Development of E.*coli* Host Cell Proteins generic ELISA and A Comparison with *generic* E.*coli* HCP ELISA available commercially.

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INTRODUCTION

New generation pharmaceuticals manufactured by the biotechnology route can be contaminated by residual host cell proteins during the cell culturing or fermentation stage. These proteins may cause an immune reaction in patients leading to manufacturers being advised to comply with these as per ICH and FDA guidelines. In the present study, we developed a generic version of an immunoassay to detect host cell proteins generated in E.*coli*. The antibodies used to manufacture the immunoassay were determined in view of the cell line frequently used for manufacturing. We also compared the sensitivity and specificity with alternate immunoassay kit available commercially.

MATERIALS AND METHODS

The antigen was prepared from non-pathogenic clinical isolates and commonly used laboratory strains K12, ATCC 37197, TOP 10F, JM109, HB101, XS127/P, C236/P3 with a cocktail blend of BL21 and DH5 alpha. Goat polyclonal antibodies were raised against the host cell proteins from this cocktail blend of strains. The identification and affinity was confirmed using SDS-PAGE and Western Blotting.

The secondary antibody was conjugated to horse radish peroxidase (HRP). The assay optimization was done using the protein available. Microtitre plate (Corning Costar, USA) were coated with the polyclonal antibodies and checkerboard titration was performed to optimize reagent concentrations. Using this design analysis of different concentrations of the two reagents (coating antibody and the labeled antibody) in each well were done to obtain the best noise : signal ratio. Proprietary blocking solution, stabilizers and substrates were used (Surmodics Inc; USA) to block non-specific binding and improve noise : signal ratio.

The best noise : signal ratio was chosen and the kit standardized. Liquid stabilizers were used (Surmodics Inc; USA) and elevated shelf life studies were conducted to establish the shelf life of the reagents (coated microtitre plate and the secondary antibody conjugated HRP.

RESULTS

Standard curve for the ELISA was performed using the quantified antigen as standards. Figure 1A shows the standard curve obtained from the E.*coli* HCP ELISA. The Standards were prepared at various concentrations in RPMI-1640 containing 10% FBS and protein stabilizers. The analytical sensitivity of developed E.*coli* HCP ELISA was investigated and standardized. The detection limit defined as minimal concentration of E.*coli* HCP that produces a signal equal to non-specific background signal + 2SD, of this ELISA, was measured to be less than 890pg/ml and the limit of quantitation as 1.33 ng/ml.

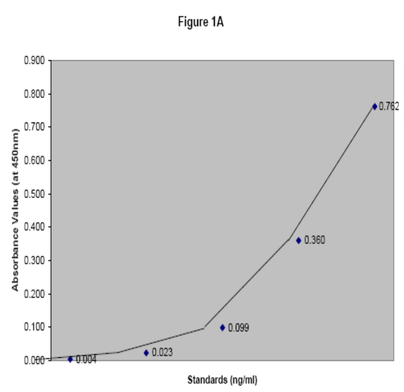

Figure 1A

Standards	Absorbance at 450nm
1 ng/ml	0.004
3 ng/ml	0.023
12 ng/ml	0.099
40 ng/ml	0.360
100 ng/ml	0.762

COMPARISON WITH ELISA COMMERCIALLY AVAILABLE

A commercially ELISA kit for E.*coli* HCP was commercially procured from Cygnus Technologies Inc., USA. Cell lysate samples were prepared for E.*coli* and HCPs generated. The cell lysate concentrate was centrifuged at 2000 rpm and the supernatant removed and tested with both the kits at various dilutions.

Analysis done by using Cygnus Technologies ELISA

Samples	OD @ 450nm	Avg OD @ 450nm	HCP ng/ml	Avg HCP ng/ml	Dilution Factor (DF)	Avg HCP ng/ml*DF	SD	%CV	Dilution Variation
Sample-(1:2560)	0.461	0.463	45.79	45.94	2560	117624.32	0.002	0.4	100.0
	0.464		46.09						
Sample-(1:5120)	0.254	0.260	25.92	26.44	5120	135390.72	0.008	2.9	115.1
	0.265		26.95						
Sample-(1:10240)	0.164	0.152	17.45	16.24	10240	166389.76	0.018	11.6	122.9
	0.139		15.04						

Analysis done by using Krishgen Biosystems ELISA

Samples	OD @ 450nm	Avg OD @ 450nm	HCP ng/ml	Avg HCP ng/ml	Dilution Factor (DF)	Avg HCP ng/ml*DF	SD	%CV	Dilution Variation
Sample-(1:2560)	1.486	1.441	>104.95	NR*	2560	NR	0.064	4.5	100.0
	1.395		>104.95						
Sample-(1:5120)	0.935	0.932	>104.95	NR*	5120	NR	0.004	0.5	NR*
	0.929		>104.95						
Sample-(1:10240)	0.564	0.585	66.417	69.50	10240	711633.92	0.029	5.0	NR*
	0.605		72.574						
Sample-(1:20480)	0.288	0.316	31.943	35.08	20480	718469.12	0.040	12.5	101.0
	0.344		38.220						

** NR = Not Reportable*

It was observed that in the Krishgen ELISA the samples of lower concentration below 1:10240 were detected even at dilution ratio of 1:20480. Furthermore the kit was more specific and sensitive in reporting higher values in these cell lysate samples. The %CV at lower concentrations were less than the other ELISA.

CONCLUSION

The Krishgen E.*coli* HCP ELISA reported a higher sensitivity compared to the other commercially available ELISA. Some differences (generally not statistically significant) were observed in the values reported as standards

Cygnus			Krishgen		
Std ng/ml	Avg OD @ 450nm	SD	Std ng/ml	Avg OD @ 450nm	SD
1.5	0.016	0.000	1	0.004	0.001
6	0.055	0.000	3	0.023	0.003
12	0.106	0.001	12	0.099	0.018
40	0.403	0.011	40	0.360	0.022
100	0.915	0.013	100	0.762	0.057

for both kits. The values reported for the samples showed an average 2 fold difference at lower concentrations. Significantly increased values were also reported at lesser %CV for the Krishgen E.*coli* HCP ELISA. From the perspective of using a generic version of an HCP assay kit for in-process controls, it is important to consider this ability to measure HCPs at lower concentrations.