

Competitive Immunoassays for Simultaneous Detection of Metabolites and Proteins Using Micromosaic Patterning

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Abstract

New high-throughput immunoassay methods for rapid point-of-care diagnostic applications represent an unmet need and current focus of numerous innovative methods. We report a new micromosaic competitive immunoassay developed for the analysis of the thyroid hormone thyroxine (T4), inflammation biomarker C-Reactive Protein (CRP), and the oxidative damage marker 3-nitrotyrosine (BSA-3NT) on a silicon nitride substrate. To demonstrate the versatility of the method, both direct and indirect format competitive immunoassays were developed and could be applied simultaneously for single samples. Signals from standard solutions were fit to a logistic equation, allowing simultaneous detection of T4 (7.7 to 257.2 nM), CRP (0.3 to 4.2 μ g/mL), and BSA-3NT (0.03-22.3 μ g/ml). Total assay time including sample introduction, washing, and fluorescence measurement was less than 45 minutes. Dissociation constants for affinity pairs in the system have been estimated using regression. This proof-of-concept experiment shows that both small and macromolecular biomarkers can be quantified from a single sample using the method, and suggests that groups of clinically related analytes may be analyzed by competitive micromosaic immunoassay techniques.