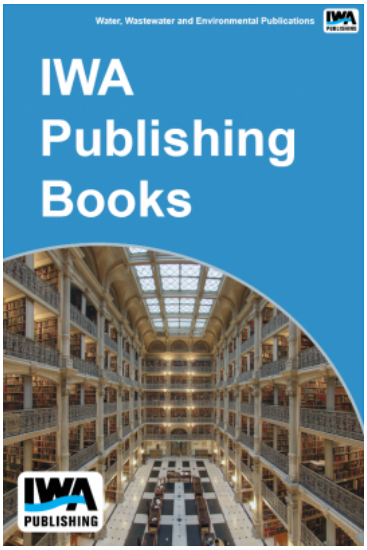
Evaluation of the Doodlebug: A Biochip for Detecting Waterborne Pathogens



Doodlebug is a new biochip technology that uses surface-enhanced Raman scattering (SERS) microscopy for label and reagent free transduction. The biochip itself comprises pixels of capture biomolecules immobilized on a SERS-active metal surface. Once the biochip has been exposed to the sample and the capture biomolecules have selectively bound their ligands, a Raman microscope is used to collect SERS fingerprints from the pixels on the chip. Because imaging Raman microscopes offer spatial resolution as fine as 250 nm, spectra can be collected from individual microorganisms captured by the pixels. This exquisite sensitivity eliminates the need for labels such as fluors or enzymes, or reagents such as those used in PCR; and, thereby, eliminates the potential for false responses from environmental constituents that either mimic or mask the signals from labels or reagents.

The WERF program showed that SERS, like other whole-organism fingerprinting techniques, is very specific. Studies with representative strains of six *Legionella* species, recently-passaged oocysts from six *C. parvum* genotype 2 strains, three genotype 1 strains, and a *C. meleagridis* strain, plus a *Giardia* sample, indicated that SERS could identify the bacteria and oocysts at the species and subspecies levels. Therefore, Doodlebug holds promise for individually identifying pathogens, even when organisms that cross-react with the capture biomolecules are present in a sample. The fingerprints that were used in oocyst identification were routinely collected from a single oocyst within 60 seconds.

Experiments involving the impact of environmental and water treatment conditions suggest that the Doodlebug approach will be able to differentiate between viable and nonviable, and possibly injured, organisms. It appears that the SERS fingerprint may even be useful in determining the ‘age’ of an oocyst; e.g., whether it is too old to be infectious. The WERF program culminated in a demonstration of the simultaneous detection of two very different microorganisms (i.e., *C. parvum* oocysts and *Bacillus* spores) present in a mixture. Although oocyst capture was selective, some spores adhered nonspecifically to the anti-oocyst MAb surface. This showcased Doodlebug’s ability to eliminate false responses due to nonspecific adherence or cross-reactivity, since the spores were readily differentiated from the oocysts on the basis of their SERS fingerprints.

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