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Real-time monitoring of glucose-6-phosphate dehydrogenase activity using liquid droplet arrays and its application to human plasma samples

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Abstract:

Glucose-6-phosphate dehydrogenase (G6PD) regulates nicotinamide adenine dinucleotide phosphate (NADPH) levels and is related to the pathogenesis of various diseases, including G6PD deficiency, type 2 diabetes, aldosterone-induced endothelial dysfunction, and cancer. Therefore, a highly sensitive array-based assay for determining quantitative G6PD activity is required. Here, we developed an on-chip G6PD activity assay using liquid droplet fluorescence arrays. Quantitative G6PD activity was determined by calculating reduced resorufin concentrations in liquid droplets. The limit of detection (LOD) of this assay was 0.162 mU/ml (2.89 pM), which is much more sensitive than previous assays. We used our activity assay to determine kinetic parameters, including Michaelis–Menten constants (K_m) and maximum rates of enzymatic reaction (V_{max}) for $NADP^+$ and G6P, and half-maximal inhibitory concentrations (IC_{50}). We successfully applied this new assay to determine G6PD activity in human plasma from normal healthy individuals (n=30) and patients with inflammation (n=30). The inflammatory group showed much higher G6PD activities than did the normal group (p<0.001), with a high area under the curve value of 0.939. Therefore, this new activity assay has the potential to be used for diagnosis of G6PD-associated diseases and utilizing kinetic studies.

Full text:
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