A Forceful New Method to Sensitively Detect Proteins

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(PhysOrg.com) -- Scientists at the Naval Research Laboratory recently reported the detection of toxins with unprecedented speed, sensitivity, and simplicity. The approach can sense as few as a few hundred molecules in a drop of blood in less than 10 minutes, with only four simple steps from sample to answer.

The sensitive new test builds on NRL's patent-pending Fluidic Force Discrimination® (FFD) assay. In a FFD assay, a chip has arrays of receptor molecules such as antibodies that capture toxins or other target molecules that have been labeled with micrometer-sized beads. By encapsulating the chip in a microflow chamber, the fluid flow can be controlled to apply just enough force to remove beads that are resting on the array but not truly labeling a toxin. "In this way," explains lead author Dr. Shawn Mulvaney, "very few molecules can be detected, because there is almost no background signal." "And because we can get the background so low," he adds, "FFD assays are very specific, with very few false positives."

In the current report, the NRL researchers have adapted FFD assays to detect a protein toxin at concentrations as low as 35 attomolar - over 1000 times more sensitive than existing commercial tests for proteins. In the new assay, dubbed "Semi-Homogeneous Fluidic Force Discrimination," the antibody-coated microbeads are mixed directly with the sample and rapidly collect the dilute toxin molecules. The toxin-coated beads are then injected into the microflow chamber where they are captured by the receptor designed for that target. Finally, beads that

don't belong are removed with fluid forces. The remaining beads are all attached by the toxin to the surface and may be counted to indicate the toxin concentration. NRL has developed both electronic and optical systems to count the beads, along with reusable plastic test cartridges.

The paper won the award for Most Original Contribution at the Tenth World Congress on Biosensors, held in Shanghai, China, May 14-16, 2008 out of 978 competing papers. The awards committee noted that it was the combination of outstanding performance and modeling that set the NRL paper above the competition. The researchers developed a detailed mathematical model that includes every step of the assay, which was critical to maximizing the capture and the overall sensitivity they thereby achieved. "When very few molecules are present in a sample, such as a drop of blood," comments NRL's Dr. Paul Sheehan, "it is critical to try and capture and count every single one." Dr. Paul Sheehan emphasized that "target capture and delivery tends to be a neglected aspect of biosensor design."

"A key advantage of the NRL platform," explains Dr. Lloyd Whitman, now at the National Institute of Standards and Technology, "is that it can be applied simply even to the most challenging samples, such as serum, blood, urine, or food." "We expect it to have broad applications in medical and veterinary diagnostics, food and water testing, and national security." Dr. Mulvaney concludes, "Based on the simplicity of the method, we envision small, portable systems for point-of-care testing, field monitoring, and use by first responders."

More information: The paper, "Attomolar protein detection in complex sample matrices with semi-homogeneous fluidic force discrimination assays," by S. P. Mulvaney, K. M. Myers, P. E. Sheehan, and L. J. Whitman, appears in the January 2009 issue of *Biosensors & Bioelectronics*.