

45. Immuno-PCR – highly sensitive protein detection: Results of the detection of native mistletoe lectin in human serum samples

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Enzyme linked immuno sorbent assay (ELISA) is nowadays a standard laboratory technique for specific antigen detection. The Immuno-PCR (IPCR) approach links this method with the highly efficient exponential signal amplification of the PCR, thus increasing conventional immunoassay sensitivity approx. 100–10.000 fold. The ImperacerTM IPCR assay format uses tailored antibody-DNA conjugates in combination with fast and quantitative real-time PCR detection. This technology is a convenient tool for (a) increasing assay sensitivity, (b) increasing assay robustness by minimization of biological sample effects, and (c) reducing sample volume.

These advantages are shown in the development and application of an ImperacerTM assay for the detection of native mistletoe lectin (*Abnobaviscum*TM *Fraxini*) in human serum samples of selected individual patients in preliminary pharmacokinetic analyses. A quantitative assay protocol was validated, revealing a linear detection range of 0.1–100 ng/ml, an average recovery of $95 \pm 8\%$ and an average inter-assay standard deviation of 3%. Individual differences between the patients were minimized by sample dilution and the use of individual calibration curves. With these, a quantitative assay was carried out for all individuals, revealing the kinetics of the lectin in patient serum following the subcutaneous or intravenous application of the drug. Highly sensitive analytics allow for the monitoring of very small amounts of the target protein in complex matrices, including the determination of antigen concentration decrease in serum during the observed time interval (0.33 h–24 h). This study demonstrates practical application of the ImperacerTM technique which opens up new possibilities for the quantification of e.g. low-dosed drugs and the detailed analytics of biomarker kinetics.

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