ELISA Based Quantitation of Human Proteins

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INTRODUCTION

Recombinant human proteins expressed in bacterial, insect or rodent cells are used in many *in-vitro* diagnostics and increasingly as *in-vivo* therapeutics for treatment of human diseases.

Yet recombinant human proteins expressed in bacterial, insect or rodent cells have characteristic species-specific post-translational modifications (PTMs), which are quite distinct from human PTMs.

The presence of non-human PTMs can result in inaccurate quantitation of serum proteins in assays and inaccurate prediction of potency and immunogenicity of these proteins *in-vivo*.

Mature human proteins are often highly glycosylated, with glycans representing 20-75% of the molecular mass (Mr).

However bacterial cells, such as *E. coli*, do not glycosylate polypeptides[1,2], thereby exposing abnormal cryptic (immunogenic) sites on recombinant human proteins.

Other non-human cells (NS0 & CHO), add non-human glycan structures[3,4] that may also be immunogenic on human recombinant proteins.

Other differences, such as in methylation and phosphorylation, resulting from expression in non-human cells, can also affect epitope structures.

Consequently, antibodies generated using non-human cell expressed human recombinant proteins may bind differently to the native forms of these same human proteins.

These differences in immunoreactivity could result in inaccuracies in quantitation of the native proteins by immunoassays, which employ these non-human cell expressed human protein standards.

Alterations in epitopes and exposure of cryptic epitopes on the non-human cell expressed proteins can also result in immunogenicity and decreased potency of the recombinant proteins *in-vivo*.

RESULTS

Representative results of two or more experiments are illustrated in Figures 1-4.

Figures 1 & 2 illustrate respectively two and eight-fold differences in quantitation of *E. coli* compared to human cell expressed recombinant human GM-CSF and IL-4, by standard ELISA testing.

ELISA results demonstrated identical immunoreactivity of *E. coli* and human cell expressed human TNF-alpha (Figure 3).

However the human cell expressed TNFRII-Fc based ELISA demonstrated significantly different binding of *E. coli* compared to human cell expressed TNF-alpha (Figure 4).

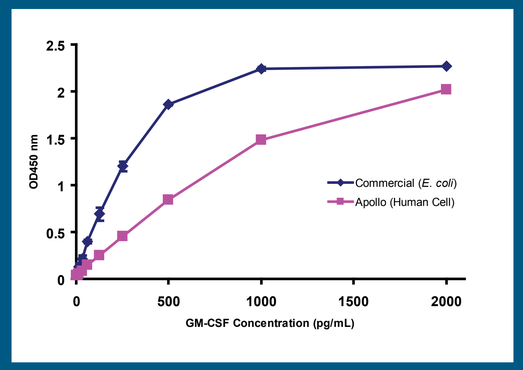

Figure 1: ELISA Assay - *E. coli* vs Human GM-CSF[hcx]

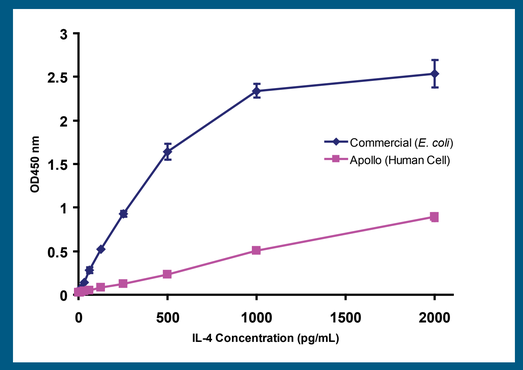

Figure 2: ELISA Assay - *E. coli* vs Human IL-4[hcx]

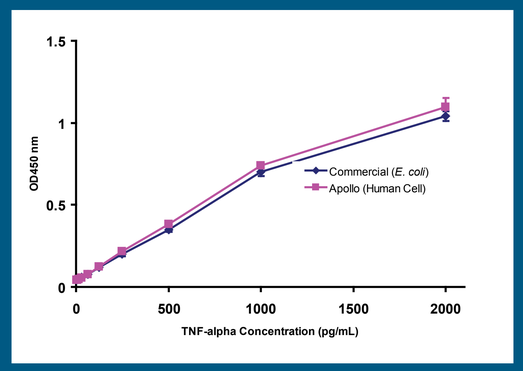

Figure 3: ELISA Assay - *E. coli* vs Human TNF-alpha[hcx]

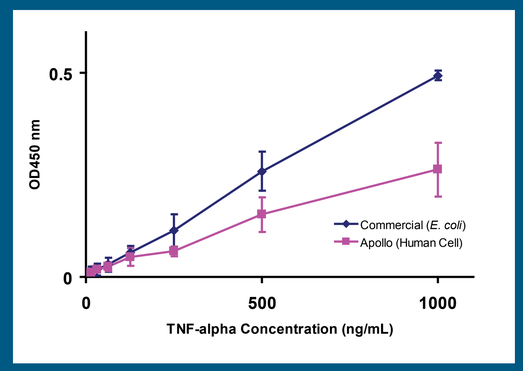

Figure 4: Receptor-ELISA Assay - *E. coli* vs TNF-alpha[hcx] Binding to TNFRII-Fc[hcx]

METHODS

Commercial kit assays were used to compare the response curves of a range of recombinant human proteins expressed from human cells to those same proteins derived from non-human cells.

Standard kit protocols were followed, according to the kit instructions.

Soluble chimeric human TNFRII-Fc expressed from human cells was also tested for differential binding of *E. coli* or human cell expressed recombinant human TNF-alpha, in the same ELISA format, by substituting TNFRII-Fc for the capture antibody.

In these assays, TNFRII-Fc was also bound to 96 well plates according to the same standard ELISA kit protocols, at a concentration of 1 μg/mL.

SUMMARY AND CONCLUSIONS

Our results have demonstrated differential binding of ELISA kit antibodies to some non-glycosylated *E. coli* expressed compared to human cell expressed recombinant human proteins.

These immunoassays, based on *E. coli* expressed human recombinant proteins would therefore be inaccurate when used for quantitation of native human GM-CSF and IL-4.

Furthermore, these immunoreactivity differences are consistent with the known immunogenicity of several non-glycosylated recombinant human proteins, *in-vivo*[5,6].

There was no difference in the immunoreactivity of human TNF-alpha expressed from *E. coli*, compared to human cell expressed human TNF-alpha in the standard ELISA kit.

However, the human cell expressed TNFRII receptor-ELISA results suggested differences in binding of the non-glycosylated (*E. coli*) compared to glycosylated (human cell) recombinant human TNF-alpha to this receptor.

These results have implications for drug potency and therapeutic dose calculation for TNFRII based compounds.

References

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