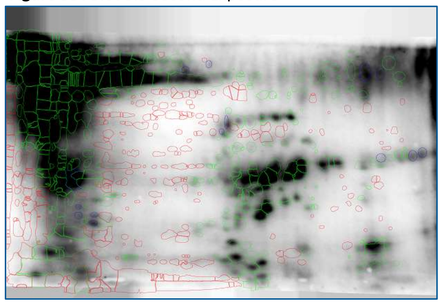


Figure 5D: Blot 5.5 Second Exposure



CONCLUSIONS

- Both spot analysis methods generated a final coverage that was between 44 – 59%. However there was much more variation in coverage between the 1.0 second exposure and 5.5 second exposure (11%) using the PD Quest Spot analysis.
- Spot analysis using Spot Map resulted in coverage that was consistent within 1% between all three blot exposure times.
- Workflow between the two analysis systems varied by about 2 hours – analysis took approximately 1 hour using the Spot Map software and approximately 3.5 hours using the PD Quest software.
- PD Quest spot analysis software offers numerous options and parameter selection criteria whereas Spot Map software has limited parameter selection but requiring fewer steps in the workflow to determine antibody coverage.

REFERENCES

1. SpotMap v2. TotalLab Ltd, Keel House, Garth Heads, Newcastle-upon-Tyne, United Kingdom
2. PDQuest 2-D analysis software. Bio-Rad Laboratories, Inc. Hercules, California 94547 USA.