Generation of Host Cell Protein Reagents for Development of a Process-Specific Host Cell Protein Assay

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ABSTRACT

Generation of pure biopharmaceuticals requires development of sensitive quantitative analytical methods for detection of process related impurities. One such analytical tool is the detection of residual host cell proteins (HCPs) in the final drug product. The industry standard for detection of residual HCPs is commercially available "generic" HCP enzyme-linked immunosorbent assays (ELISAs). Regulatory agencies suggest that biopharmaceutical companies develop "process-specific" HCP assays in order to increase assay sensitivity to detect HCPs that are specific to a particular production cell line or process. We developed a process-specific HCP ELISA for a product that is currently in a Phase III clinical trial. The source of the HCPs for use as the antigen to generate anti-HCP antibodies is essential to developing a sensitive assay with broad coverage. Here we compare the HCP profile from culture supernatant after a full production run and after the first capture step. After careful analysis, HCPs from a production run were used to immunize rabbits for generation of anti-HCP antibodies. During immunization, rabbit sera were tested by ELISA for anti-HCP titers and western blot to assess the range of HCP reactivity. HCP affinity purified and total rabbit IgG were then analyzed by 2D western blot for HCP reactivity.

RESULTS

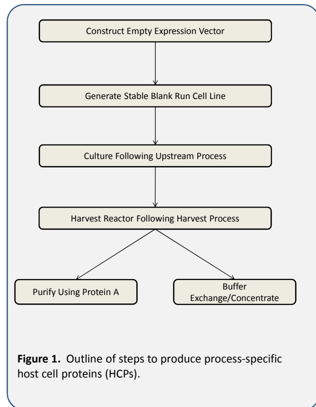

Figure 1. Outline of steps to produce process-specific host cell proteins (HCPs).

Figure 2. SDS-PAGE of harvest and protein A eluate. Harvest and protein A eluate were compared reduced and non-reduced on an Bis-Tris 4-12 % gel stained with Coomassie Blue. Lane 1 and 2: harvest at 2.5 and 5 ug. Lane 3: MabSelect eluate at 1.22 ug. Lane 4 and 5: blank. Lane 6: molecular weight standard. Lane 7: harvest. Lane 8: MabSelect eluate. Lanes 1-3 are non-reduced and lanes 7 and 8 are reduced.

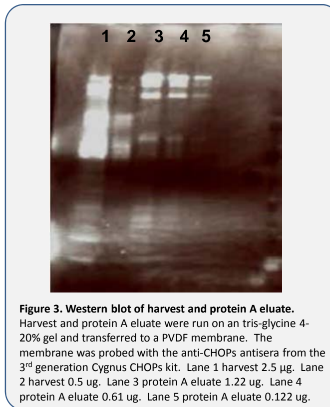

Figure 3. Western blot of harvest and protein A eluate. Harvest and protein A eluate were run on an tris-glycine 4-20% gel and transferred to a PVDF membrane. The membrane was probed with the anti-CHOPs antisera from the 3rd generation Cygnus CHOPs kit. Lane 1 harvest 2.5 µg. Lane 2 harvest 0.5 ug. Lane 3 protein A eluate 1.22 ug. Lane 4 protein A eluate 0.61 ug. Lane 5 protein A eluate 0.122 ug.

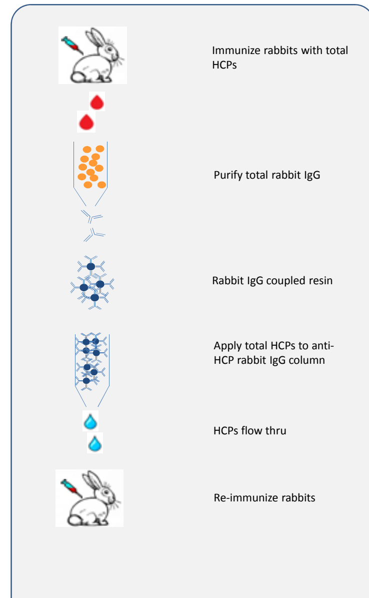

Figure 4. Cascade immunization of rabbits. Rabbits were immunized several times with total HCPs. Total IgG was purified from pooled rabbit sera and conjugated to resin. HCPs were then loaded onto the column. HCPs that flowed through the column were collected and used to immunize the rabbits to increase the chance of generating rabbit antibodies to these HCPs.

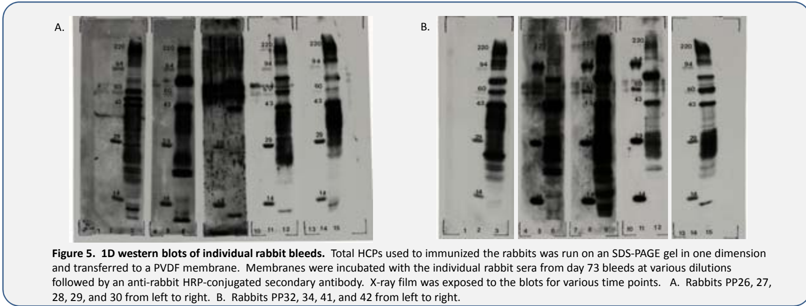

Figure 5. 1D western blots of individual rabbit bleeds. Total HCPs used to immunized the rabbits was run on an SDS-PAGE gel in one dimension and transferred to a PVDF membrane. Membranes were incubated with the individual rabbit sera from day 73 bleeds at various dilutions followed by an anti-rabbit HRP-conjugated secondary antibody. X-ray film was exposed to the blots for various time points. A. Rabbits PP26, 27, 28, 29, and 30 from left to right. B. Rabbits PP32, 34, 41, and 42 from left to right.

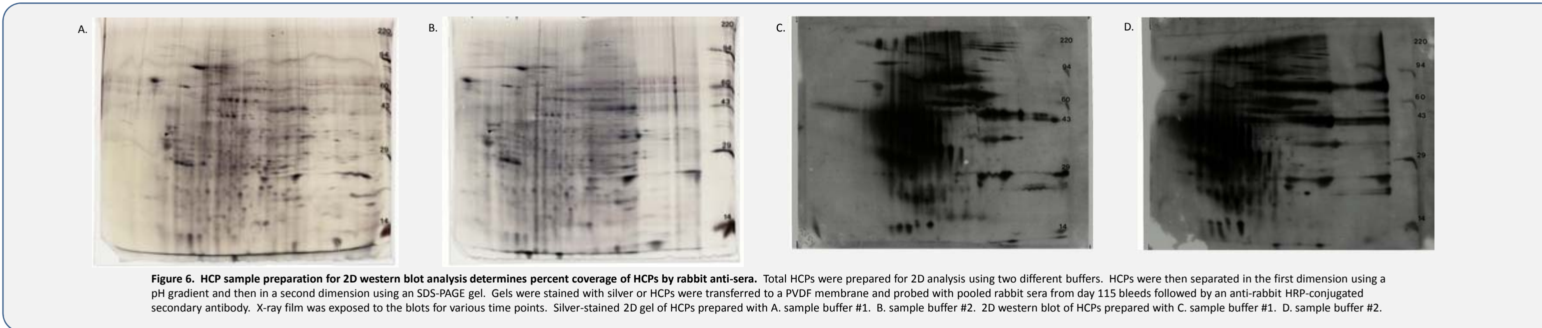

Figure 6. HCP sample preparation for 2D western blot analysis determines percent coverage of HCPs by rabbit anti-sera. Total HCPs were prepared for 2D analysis using two different buffers. HCPs were then separated in the first dimension using a pH gradient and then in a second dimension using an SDS-PAGE gel. Gels were stained with silver or HCPs were transferred to a PVDF membrane and probed with pooled rabbit sera from day 115 bleeds followed by an anti-rabbit HRP-conjugated secondary antibody. X-ray film was exposed to the blots for various time points. Silver-stained 2D gel of HCPs prepared with A. sample buffer #1. B. sample buffer #2. 2D western blot of HCPs prepared with C. sample buffer #1. D. sample buffer #2.

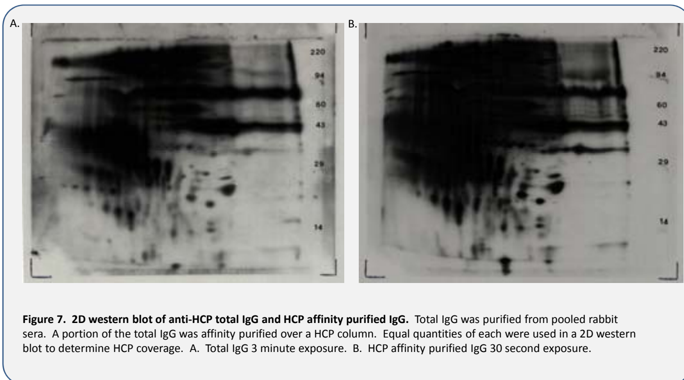

Figure 7. 2D western blot of anti-HCP total IgG and HCP affinity purified IgG. Total IgG was purified from pooled rabbit sera. A portion of the total IgG was affinity purified over a HCP column. Equal quantities of each were used in a 2D western blot to determine HCP coverage. A. Total IgG 3 minute exposure. B. HCP affinity purified IgG 30 second exposure.

Animal ID	Day 0	Day 31	Day 53	Day 73	Day 94	Day 115	Day 165	Day 193	Day 221	Day 249	Day 291
PP 26	<100	800	2,450	11,600	7,350	14,200	9,610	10,200	8,460	6,380	7,770
PP 27	425	1,130	2,490	8,840	4,790	7,590	9,740	14,000	11,600	11,900	9,730
PP 28	<100	309	520	2,550	2,090	5,020	6,490	Exsanged	Exsanged	Exsanged	Exsanged
PP 29	156	1,480	963	2,550	2,130	4,730	4,110	5,410	7,600	4,910	6,210
PP 30	<100	1,250	1,960	4,340	3,830	10,600	8,420	10,500	9,450	7,030	8,820
PP 32	<100	280	2,980	4,490	4,770	10,400	6,370	9,440	9,660	8,830	8,740
PP 34	<100	608	2,260	16,800	9,990	10,300	7,850	12,500	11,800	10,100	13,300
PP 38	<100	2,890	4,260	10,500	8,050	12,700	5,060	7,720	5,990	4,900	6,100
PP 41	<100	1,840	2,080	1,640	1,780	2,710	1,870	Exsanged	Exsanged	Exsanged	Exsanged
PP 42	<100	100	925	3,090	2,940	7,540	6,170	5,240	6,660	8,830	8,960

Table 1. 50% ELISA titers of individual rabbit sera to HCPs. ELISA plates were coated with total HCPs, washed, and blocked. Plates were then incubated with rabbit sera at various serial dilutions followed by an anti-rabbit HRP-conjugated secondary antibody. 50% titer is the value midway between the highest signal and background fore each rabbit sera. Based on antibody titer and HCP coverage rabbits PP28 and PP41 were terminated from the study.

CONCLUSIONS

- The source of HCPs is important in generating anti-sera with broad HCP coverage. Culture harvest was selected over protein A eluate as the former would potentially result in the ability to detect more residual HCPs in the final product.
- 1D western blots showed that individual rabbits had distinctive immunological responses to the HCPs with most having a broad range of coverage.
- HCP sample preparation for 2D western blots can alter the results of the coverage of the anti-HCP rabbit sera. Sample prep buffer #1 resulted in 53% coverage while sample prep buffer #2 resulted in 74% coverage.
- ELISA titer data over the duration of the immunization demonstrated a range of immunological responses from each rabbit with some having significant anti-HCP responses lasting the duration of the study.
- 1D western blot and ELISA titer data were used to select the animal sera to pool to generate the final anti-HCP antibody reagents.
- Total IgG and HCP affinity purified IgG resulted in different levels of HCP reactivity with the affinity purified IgG having better coverage and greater sensitivity.

ACKNOWLEDGEMENTS

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