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We report calorimetric lab-on-a-chip immunoassay for rapid quantification of analytes in biological samples. The feasibility of the technology was demonstrated via accurate measurement of the concentration of the inflammatory cytokine TNF-α in human astrocytes cell culture media. This microfluidics technology offers multiple advantages over traditional immunoassays. Since the method is based on detection of the heat released during an enzymatic reaction, the assay can be performed using different enzymes. The method permits the substrate to be introduced multiple times after the thermoelectric signal returns to baseline level that increases the statistical significance of the results. The immunoassay was performed in a microfluidic device with an integrated antimony/bismuth thermopile sensor that has 60 thermocouple pairs. The device had two inlets and single outlet and was fabricated using xurography technique. The fluid flowing through inlet 2 was hydrodynamically focused within the reaction zone located above the measuring junctions of the thermopile. Anti-TNF-α monoclonal antibody was used to capture the analyte that was followed by detection with glucose oxidase-conjugated secondary antibody. Glucose (55mM) was injected through a sample loop into the fluid flowing within the microfluidic device. Nanovolt meter connected to the thermoelectric sensor recorded the voltage change caused by the enzymatic reaction. COMSOL simulations were performed to analyze the effect of flow velocity of inlet 2 on the glucose concentration within the reaction zone. The amount of glucose decreased as inlet 2 flow rate was reduced. The magnitude of the thermoelectric signal was proportional to the concentration of TNF-α in the biological sample. Standard calibration curve was created using serial dilutions of synthetic TNF-α (0-2000 pg mL^{-1}) by plotting the area under the curve of the signal versus the concentration of the analyte. The equation of the calibration curve was $y=0.0314x+2.6927$, $R^2=0.9942$. The concentration of TNF-α was quantified using cell culture medium from lipopolysaccharide (100 ng mL^{-1}) treated and non-stimulated human astrocytes. The limit of detection of the microfluidics calorimetric assay was 9.88 pg mL^{-1} and the limit of quantification was 30 pg mL^{-1}. The estimated concentration of TNF-α was 165 pg mL^{-1} and 170 mg mL^{-1} using microfluidic system and conventional absorbance plate reader respectively.