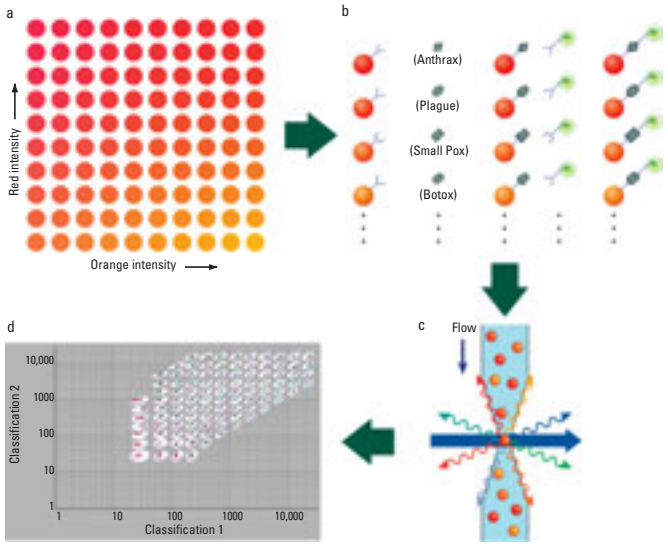
RESEARCH PROFILES

Liquid array single-handedly detects bounty of BW agents

After researchers at Lawrence Livermore National Laboratory (LLNL) in California and Tetracore, Inc., in Gaithersburg, Md., joined forces in the summer of 2001, the September 11 terrorist hijackings and the anthrax attacks that followed spurred scientists across the country into building the best biological warfare (BW) detection systems possible for civilian populations.



(a) Varying ratios of red and IR dyes in polystyrene latex microspheres generate a 100-plex liquid array, (b) beads are coated with capture antibodies specific for target antigens, (c) a flow cytometer analyzes the beads, and (d) a dot plot of a 100-plex bead analysis is computed.

In the April 15 issue of *Analytical Chemistry* (pp 1924–1930), Kodumudi Venkateswaran of LLNL and colleagues introduce a multiplexed liquid array immunoassay that can discriminate between strains of closely related pathogens in a single sample. Their multiplexed liquid array immunoassay, which can be automated, uses 100 polystyrene microbead sets embedded with precise ratios of red and IR fluorescent dyes—each bead having a unique spectral address. The immunoassay can simultaneously detect pathogens, from viruses to vegetative cells.

Their liquid array technology is to be used in a device called the autonomous pathogen detection system (APDS). The APDS is a stand-alone instrument about the size of a vending machine that continuously monitors for multiple, airborne BW agents. Despite its size, says Mary McBride of LLNL, "it is smaller than any other autonomous system that's out there right now."

The researchers obtained a maximum assay sensitivity in ~1 h that rivaled other methods, with good sensitivity reached in 30 min. In one example, they obtained a limit of detection (LOD) of 3 μg/L for *Bacillus globigii* (Bg), which simulated anthrax spores, compared with an LOD of 39 μg/L using an enzyme-linked immunosorbent assay (ELISA).

LLNL researchers have been working on the APDS for five years. In 2001, they partnered with Tetracore, which supplied them with the protein-G purified capture and biotinylated detector antibodies for the four simulated agents used: Bg to simulate anthrax spores; MS2 for smallpox virus; ovalbumin to cover protein toxins such as ricin, botulinum toxin, or staphyloccocal enterotoxins; and *Erwinia herbicola* to stand in for plague bacteria. The researchers developed the assays on a commercially available ~23-kg flow cytometer.

The APDS uses a typical sandwich immunoassay format in which capture antibodies that are antigen-specific are immobilized on the polystyrene beads. The researchers covalently coupled each capture antibody to a unique carboxylated bead set with a particular spectral address (1.25×10^6 microspheres in 100 μL). This allowed them to look for up to 100 different analytes in a single sample. After the beads were incubated with antigens, the bound analyte was detected using secondary, or "detector", antibodies. The fluorescent reporter phycoerythrin was then added and indirectly labeled the detector antibodies, which completed the "antigen sandwich", say the researchers.

Venkateswaran's group then used the flow cytometer to interrogate each optically encoded and fluorescently labeled bead—one at a time. A red laser excited the dye molecules inside each bead, determining the unique set, and a green laser quantified the assay at the bead surface. The flow cytometer read several thousand beads per second, with the signal a function of antigen concentration. The researchers were able to complete an analysis in <15 s.

The assay includes internal control beads that continually test the assay's performance, says McBride. To help avoid false positives and false negatives, the control beads not only show whether the system is performing properly but also if the correct amount of reagents has been added, says McBride. "The internal controls built into the benchtop assays that monitor instrument performance, reagent addition, and reagent stability are critical when those assays are transitioned to automated systems."

The liquid array offers advantages that other detection systems don't, says Venkateswaran. For example, polymerase chain reaction (PCR) has been effectively used to detect biological agents, but conventional, real-time PCR can take ~2 h. Also, says Venkateswaran, PCR isn't multiplexed and requires expensive reagents. Time-resolved fluorescence and the ELISA can only analyze one analyte per well, and running a single ELISA can take at least 4 h, he adds.

The researchers have obtained more promising results from using the device. For now, they say, they're happy with one prospect: The liquid array shows excellent assay specificity and sensitivity and rivals "the current gold standard"—the ELISA. ■

–Cheryl M. Harris