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Centrifugal Microfluidic Platform for Multiplexed Detection of Enteric Bacteria in Ground Water

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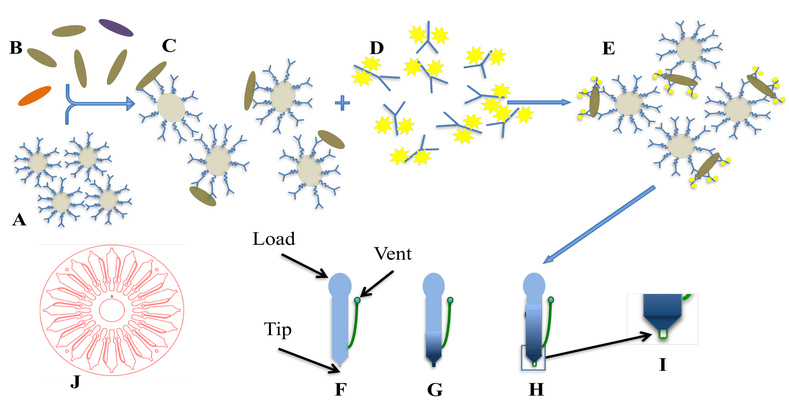
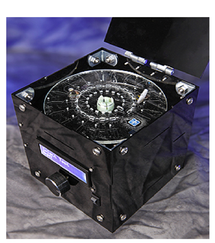
Abstract

Water-born pathogens pose a significant threat to the global population. Their timely detection provides an advantage in early diagnostics and treatment of water to insure its potability. We present an innovative centrifugal microfluidic platform (SpinDx)[1] for detection of bacterial pathogens using bead-based immunoassays. Our approach is based on binding of pathogens to antibody-functionalized capture particles followed by sedimentation of the particles through a density media in a microfluidic disk and quantification by fluorescence microscopy. Our platform is fast (20 min), sensitive (10^3 CFU/mL), requires minimal sample preparation, and can detect multiple pathogens simultaneously with sensitivity similar to that required by the EPA[2]. We demonstrate detection of a panel of enteric bacteria (*Escherichia coli*, *Salmonella* Typhimurium, *Shigella*, *Listeria*, and *Campylobacter*) at concentrations as low as 10^3 CFU/mL or ~30 bacteria per reaction.

Assay Design

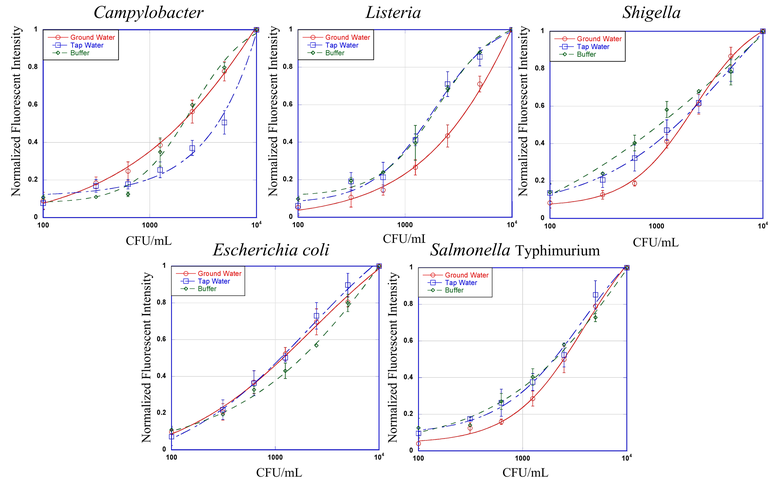


Carboxylated silica beads with anti-bacterial antibodies connected with a PEG-linker (A) are mixed with analyte of interest (B) and allowed to incubate (C). Anti-bacterial antibodies labeled with Alexa 488 fluorophore (D) are added and incubated (E). To an empty well on the disk (F), density gradient is added (G), after which the reaction from (E) is added to the well. The disk is spun to separate beads with specifically bound bacteria and fluorophore-labeled antibody from the rest of the reagents (H), after which the pelleted beads are imaged with fluorescent microscope (I). Not up to scale. (J) Schematics of microfluidic disk.



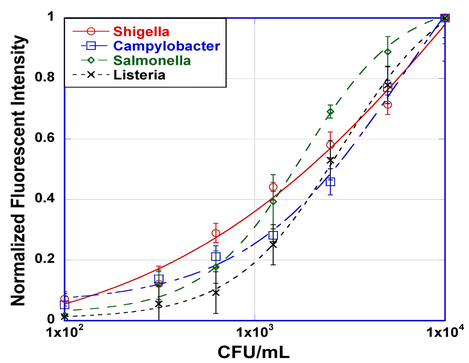
SpinDx™: Point-of-Care Diagnostics
Using Centrifugal Microfluidics,
Sandia National Laboratories
The technique is based on centrifugal microfluidics, or "lab-on-a-disk' technology, which uses centrifugal forces to manipulate samples and reagents through density media on a disk and miniaturized fluorescence optics are used for detection.

Sample Recovery in Buffer, Tap Water and Ground Water



- 20 mL of buffer, ground or tap water spiked with 10^6 bacteria/mL and concentrated with Vivaspin 20 centrifugal concentrators to 100 µL final volume
- Antibody-labeled silica beads incubated with concentrated samples and fluorescently-labeled antibodies
- Mixture added to well with pre-loaded density media
- The disc is spun, bead-bound bacterial target with fluorophore-labeled antibody pass through the density media, leaving behind unbound analyte and/or fluorophore-labeled antibody [3]
- Normalized fluorescence for negative control sample is represented by concentration 100 CFU/mL.

Multiplexing Assay



- Bacteria at concentration $5x10^6$ CFU/mL each was spiked into the pre-filtered 20 mL ground water sample
- The concentration and assay steps were performed as described in the section above

Limits of Detection and Limits of Quantification

		LOD (CFU/mL)	LOD (bacteria/ assay)	LOQ (CFU/mL)	LOQ (bacteria/ assay)
Buffer	Campylobacter	$3.58x10^3$	12	$1.54x10^4$	51
	Listeria	$5.80x10^3$	19	$6.65x10^4$	222
	Shigella	$9.09x10^2$	3	$5.28x10^4$	176
	E. coli	$2.33x10^3$	8	$4.01x10^4$	134
	Salmonella	$6.72x10^3$	22	$1.34x10^5$	447
Ground water	Campylobacter	$5.63x10^3$	19	$2.18x10^4$	73
	Listeria	$9.19x10^3$	31	$2.70x10^4$	90
	Shigella	$4.67x10^3$	16	$1.16x10^4$	39
	E. coli	$1.14x10^3$	4	$3.69x10^3$	12
	Salmonella	$4.58x10^3$	15	$1.40x10^4$	47
Tap water	Campylobacter	$9.55x10^3$	32	$3.51x10^4$	117
	Listeria	$4.51x10^3$	15	$1.52x10^4$	51
	Shigella	$4.97x10^3$	17	$2.13x10^4$	71
	E. coli	$3.57x10^3$	12	$1.39x10^4$	46
	Salmonella	$9.43x10^2$	3	$3.8x10^3$	13
Multiplexed	Campylobacter	$2.41x10^3$	8	$8.09x10^3$	27
	Listeria	$5.13x10^3$	17	$2.06x10^4$	69
	Shigella	$4.69x10^3$	16	$1.37x10^4$	46
	Salmonella	$5.61x10^3$	19	$1.40x10^4$	47

Summary & Future work

- Low detection limits for enteric bacteria (*Escherichia coli*, *Listeria*, *Salmonella* Typhimurium, *Shigella*, and *Campylobacter*) were achieved using centrifugal platform for single bacteria in buffer, tap water, and ground water, as well as for multiple bacteria in ground water
- Comparing the sensitivity of this assay to ELISA, where the detection limit for most bacteria ranges from 10^4 to 10^6 CFU/mL, lower concentrations can be detected (10^3 CFU/mL, or, as low as ~30 bacteria per reaction)
- SpinDX technique is highly adaptable for rapid development of new immunoassays by substituting target-relevant antibodies
- Our goal is to develop a rapid, reliable, cost-effective device for testing of water for presence of pathogenic bacteria

Acknowledgements

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References

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