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TITLE

Validation and quantitative determination of interferon-alpha in human serum using high sensitivity ELISA

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ABSTRACT

Purpose: To develop and validate a high sensitivity ELISA method adapted from a commercial kit for quantification of human interferon alpha (IFN-α) in human serum.

Methods: The assay is a two-site sandwich ELISA using two selected antibodies that bind to different epitopes of the human IFN-α. The plates were pre-coated with an IFN-α specific antibody. Samples were incubated and the immobilized anti body binds any analyte present. After washing away any unbound substances, a biotin-conjugated antibody specific for IFN-α is added to the wells and followed by a horseradish peroxidase-conjugated streptavidin. The immobilized immunocomplex sandwich is detected by incubation with a substrate solution in a timed reaction. The color development is measured on a SpectraMax 384 Plus microplate reader. The enzymatic activity of the immunocomplex bound to the IFN-α on the wall of the microplate well is directly proportional to the amount of IFN-α in the sample. Plotting the absorbance versus the respective human IFN-α concentration for each standard using a 4-parameter curve fit generates a standard curve. The concentration of human IFN-α in human serum samples is determined directly from this standard curve.

Results: The ELISA method was validated over a range of 45 to 1000 pg/mL for IFN-α in human serum. Quality control samples at 5 concentrations were used to de termine precision and accuracy. Intra-assay precision ranged from 1.8 to 9.3%, while inter -assay precision ranged from 5.0 to 11.9%. Intra-assay accuracy (defined as % error from nominal) ranged from –9.8 to 7.7% and the inter-assay accuracy ranged from –6.4 to 1.3%. The analyte has been shown to be stable in human serum for 24 hours at room temperature and after 5 freeze/thaw cycles at –20°C or –70°C. The analyte is also stable in processed sample for 117 days at –20°C or -70°C. The dilution reproducibility, matrix effect and recovery were also demonstrated.

Conclusion: A highly sensitive ELISA method has been validated for the quantitation of IFN-α in human serum from 45 to 1000 pg/mL. The assay was shown to be sensitive, selective, reproducible, and reliable as a bioanalytical measurement..