INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Simplification And Automation Of A Biological Sequencing Workflow For Threat-agnostic Detection In Field Settings

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Traditional nucleic acid amplification tests and immunoassays require a priori knowledge of the threat being targeted, whereas nucleic acid sequencing can be done in a non-targeted fashion that allows detection of any and all biological threats, both known and unknown. For this reason, incorporation of biological sequencing technology into the DoD's threat detection infrastructure is critical to address the rise in novel, emerging, and engineered biothreats. Recent advances in field portable sample preparation and sequencing devices have created an opportunity to revolutionize the DoD's capability to detect, identify, and characterize biological threats via sequencing at the point of need, thereby accelerating the timeline from sample collection to actionable intelligence. Despite these advancements, instrument size, method complexity, turnaround time, reagents that require cold-storage, and data analysis requirements are still hurdles limiting successful deployment of sequencing capabilities to field environments.

To address this gap, we performed feasibility testing of a simplified workflow that includes highly-portable sequencing devices, automated sample and library preparation modules, room-temperature stable reagents, and push-button data analysis for in-field detection, identification, and characterization of known, emerging, and engineered biological threat agents with minimal training. Results from this testing have proven the feasibility of a rapid, simplified, automated workflow for whole genome sequencing (WGS) in austere field settings. The workflow developed provided high-confidence, species-level detection of a simulant bacteria (Bacillus thuringiensis subsp. kurstaki) in <1 hour from sample to answer, and is fully automatable. We also performed studies to assess the feasibility of this workflow with additional sample/pathogen types, yielding valuable information on the current potential of the developed workflow and underlying technologies, as well as the remaining gaps and challenges that must be addressed.

After performing feasibility testing with the air-gapped workflow, we initiated development of a fully integrated, sample-to-answer sequencing device for agnostic detection, identification, and characterization of known, emerging, and engineered biological threat agents. This revolutionary low size, weight, and power (SWaP) device will integrate and automate nucleic acid extraction, sequencing library preparation, sequencing, data analysis, curation, and reporting into a portable, ruggedized, and battery-operated device. The device in development would be approximately 8"L x 6"W x 5"H, and weigh approximately 8 lbs. Furthermore, it would require no user intervention after loading a sample, making it an ideal technology for users that are not professional molecular biologists and/or have other duties to perform while the sample is being prepared for sequencing. Complete sample-to-answer automation will also facilitate future integration into autonomous environmental monitoring systems for aerosols, water, and other matrices, enabling early warning systems to detect novel threats.