

VALIDATION OF A SEMI-QUANTITATIVE BIOASSAY WITH AN ELISA ENDPOINT FOR THE DETECTION OF NEUTRALIZING ANTI-PROTEIN X ANTIBODIES IN HUMAN SERUM

G. Pinard¹, M. Poirier¹, B. Ruelland¹, H Harouchi¹, L.LeSauter¹, D. Finco-Kent², X. Guo² and T. Kawabata²

¹Immunology, Laboratory Sciences, Charles River Laboratories Preclinical Services Montreal Inc., Senneville (Montreal), Quebec, Canada

²Safety Sciences, Immunotoxicology Laboratory, Pfizer Global R&D, Groton, Connecticut, USA

Pharmacologic activity of protein drugs may be neutralized with the development of anti-drug antibodies. A semi-quantitative bioassay for the detection of anti-Protein X neutralizing antibodies was developed and validated. Binding of Protein X to the cell-surface receptor results in a concentration-dependent receptor internalization followed by degradation and/or sequestering. Receptor expression of cell lysates was measured using an ELISA specific for the receptor. If neutralizing antibodies were present, it would inhibit Protein X binding to the receptor and attenuate receptor internalization.

Validation parameters included: cut-point determination in the presence and absence of Protein X, specificity and recovery, precision, defining the linear region for neutralizing activity, sensitivity, and sample stability. The cut-point factor was calculated based on the 95th percentile of the normal distribution of the mean response of 25 lots of normal serum and 25 lots of serum from the target population tested on three occasions. The intra- and inter-assay precision of the dose-response curve met the acceptance criteria ($CV \leq 25\%$) for the concentrations in the linear portion of the curve. The inter-assay precision of the low, medium and high positive control (%CV) was of 36.3%, 31.2% and 28.9% respectively. The linear region of the neutralizing activity of the monoclonal antibody positive control (PC) was between 16 to 33 ng/mL of neutralized Protein X. Based on the PC, the assay sensitivity was 2.5 $\mu\text{g}/\text{mL}$ human serum.

Validation results demonstrated that this cell-based method is suitable for the detection of neutralizing anti-Protein X antibodies in human serum.