Novel Immunocapture Technology for Field Deployable Nucleic Acid-Based Detection of Citrus Pathogens

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The primary hypothesis guiding this project is that a highly simplified and field deployable sample preparation system can be realized using low cost and easily used lateral flow chromatography technologies.

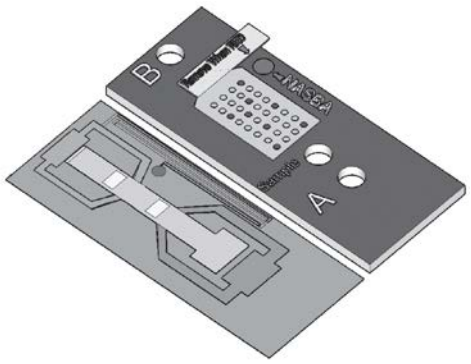
Specifically, we proposed the development of a nucleic acid analysis system making use of Lateral Flow Microarray technology (LFM) and supporting field deployable sample preparation and amplification methods. Toward this end we are developing and testing lateral flow chromatographic immuno-capture methods, novel passive fluid exchange systems, and integration of these systems to form the basis of a field deployable diagnostic platform.

In this period of performance, we have developed technologies critical to the successful realization of the project's primary deliverable: a facile sample preparation system suitable for supporting the detection of citrus pathogens. Moreover, we have demonstrated integration of these technologies into a sample preparation system offering inhibitor depletion, immuno-capture target enrichment and sample washing.

The passive device has been tested on a highly inhibitory and complex sample matrix. Using this newly developed approach, sample preparation steps, prior to isothermal amplification, are accomplished in 5-10 minutes with no user intervention and no laboratory instrumentation or external electrical requirements.

This method will form the foundation for a first generation prototype that will enable a rigorous evaluation of assay performance in the context of real-world samples. Indeed, in the current period of funding we have already demonstrated a breadboard implementation of a fully integrated immuno- capture and buffer wash device for tobacco mosaic virus (TMV) detection.

This breadboard system will form the basis for the development of a first generation prototype designed to support the detection of citrus pathogens. Additionally, this first generation system will allow exploration of varying approaches to engineering challenges associated with integrating additional assay subsystems.



A schematic representation of the nucleic acid detection platform currently under development. The internal components of the device are fabricated from thin absorbent substrates that enable sample processing as well as rapid nucleic acid amplification and detection. The low materials and manufacturing costs will allow the disposable device to provide cost effective testing for pathogens and genetic traits of interest. The integration of all steps from crude sample to DNA or RNA detection without user intervention or laboratory instrumentation offers an easily used solution to nucleic acid detection in the field.

Significantly, it should be noted that this first generation prototype will allow assays to be accomplished free of the instrumentation requirements associated with polymerase chain reaction (PCR) and enable sample preparation, amplification and detection to be conducted without dedicated laboratory instrumentation.

The methods developed to date have been demonstrated with complex samples containing significant concentrations of amplification enzyme inhibitors. To facilitate development under biosafety level-1, the technologies were tested using pathogen-specific reagents designed to detect tobacco mosaic virus. Sequence specific nucleic acid reagents can be readily redesigned for the detection of citrus pathogens of interest. However, the development of immunological reagents for immuno-capture methods, should no suitable ligands be commercially available, is more time consuming and costly. For this reason we are now developing methods that obviate the need to use immunological reagents to capture and concentrate dilute targets from complex specimens. These methods will significantly reduce the complexity associated with immuno- capture based approaches. Moreover, this approach, based upon nucleic acid hybridization-based template capture, will allow sample preparation systems and protocols to be further refined for more highly integrated devices requiring little or no user intervention.