A Rapid Test for Malaria Utilizing a "Smart" Microfluidic Concentrator and Immunoassay

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Statement of Purpose: Detecting dilute biomarkers from complex human samples remains a challenge in the field of rapid diagnostics[1]. A microfluidic concentrator to capture and concentrate antibody-bound antigen through aggregation of a covalent antibody conjugate of poly(N-isopropylacrylamide), pNIPAAm, at a porous membrane surface above the lower critical solution temperature (LCST) is reported. This technique is applied to the capture and detection of malarial antigen *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) from spiked human plasma for detection of clinically-relevant concentrations of antigen. Using pNIPAAm-antibody conjugates and a gold colloid detection reagent, a stable, visually-detectable signal is generated at the membrane surface. It is proposed that this technique can expand limits of detection from larger samples by concentrating the immunocomplex at the membrane.

Methods: Semi-telechelic poly(N-isopropylacrylamide), pNIPAAm, was prepared using reversible addition fragmentation chain transfer (RAFT) polymerization and end carboxyl groups were modified with tetrafluorophenol to yield amine-reactive ester groups. Active ester pNIPAAm was combined with anti-PfHRP2 IgM antibodies and conjugated antibodies were purified. Modification and binding affinity of the antibody were verified. Capture antibody conjugates of pNIPAAm, PfHRP2 antigen-spiked human plasma, and 40 nm gold colloid-conjugated detection antibodies were combined and pumped through a commercially-available membrane piece, adhered in a microfluidic card. Cards were constructed of poly(methyl methacrylate), poly(ethylene terephalate) and adhesive layers.

Results: Antibody conjugates are efficiently captured at the membrane surface, even from such complex fluids as human plasma. Conjugation and purification of the IgM antibody against PfHRP2 antigen did not adversely affect binding of antigen as compared to an identical non-conjugated antibody. When conjugates against PfHRP2 antigen are applied to a full-stack immunoassay from 50% human plasma, the limit of detection is comparable to ELISA and is achieved in a total time of less than 10 minutes from a 50 µl sample. Using larger sample volumes, visual detection of dilute antigen samples is enhanced and color intensity increases as the immunocomplex-bound gold colloid is accumulated at the membrane.

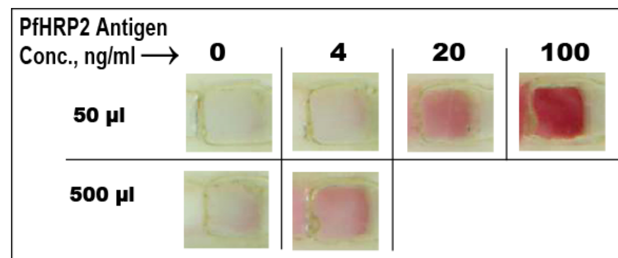

Figure 1. Gold colloid (40 nm) accumulated at membrane is dependent on PfHRP2 antigen concentration and total sample volume. A 50 or 500 µl sample of 50% human plasma, containing antigen-spiked plasma, gold-conjugated detection antibodies, and pNIPAAm-conjugated capture antibodies was pumped through a membrane piece affixed in a microfluidic card. Membrane area shown is 0.5 mm by 0.5 mm. A typical ELISA detection limit, using identical antibodies, is approximately 4 ng/ml.

Conclusions: A system of stimuli-sensitive antibody conjugates and porous membranes was developed for the purpose of separating and concentrating immunocomplexes within a simple microfluidic device. Reactive esters of pNIPAAm provide a facile way of conjugating pNIPAAm to amine groups on an antibody to yield phase-transitioning, 'smart', reagents suitable for capture of antigen. Full stack detection immunocomplexes for detection of PfHRP2 malarial antigen were separated from 50% human plasma samples. This technology has a wide range of applications for enrichment of target from samples, but can also be applied to sample preconditioning to remove specific unwanted elements from complex mixtures without further diluting the sample. Concentration of antigen and full-stack immunocomplexes from complex fluids using such system is demonstrated, providing opportunities to concentrate dilute biomarkers, thus improving detection ranges and expanding diagnostic options in microfluidic immunoassays.

References:

Yager, P., et al., NATURE. 2006; 442: 412-418.

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