# REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

## Rapid Colorimetric Sensors To Detect Toxic Chemicals In Environmental Samples

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The World Health Organization predicts that by 2025 half of the world's population will reside in water-stressed regions, with over a million deaths annually due to tainted drinking water, inadequate sanitation, and a lack of regulatory testing. For the DoD this means that Warfighters conducting multi-domain operations OCONUS are at significant risk of detrimental health effects due to consumption of contaminated water from indigenous sources. Especially given current testing methods that require shipping samples to a central lab with wait times ranging from 2 days to 8 weeks for analysis. The lack of rapid, easy to use, low size/weight/power/cost (SWaPC) tests for resource limited environments represents a significant health hazard that minimizes Soldier Lethality.

Here we describe development of paper-based devices in conjunction with capillary-flow-driven microfluidics to create a multiplexed colorimetric sensor that is rapid (mins) and sensitive (ppm-ppb). Specifically, we fabricated and optimized a multiplexed device for simultaneous testing of three heavy metal ions: nickel, copper, and iron. The device consists of three capillary flow channels created by stacking PET film with double-sided adhesive. Each flow channel transports liquid sample to a 5mm sized detection zone prepared by wax printing Whatman 1 grade paper. The detection zone contains two stacked detection layers with pre-stored reagents above two layers of waste pad that pull sample through the detection zone. Nickel is tested using Dimethylglyoxime as a bidentate chelating ligand that forms a clear pink color when reacted with Ni2+ under alkaline conditions. Copper is detected using Bathocuproine, a highly sensitive and selective Cuproine that instantly forms a brown color when reacted with Cu+ under acidic pH. Similarly, Iron is detected using Bathophenanthroline that forms a red color when it reacts with Fe2+ under acidic pH. The detection pads are fabricated at optimum pH for each assay using buffer solutions. The device also contains masking reagents like hydroxylamine, ammonium acetate, and sodium fluoride in pre-treatment zones to minimize interferences from other heavy metal ions. As constructed the sensor only requires one user input step (sample addition) and does not require accurate pipetting facilitating adoption by minimally trained users. The device can hold up to 300µl of water sample without the reagent being washed away. This stacking approach allows a high volume of sample to reach the detection zone faster compared to single layer paper microfluidic sensors increasing sensitivity.

The concentration is quantified in terms of relative grayscale intensity acquired with a flatbed scanner using ImageJ software. Thus far, the device has achieved detection limits as low as 2ppm for Nickel, 300ppb for copper, and 1.1ppm for Fe (within 5 mins) with minimal matrix effect on the signal. With further optimization we anticipate detection of metal contaminants in drinking water at or below military exposure guidelines (ppb levels). Future work includes assay development for colorimetric detection of organophosphates and perfluorinated (PFAS/PFOA) compounds with potential for multiplex detection of CBRN targets in various sample types.

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