

### **Self-contained Microelectrochemical Immunosensor for Small Volumes**

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A self-contained, microelectrochemical immunosensor on the smallest volumes reported to date (1  $\mu\text{L}$  for the antigen and 0.2  $\mu\text{L}$  for the p-aminophenyl phosphate which is the substrate source of the electrochemically detected species) has been developed using mouse IgG as a model system in a sandwich type enzyme-linked immunosorbent assay (ELISA). It takes less than 30 minutes to complete the immunoassay from the capture of the antigen to the detection of the electrochemical signal. At only 30 s after the 0.2  $\mu\text{L}$  drop of 4 mM PAPP was placed on top of the immunosensor, a measurable current of the enzymatically-generated PAP was recorded at a scan rate of 50 mV/s. There is no incubation time needed for the PAPP on the immunosensor which demonstrates the advantage of the close proximity of the electrodes to modified surfaces and their application in the analysis of small volumes.

Using a 50  $\mu\text{m}$ -diameter x 8  $\mu\text{m}$  deep cavity with individually addressable electrodes on a microfabricated chip, the primary antibody was selectively and covalently attached at a gold, recessed microdisk (RMD) at the bottom of the microcavity to the free end of SAMs of either 11-mercaptoundecanoic acid or 11-mercaptoundecanol using 1-ethyl-3-[3-(dimethylaminopropyl)-carbodiimide hydrochloride. Non-specific adsorption to the insulating material, polyimide, of the microcavity device was eliminated using a mixture of tween, bovine serum albumin, and mercaptooctadecane. Electrochemical desorption was used to selectively pattern the immunoassay activity at the RMD. The enzyme label on the secondary antibody, alkaline phosphatase is used for the enzymatic conversion of the substrate p-aminophenyl phosphate to p-aminophenol (PAP) that is detectable in less than 30 s using cyclic voltammetry at a gold, tubular nanoband electrode that is located on the wall of the microcavity and immediately adjacent to the modified RMD. This presumably results from the geometry of the microcavity where the PAP can only escape from the microcavity by passing the TNB, although collection efficiency is not 100 %. A third layer of gold, also within the confines of the microcavity, served as the pseudo reference/auxiliary electrode.

A calibration curve for IgG was obtained for 1  $\mu\text{L}$  solutions of 5 ng/mL to 100 ng/mL and one for PAP<sub>R</sub> was obtained for 200 nL solutions of  $5 \times 10^{-6}$  M to  $4 \times 10^{-3}$  M. Detection limits, which have not yet been optimized, are  $2.86 \times 10^{-9}$  M or 570 attomole for PAP<sub>R</sub> and 15 pg/ml, or 92 zeptomole for IgG. Research is ongoing for the application of the microelectrochemical immunosensor for the detection of *Cryptosporidium* oocysts in drinking water.