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A Multiplexed Diagnostic Platform for Point-of-Care Pathogen Detection

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Disclaimer

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Abstract

We developed an automated point-of-care diagnostic instrument that is capable of analyzing nasal swab samples for the presence of respiratory diseases. This robust instrument, called *FluIDx*, performs autonomous multiplexed RT-PCR reactions that are analyzed by microsphere xMAP technology. We evaluated the performance of *FluIDx,* in comparison rapid tests specific for influenza and respiratory syncytial virus, in a clinical study performed at the UC Davis Medical Center. The clinical study included samples positive for RSV (n = 71), influenza A (n = 16), influenza B (n = 4), adenovirus (n = 5), parainfluenza virus (n = 2), and 44 negative samples, according to a composite reference method. *FluIDx* and the rapid tests detected 85.9% and 62.0% of the RSV positive samples, respectively. Similar sensitivities were recorded for the influenza B samples; whereas the influenza A samples were poorly detected, likely due to the utilization of an influenza A signature that did not accurately match currently circulating influenza A strains. Data for all pathogens were compiled and indicate that *FluIDx* is more sensitive than the rapid tests, detecting 74.2% (95% C.I. of 64.7 - 81.9%) of the positive samples in comparison to 53.6% (95% C.I. of 43.7 - 63.2%) for the rapid tests. The higher sensitivity of *FluIDx* was partially offset by a lower specificity, 77.3% versus 100.0%. Overall, these data suggest automated flow-through PCR-based instruments that perform multiplexed assays can successfully screen clinical samples for infectious diseases.

Introduction/Background

This project was funded by the LDRD committee to address the lack of rapid and accurate diagnostic tools at our healthcare facilities. The diagnostic tools currently available to point-of-care physicians are limited to immuno-assay based rapid test strips, which are only approximately 70% sensitive and screen for only one or two pathogens at a time. RT-PCR assays are approximately 100-fold more sensitive than immuno-assays and can be multiplexed to detect many pathogens in one assay. Currently, RT-PCR analysis requires a skilled technician working in a fully equipped clinical microbiology laboratory. The purpose of this LDRD project was to develop an automated instrument that would essentially replace the requirement of having a skilled technician perform manual RT-PCR assays to diagnosis a patient's sample. During the first two years of this LDRD project, we developed the first-ever bead-based multiplex instrument that is fully automated. The last year of the LDRD project focused on the evaluation of this new instrument against the immuno-assay tests. The positive data generated during the clinical trial demonstrates the proof-of-principle behind automating these types of assays and will hopefully lay the groundwork to bringing bead-based multiplex assays out of the laboratory and to the point-of-care where they will offer a better diagnostic alternative than immuno-assay tests. The arrival of this type of technology to the clinic will change

the manner in which care is delivered, including decisions surrounding prescribing anti-microbial drugs and admitting or discharging patients.

Results

Detailed descriptions of both the automated instrument and the clinical study are provided in the attached manuscripts, which at the time of writing this report, are being submitted for publication to the Journal of Clinical Microbiology. The first manuscript details the construction and operation of the instrument, and an analytical study that evaluated the ability of the instrument to reliably analyze samples in a reproducible manner. The second paper details the results of a clinical study using *FluIDx* and rapid tests to diagnose patient samples collected at the UC Davis Medical Center. The clinical study involved screening 141 nasal swab samples for respiratory viruses. Analysis of these data indicates that *FluIDx* is more sensitive, but less specific than the rapid tests. *FluIDx* has the added advantage of being able to screen for multiple pathogens at a time, thereby reducing overall cost and labor associated with determining the diagnosis of a sample.

Exit Plan

LLNL's Industrial Partnership Office has licensed *FluIDx* intellectual property to Tassajara Technologies and is currently negotiating a CRADA agreement to facilitate the commercialization of this technology in the private sector.