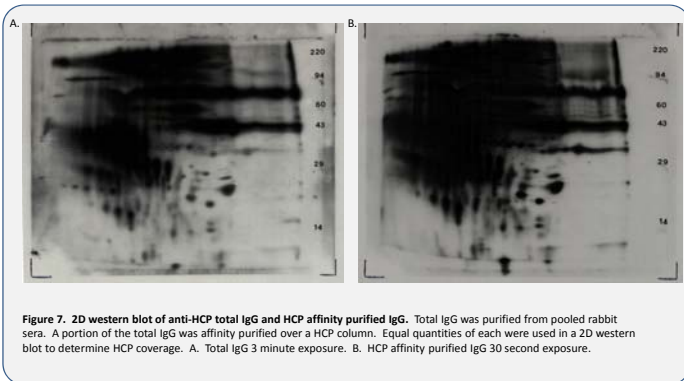
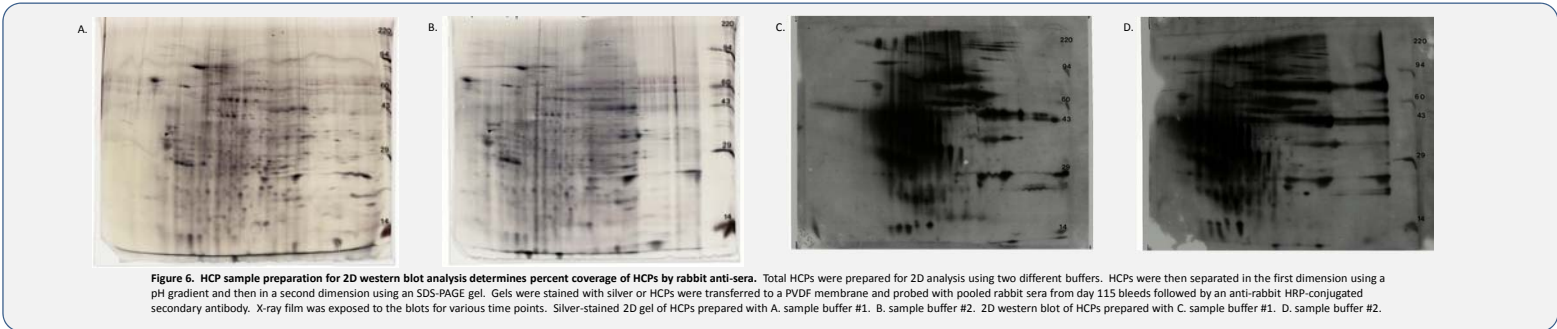
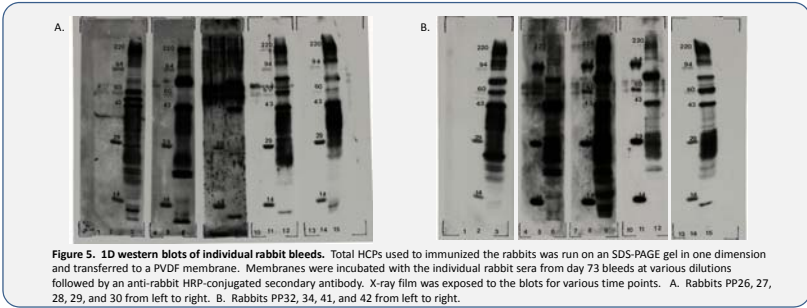
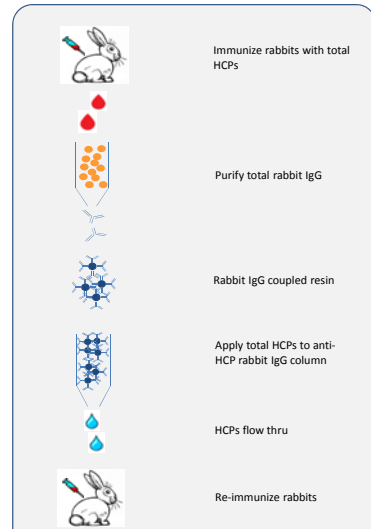
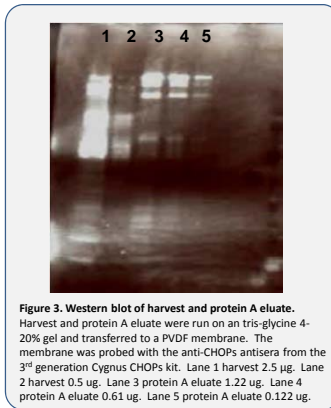
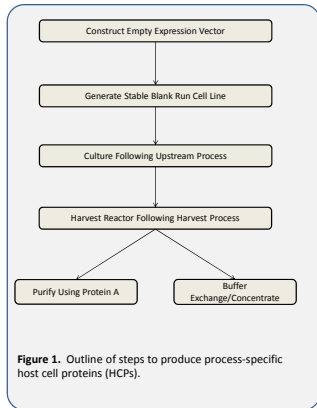


ABSTRACT

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



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 for 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

- Covance Research Products generated the HCP anti-sera.
- Kendrick Laboratories performed the 1D and 2D western blots.
- We would like to thank the Process Science group for their technical and editorial support.
- We would also like to thank Steve King, Rob Garnick, and Larry Dumont for their insightful discussions and technical guidance with this project.