Title:

SiMoAs for the Ultra-Sensitive Detection of the Host Immune Response to Microbial Infections

Authors:

Shonda Gaylord, Danlu Wu, Trinh Dinh, Milena Milutinovic, Sama Abdul-Aziz, David R. Walt

Presented by:

Shonda Gaylord and Danlu Wu

Departments:

Department of Chemistry Department, School of Arts and Sciences; Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine

Abstract:

Advances in diagnostic sensitivity, enabling reliable diagnosis in the acute stage of infection, can aid in clinical precision, patient care, disease control and prevention, and outbreak surveillance. The potential for early diagnosis of infections relies on the ability to detect ultra-low concentrations of circulating pathogens or related biomarkers in clinical samples. Both cytokines and immunoglobulins are secreted by the host immune system in response to a foreign agent and can be used as diagnostic markers of infection. Sub-femtomolar detection of prostate specific antigen (PSA), botulinum toxin, HIV p24 protein, and bacterial genomic DNA has recently been demonstrated by capturing single protein molecules or fragmented DNA onto magnetic beads using specific capture agents. These assays, coined single-molecule arrays (SiMoAs), are identical to conventional sandwich and indirect ELISAs in that they use beta-galactosidase as a reporter enzyme. Each magnetic bead isolated into individual 46-fL reaction wells on a 50,000 microwell array in the presence of substrate, which generates a high local concentration of fluorescent product if the bead carries a labeled immunocomplex. This technique provides ultra-sensitive detection technology with sub-milliliter clinical sample volumes.

Here we present the development and application of SiMoAs for nine cytokines and IgG/IgM for dengue infection. We are able to detect the target cytokines at sub-femtomolar concentrations, i.e., 200- to 1000-fold more sensitive than conventional ELISA technology. Currently, six previously undetectable cytokines including GM-CSF, TNF-alpha, IFN-gamma, IL-2, IL-4 and IL-10 are detectable in healthy human serum samples using the SiMoAs. We are also able to detect dengue immunoglobulin in samples, positive for DEN-1 infection by qRT-PCR, but negative using conventional ELISAs. Our findings demonstrate how the ultra-sensitivity provided by SiMoAs can be used for the early diagnosis of infectious disease, more specifically, for the presence of biomarkers that were previously undetectable. This research may allow for the identification of different infectious diseases by observing unique cytokine fingerprints. It may reduce the “transient window period” in diagnosing acute infections using serological assays.