

RAPID, SENSITIVE DETECTION AND QUANTIFICATION OF TOXINS FROM COMPLEX BIOLOGICAL MATRICES

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ABSTRACT

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 to detect several high priority potential bioterrorism agents including ricin, Shiga-like toxin 1 (SLT-1), and anthrax. The platform allows the development of immunoassays which are rapid (<20 minute) and sensitive (subpicomolar limit of detection) 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 increasing signal-to-noise.

KEYWORDS: Toxin detection, Rapid diagnostics, Immunoassay, Centrifugal platform

INTRODUCTION

Lab-on-a-disc devices can be a convenient method by which to control fluidic transport in a microscale device. Instead of relying on complex pumping modalities, such as pressure, acoustic, or electrokinetic pumping, fluids are driven by centrifugal forces derived from a single rotating motor. This does not, however, preclude highly complex disc devices. The literature has shown a variety of fluidic handling modalities such as valving, internal calibration, mixing, sample splitting and recombination, metering, and decanting. Recently, we published device fabrication and initial results for our own design of a centrifugal microfluidic device in Clinical Chemistry [1] examining cell sorting and an immunoassay. In this current work, we extend the early proof-of-principle experiments to detection of several high priority potential bioterrorism agents: Shiga-like toxin 1 (SLT-1), ricin, and anthrax. The advantages of this platform over conventional immunoassay formats are relatively fast assays (<20 minute) while maintaining sensitivity (subpicomolar limit of detection) in a simple one-step assay format. Microparticles are used as the assay surface both to increase the available surface area for detection and to provide a substrate which can travel through the density medium. By removing the assay particles from the mixing zone of the device, the assay format is greatly simplified compared to other disc-based immunoassays [2,3] which rely on multiple washing/incubation steps and thus require extensive valving.

THEORY

This technique utilizes the principle that low density fluids will float on top of higher density fluids; mixing between the density-defined layers is limited to relatively slow diffusional processes. In centrifugal microfluidics, density layers are maintained orthogonal to the axis of rotation with the densest fluids closest to the periphery of the disk. The sedimentation rate of spherical particles (equation 1) is based on Stoke's law.[4]

$$U_s = \frac{2(\rho_p - \rho_f)}{9\mu} g R^2 \quad (1)$$

U_s is the sedimentation velocity, μ is the fluid viscosity, ρ_p is the density of the particle, ρ_f is the density of the fluid, g is acceleration due to gravity or centrifugation, and R is the particle radius. Although this equation only applies to isolated particles, it is a reasonable guide to the relative sedimentation speed of a mixture of particles in dilute suspension. Notably, sedimentation velocity depends on the square of particle radius. For this reason, nanometer-sized particles such as proteins are essentially unmoved by the forces typically achieved by centrifugation despite their substantial density while micron-sized particles are sedimented after minutes of centrifugation at thousands of RPM. Furthermore, at low values of g micron-sized particles can stay suspended in aqueous media for minutes or hours, allowing them to be processed by conventional microscale fluid handling techniques. Therefore, beads can be incubated with a complex sample in suspension, then be used to specifically transport analyte to the bottom of a container while leaving behind any nanometer-sized contaminant molecules such as excess labeling agents. Any loosely bound proteins will be detached and left behind in the density media as each microsphere is effectively washed with hundreds of times its volume by Stoke's flow.

EXPERIMENTAL

Microfluidic disks were constructed from double-sided pressure sensitive adhesive (Fralock, USA) sealed between layers of rigid plastic. Channel designs were cut using a computer-controlled plotter-cutter (Graphtec America Inc, USA). Disk materials were cleaned, assembled by alignment, and sealed with pressure directly applied to the disk surface. All samples and density media were loaded into the disks by access ports. Liquid movement during loading was driven by capillary action.

Antibodies (Toxin Technologies, USA) were conjugated to the microparticles (Bangs Laboratories Inc, USA) via standard amide bond formation chemistry. Carboxylic acids were first activated using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and converted to succinimidyl esters with N-hydroxysuccinimide. A schematic of the assay design and workflow is shown in Figure 1. To a suspension of antibody-conjugated microparticles, fluorescently labeled secondary antibodies were added to create the detection suspension. Antigen-containing samples were introduced and allowed to bind for 10 minutes in the detection suspension and 4 μ L of the mixture were layered on top of the density medium. The samples were spun to allow the microparticles to sediment through the density medium. After 45 seconds of centrifugation, the pellets were imaged on an Olympus inverted fluorescent microscope with a 10X air objective. Images were analyzed by ImageJ; background-subtracted intensities were averaged, plotted, and fitted with Origin.

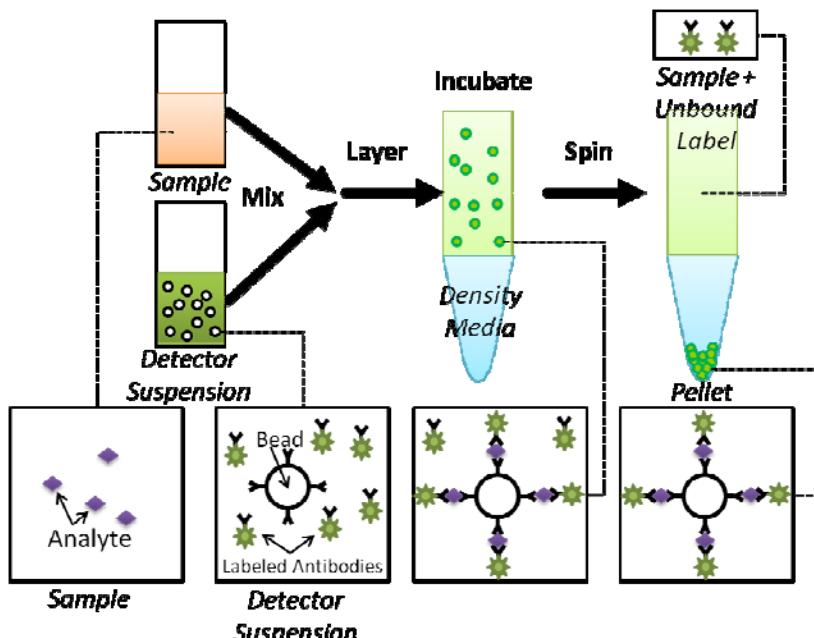


Figure 1: Schematic of assay design.

RESULTS AND DISCUSSION

We demonstrated the sensitive detection of three high-priority potential bioterrorism agents: ricin, Shiga-like toxin 1, and anthrax. Each sample was analyzed in triplicate and error was plotted as the standard deviation. The data were fit to a four-parameter logistic fit with Origin (Figure 2). The limits of detection (LoD), defined as the zero point plus three standard deviations were calculated for the three analytes from the fit of the curve. LoD for anthrax PA in serum was found to be 1.9 pM, LoD for SLT-1 in serum was 0.8 pM, and LoD for ricin in serum was 1 pM. System performance may also be expressed in terms of antigen mass: 2 μ L of a 1 pM solution of ricin (a 65 kDa protein) is detection of 130 fg of ricin. Similarly, the platform detected 315 fg of anthrax protective antigen and 108 fg of SLT-1.

Detection of biological toxins is a difficult task due to the acutely toxic nature of the proteins. For this reason, limits of detection must be very sensitive in order for an assay to be clinically relevant. For SLT-1, the LD₅₀ has been found to be 0.02 μ g/kg, for ricin 2.7 μ g/kg.[5] Anthrax intoxication occurs primarily through the introduction of spores rather than through the use of the proteins which make up the anthrax toxin. There have been studies, however, showing that 12.5 μ g/kg of the protective antigen in combination with the lethal factor is a reasonable estimate for the LD₅₀.[6]

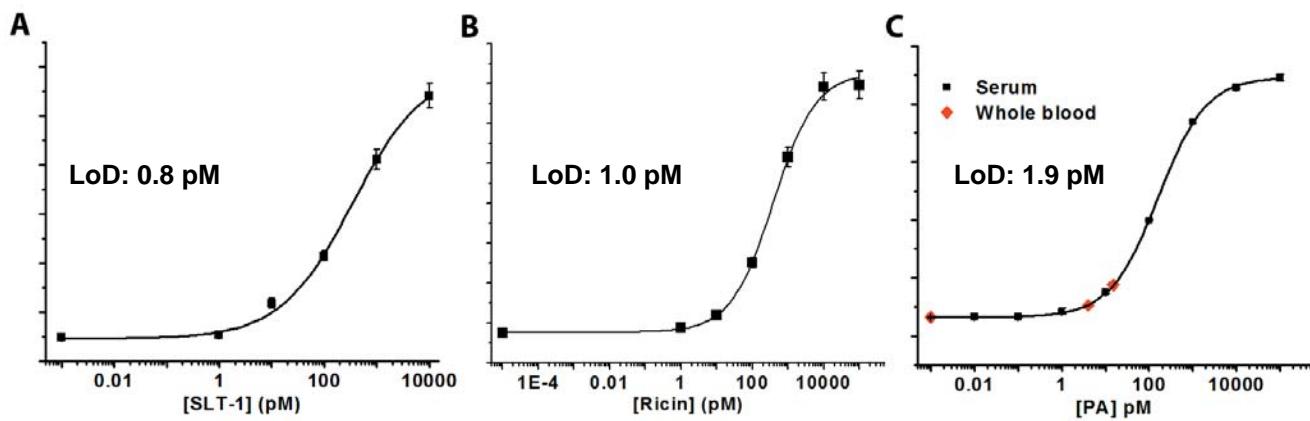


Figure 2: Detection of (A) Shiga-like toxin 1 in serum, (B) ricin in serum, (C) anthrax protective antigen in serum and whole blood. Each point was performed in triplicate. Error bars are standard deviation. For SLT-1, $r^2=0.96$ and $\chi^2=1.538$; for ricin $r^2=0.99$ and $\chi^2=0.478$; for anthrax PA $r^2=0.98$ and $\chi^2=1.894$.

CONCLUSION

Sensitive detection limits coupled with a simple, one-step assay format make this platform an attractive solution for rapid diagnostics in the event of mass release of biological toxins. Further development toward a fully automated device is ongoing.

ACKNOWLEDGEMENTS

This work was funded by NIH-NIAID award 1U01AI075441-0. Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

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