

VALIDATION OF A QUALITATIVE ELISA METHOD FOR THE DETECTION OF ANTI-PROTEIN X IgG AND IgM ANTIBODIES IN HUMAN SERUM

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Objective: Immunogenicity testing for peptide and protein therapeutics is an important component of drug safety and efficacy testing. A qualitative ELISA method for the detection of anti-Protein X IgG and IgM antibodies was developed and validated for a protein therapeutic. Immunodepletion, initially validated using the surrogate positive control, was re-optimized with the positive study samples in light of the results obtained during sample analysis.

Method: In this sandwich ELISA, the human serum samples, diluted 1/30, were incubated onto a plate coated with Protein X. The detection system was a mixture of goat anti-human IgG and goat anti-human IgM antibodies conjugated to horseradish peroxidase. A rabbit antiserum containing rabbit anti-Protein X polyclonal antibodies was used as a positive control (PC) in the assay. Purified human IgG and IgM served as immunoglobulin positive controls (IPC) for binding of the detection antibodies. Validation parameters included: negative cut-off (NCO) determination, intra-assay and inter-assay precision, specificity and recovery, assay sensitivity, drug interference, immunodepletion, prozone effect and PC stabilities.

Results: The NCO, set at 0.218 A_{450} , was determined using 25 individual lots of normal serum and 25 individual lots of serum obtained from the patient population. The intra-assay precision of the PC and IPC were within the acceptance criteria ($CV \leq 25\%$). The inter-assay precision criteria ($CV \leq 25\%$) were met for the IPC but LPC, MPC and HPC %CV were 29.7%, 27.5% and 27.8%, respectively. The LPC, MPC, HPC were considered acceptable since the higher % CV was mostly due to one occasion out of the seven considered for precision assessment. The specificity and recovery, tested with 10 lots of normal serum and 10 lots of serum from the patient population, met the acceptance criteria. In the absence of purified PC, the assay sensitivity was confirmed as below the recommended 500 ng/mL based on the IgG and IgM IPCs. Another indicator of method sensitivity was the detection of 5 false positive and 1 true positive lots of serum out of the 50 lots tested during the NCO determination. Protein X interfered with the detection of the LPC at a concentration of 15 $\mu\text{g/mL}$ in neat human serum. Re-optimization of the immunodepletion conditions with the positive study samples was required; the immunodepletion was performed in the presence of 100 $\mu\text{g/mL}$ of Protein X. No prozone effect was observed when testing the PC more than two times more concentrated than the HPC level.

The validation data demonstrated that the method was suitable for the detection of anti-Protein X IgG and IgM antibodies in human serum and highlighted the need for appropriate confirmatory assays.