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Rapid, Sensitive Detection and Quantification of Toxins from Complex Biological Matrices

Chung-Yan Koh*, Ulrich Y. Schaff*, Larry H. Stanker†, Bal Ram Singh‡, Anson V. Hatch*, Anup K. Singh*, Greg J. Sommer*
*Sandia National Laboratories, Livermore CA, USA
†United States Department of Agriculture, Albany CA, USA
‡University of Massachusetts-Dartmouth, North Dartmouth MA, USA

In scenarios of mass biotoxin release, either intentional or accidental, rapid diagnosis is vitally important to delivering effective treatment to affected individuals. Here, we present our latest results utilizing a microfluidic disc-based immunoassay. Device fabrication and initial results were recently published in Clinical Chemistry [1]. In this current work, we extend the early proof-of-principle experiments to detection of several high priority potential bioterrorism agents including botulinum neurotoxin and anthrax. The platform allows the development of immunoassays which are rapid (<20 minute) and sensitive (~500 attomolar limit of detection for BoNT/A) while maintaining a simple one-step assay format. Precipitation of microparticles through the density medium and into the focused channel tip both concentrates the signal and thoroughly washes the particles thereby dramatically reducing background while increasing signal. This novel one-step approach greatly simplifies the assay compared to other disc-based immunoassays [2,3] which rely on multiple washing/incubation steps and thus require extensive valving.

An overview of the assay is shown in Figure 1. Antibody-conjugated microparticles are incubated with the analyte of interest as well as a fluorescently-labeled reported antibody. This suspension is layered above a density medium pre-loaded on the disk. Upon application of centrifugal force, the resulting sandwich immunocomplex travels through the density medium. Any antigen, detection antibody, or matrix component that is unbound remains above the density medium. The microparticles pellet to the bottom of the channel where the signal is read. We demonstrate, in Figure 2a, sensitive detection and quantification of several toxins spiked into sample matrices such as serum and whole blood. Analysis of samples from whole blood is simplified by using a medium with a density greater than that of red blood cells such that the cells accumulate at the interface of the layers and do not interfere with analysis of the signal pellet (Figure 2b).

The simplified disc-based assay architecture facilitates ready incorporation into an integrated point-of-care diagnostic platform. This assay's rapid (<20 min) sample-to-answer time, low limits of detection (sub-picomolar) and compatibility with complex biological samples provide vast improvements over current state-of-the-art approaches for diagnosis of toxin infection, particularly for potential mass exposure scenarios.

Word Count: 349

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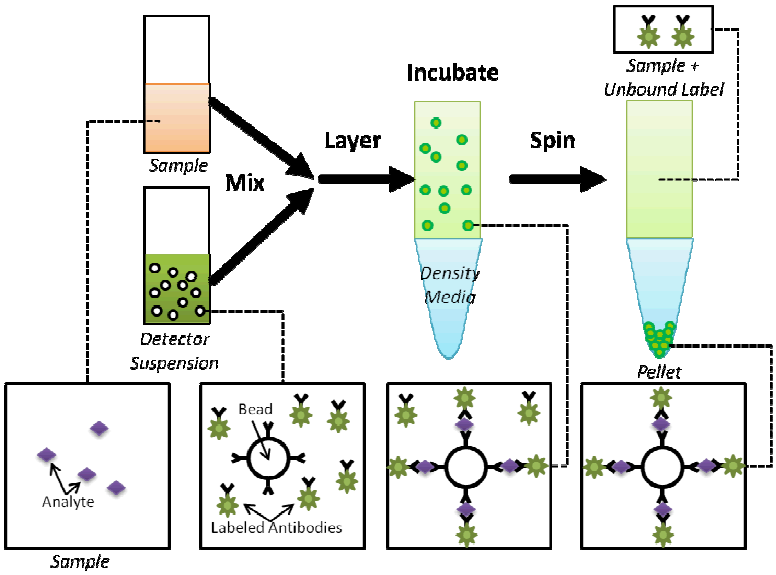
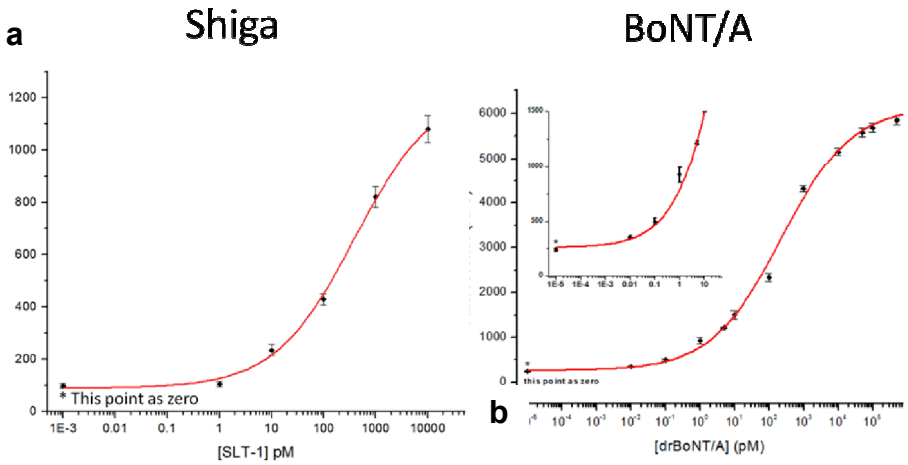


Figure 1: Overview of microfluidic disc-based immunoassay. An analyte-containing sample is mixed with the detector suspension and layered over the density media. The immunocomplex is precipitated through the density media by application of centrifugal force, leaving the sample and unbound label in the supernatant.



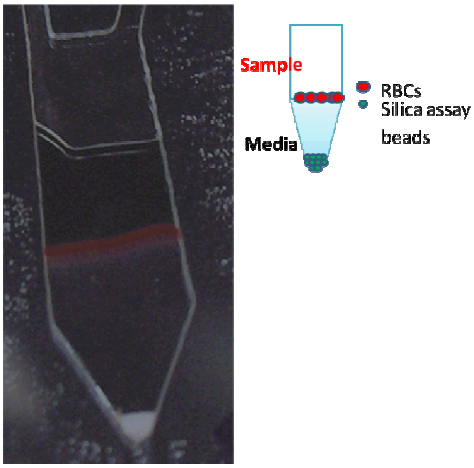


Figure 2: (a) Detection of shiga-like toxin 1 and botulinum neurotoxin A in serum samples. Limit of detection for SLT-1 is 0.8 pM and limit of detection for BoNT/A is 0.5 fM. (b) Blood cells from whole blood layer at the interface between the sample buffer and the density medium, thus reducing sample complexity and simplifying analysis.