

Development of a Direct Immunoassay for Human Ferritin Using Diffraction-Based Sensing Method

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Abstract : Diffraction-based sensing was utilized in the quantification of human ferritin in blood serum to provide an alternative to label-based immunoassays currently used in clinical diagnostics and researches. The diffraction intensity was measured by the diffractive optics technology or dotLab™ system. Two methods were evaluated in this study: direct immunoassay and direct sandwich immunoassay. In the direct immunoassay, human ferritin was captured by human ferritin antibodies immobilized on an avidin-coated sensor while the direct sandwich immunoassay had an additional step for the binding of a detector human ferritin antibody on the analyte complex. Both methods were repeatable with coefficient of variation values below 15%. The direct sandwich immunoassay had a linear response from 10 to 500 ng/mL which is wider than the 100-500 ng/mL of the direct immunoassay. The direct sandwich immunoassay also has a higher calibration sensitivity with value 0.002 Diffractive Intensity (ng mL⁻¹)-1 compared to the 0.004 Diffractive Intensity (ng mL⁻¹)-1 of the direct immunoassay. The limit of detection and limit of quantification values of the direct immunoassay were found to be 29 ng/mL and 98 ng/mL, respectively, while the direct sandwich immunoassay has a limit of detection (LOD) of 2.5 ng/mL and a limit of quantification (LOQ) of 8.2 ng/mL. In terms of accuracy, the direct immunoassay had a percent recovery of 88.8-93.0% in PBS while the direct sandwich immunoassay had 94.1 to 97.2%. Based on the results, the direct sandwich immunoassay is a better diffraction-based immunoassay in terms of accuracy, LOD, LOQ, linear range, and sensitivity. The direct sandwich immunoassay was utilized in the determination of human ferritin in blood serum and the results are validated by Chemiluminescent Magnetic Immunoassay (CMIA). The calculated Pearson correlation coefficient was 0.995 and the p-values of the paired-sample t-test were less than 0.5 which show that the results of the direct sandwich immunoassay was comparable to that of CMIA and could be utilized as an alternative analytical method.

Keywords : biosensor, diffraction, ferritin, immunoassay

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