Multi-assay Functional Antibodies in HCP Detection

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ABSTRACT

Process-related impurities from host cell proteins (HCP) potentially contaminating large molecule biopharmaceutical products must be identified and monitored to guarantee the safety of the material and health of the drug recipient. HCPs may be residual contaminants left behind during the purification process from the expression hosts, such as *E.Coli*, insect, or mammalian cells and may potentially result in adverse events in patients. To investigate the presence of residual contamination 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. Highly immunogenic proteins can disguise the effects of other, similar size proteins. Choosing the proper immunization strategy in combination with the validation of the HCP antibody at every step of development are 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 with respect to immunocoverage of protein components of the host cell lysate. We present generic anti-HCP polyclonal antibodies (generated for bacterial and mammalian proteins) as an alternative to customized reagents that are functional for 2D WB and ELISA.

These HCP antibodies display sensitivity and specificity against a broad range of HCPs, measured by a robust 2D electrophoresis assay, to provide efficient immunocoverage against potential contaminants of biopharmaceutical products.

INTRODUCTION

To prepare an anti-HCP antibody a representative sample of the HCP extract is required. This sample can be taken from many stages of the bioprocess and the sample selection point can be critical for obtaining the correct antibody reagent. Often sample points are selected early in bioprocessing but downstream from the crude sample. Extracts or samples are derived from a number of host organisms including *E.coli*, mammalian host systems (i.e.CHO, HEK293), yeast and others. In general, the HCP immunogen is a complex mixture of either a crude sample or in-process sample. In certain cases poorly immunogenic antigens can be either enriched or chemically modified (i.e. alkylation) to increase the immunogenicity of the HCP extract. Mammalian HCP target proteins are not as immunogenic as bacterial host proteins and require a more intensive immunization strategy to achieve reasonable coverage in 1D and 2D Western blot.

Anti-HCP antibodies may be generated using a variety of immunization protocols. The use of complementary immunization strategies offers the advantage of overcoming the limitations found in any individual methods. Several effective strategies can be deployed individually or in combination when raising antibodies to HCPs including:

a. Fractionation of HCP proteins into low molecular weight (LMW), high molecular weight (HMW) and secreted protein components for separate immunizations

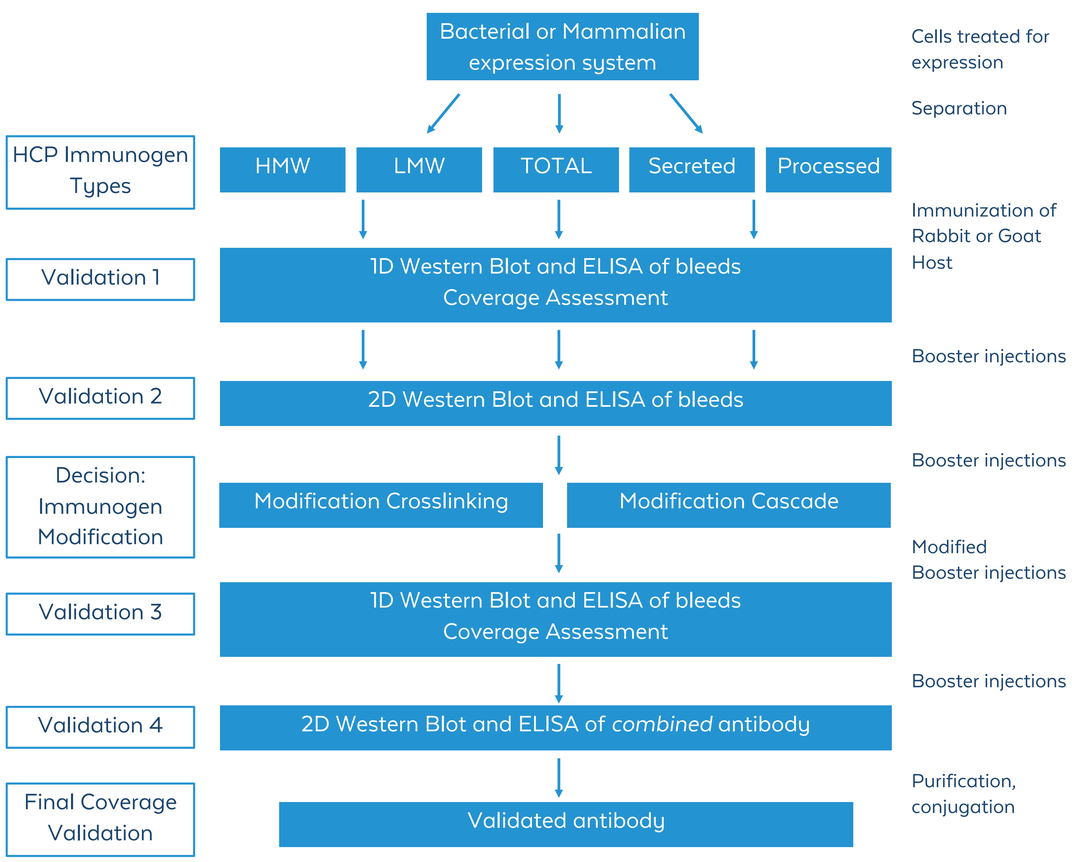
b. Cascade immunization where an initial immunization with TOTAL HCPs is followed by booster injections with a subset of less abundant or immunorecessive HCPs prepared by subtractive immunoprecipitation using initial antibodies raised against the most abundant and immunodominant HCPs present in the total HCP lysate

c. Chemical modification or crosslinking of antigen sample for booster injections

d. The use of multiple host species to control for differences in host animal immune responses, where typically mammalian and avian host animals are used

Here we present methods and data for HCP anitibody production using antigens from different hosts using different HCP sample types. These immunogens were used to immunize and boost groups of rabbit hosts animals. The antibodies were validated by a robust 2D electrophoresis assay and display sensitivity and specificity against a broad range of HCPs to provide efficient immunocoverage against potential contaminants of biopharmaceutical products.

HCP SAMPLE PREPARATION STRATEGIES



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ANTI-HCP ANTIBODY DEVELOPMENT & 2D HCP SAMPLE ANALYSIS

One of the pitfalls of 2D SDS-PAGE is the resolution of all proteins in the first dimension. We have developed a protocol to maximize the resolution and validate our HCP antibodies. To investigate the complexity of the total HCP lysate, the sample was resolved by 2D SDS-PAGE gel and stained using the fluorescent dye Oriole stain (LOD ~0.5ng) (Figure 1).

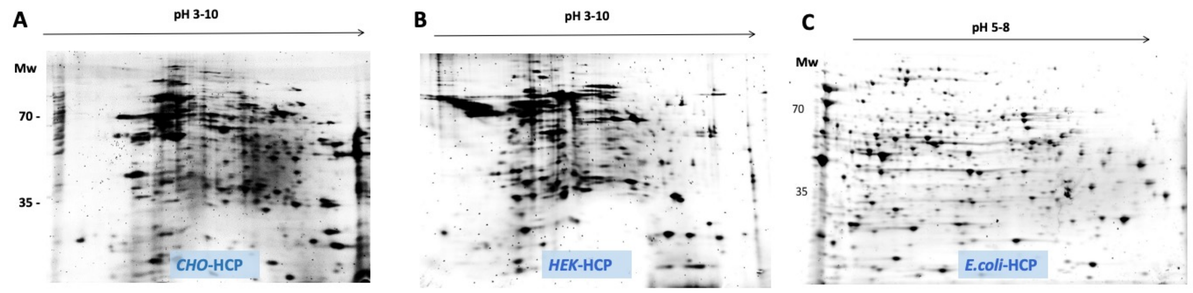

Figure 1: 2D SDS-PAGE analyses of total HCP lysate stained with Oriole protein stain. (A) CHO HCP total protein, (B) HEK293 HCP total protein and (C) *E.Coli* total protein sample.

IMMUNOCOVERAGE CHANGE OVER TIME AND BOOSTING

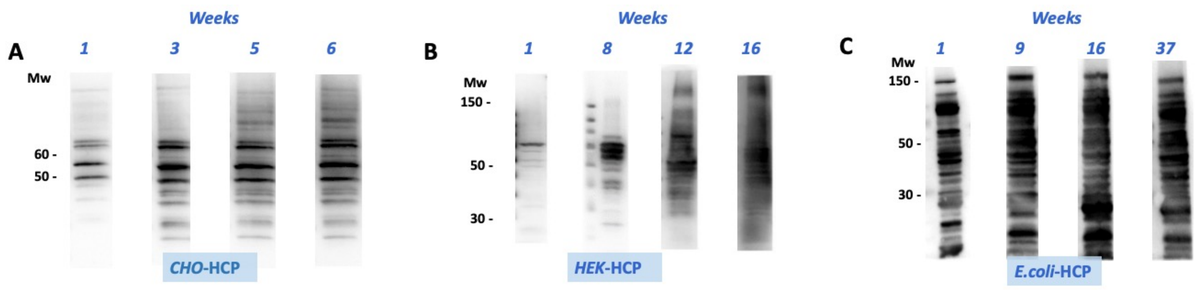

Figure 2: 1D Western blot analysis of anti-HCP antibodies against total HCP lysates. Lysates were separated on 1D SDS-PAGE and blotted on PVDF to analyze the increase of immunocoverage by time and boosting type. (A) CHO HCP total protein, (B) HEK HCP total protein and (C) *E.Coli* total protein sample.

ANTIBODY VALIDATION BY 2D ELECTROPHORESIS

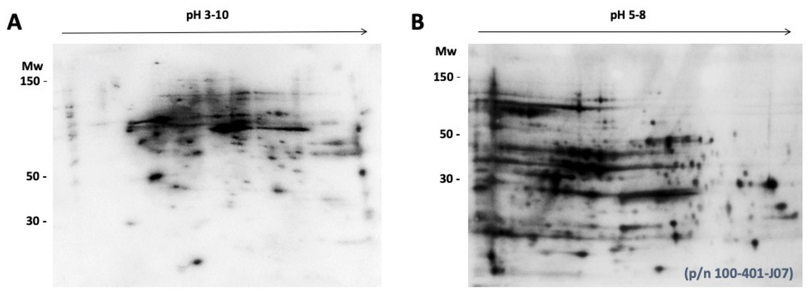

Figure 3: Western blot analysis of anti-HCP antibodies against total HCP lysates. Lysates were separated on 2D SDS-PAGE and blotted on PVDF to analyze immunocoverage of the best antibody. Antibodies were purified by Protein A and used to assay for coverage levels. (A) CHO HCP total protein, (B) *E.Coli* total protein sample.

ADVANCED VALIDATIONS

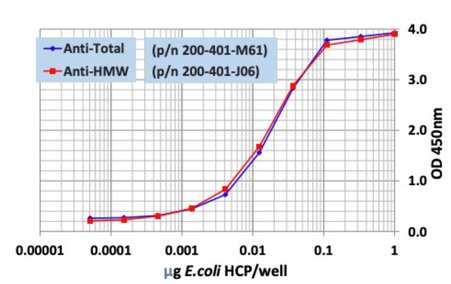

Figure 4: ELISA analysis of anti-TOTAL *E.coli* HCP antibody (p/n 200-401-M61) and anti-HMW *E.coli* antibody (p/n 200-401-J06). Total *E.coli* HCP proteins were coated on Immulon HBX ELISA plates and titered 3-fold. The plates were blocked with TMB (p/n TMBE-0100), and probed with each antibody. The LOD of both antibodies is ≥ 5ng of HCP protein.

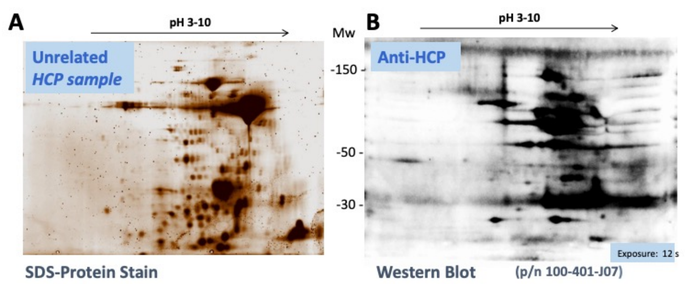

Figure 5: Western Blot analysis of anti-HCP *E.coli* (cocktail) antibody (p/n 100-401-J07) on a process unrelated *E.Coli* protein. Total HCP proteins of an unrelated process using an *E.coli* strain were resolved by 2D electrophoresis stained with Oriole protein stain (A) or probed with Rockland's anti-HCP *E.coli* (cocktail) antibody (p/n 100-401-J07) (B). By visual assessment it is evident that the generic antibody has very good coverage. This demonstrates that this generic HCP antibody can be integrated into the early stage analysis of multiple *E.coli* bioprocessing projects.

CONCLUSION & FUTURE DEVELOPMENT

Here we present various possibilities for the development of effective HCP antibodies validated using a robust 2D electrophoresis assay to provide efficient immunocoverage of HCPs from various hosts. We demonstrate that 2D electrophoresis is a necessary quality control assay in the development of anti-HCP antibodies. Relying solely on 1D blotting can yield misleading assessments of antibody coverage that in contrast are fully resolved in two dimensions.

Rockland offers a variety of generic HCP detection products like KCA-J07, an *E.coli* HCP Western Blot kit that contains 2D validated antibodies, HRP conjugated secondary antibodies, blocking and washing solutions and *E.coli* control proteins. Rockland's 2D validated antibodies have been shown to react to over 300 *E.coli* proteins from SDS/DTT solubilized *E.coli* cells.

For mammalian HCP antibody generation we recommend modification of the immunogen for booster immunization to improve the detection coverage. Rockland is currently in the process of developing additional HCP antibodies against other species validated using the methods outlined here.

Rockland offers custom HCP production and provides multiple customizable options for HCP antibody development. These options allow flexibility to the researcher to develop early stage through late stage HCP detection reagents.