Simplification and Automation of a Biological Sequencing Workflow for Threat-Agnostic Detection in Field Settings

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Background

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.

Objectives

The end goal of this work is development of a fully integrated, sample-to-answer sequencing device (**Figure 1**). This revolutionary device will:

- Automate nucleic acid extraction, sequencing library preparation, sequencing, data analysis, curation, and reporting
- Be portable, ruggedized, and battery-operated
- Have low size, weight, and power requirements (SWaP)
- Approximately 8"L x 6"W x 5"H, and weigh ~8 lbs.
- Require no user intervention after loading a sample
- Ideal for users with limited training and/or other tasks to perform
- Enable future integration into autonomous monitoring systems

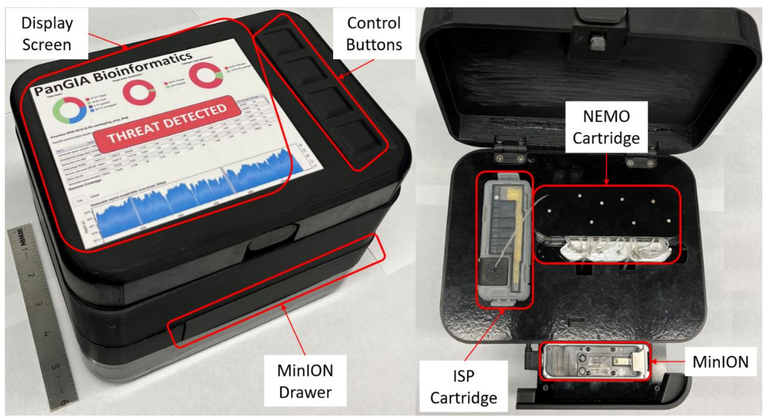

Figure 1. Conceptual Prototype of the Integrated Device

Feasibility Testing

Towards a solution for automated sequencing, we performed feasibility testing of a simplified workflow that includes a highly-portable sequencing device, automated sample and library prep 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. The current air gapped workflow (**Figure 2**) was used for feasibility testing of a fully-automated, integrated device now in development. This device will include several mature subcomponents to accelerate development and reduce technical risk.

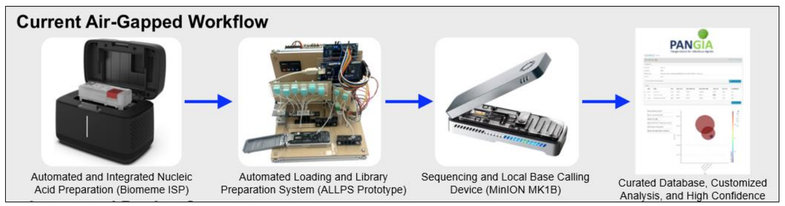

Figure 2. Air-Gapped Workflow Used for Initial Feasibility Testing

The air-gapped workflow for DNA targets started with sample addition into the Biomeme ISP for automated nucleic acid extraction. The extract was then manually transferred to our prototype for automated library preparation. After library prep, an operator connected the device outlets to a MinION flow cell for automated loading, and initiated sequencing. Finally, sequence data was processed using MRIGlobal's PanGIA bioinformatics tool to identify sequences of interest.

For RNA targets, a manual reverse transcription and non-targeted amplification process was performed after ISP extraction and prior to automated library prep.

Table 1. Overview of Feasibility Testing Results

Method	Agent	Sample description	Sequencing Result
Air-gapped system	BtK (vegetative bacteria)	Culture (210ng)	Positive detection ~15 minutes after MinION run start
	BtK (vegetative bacteria)	Culture, diluted 1:10 (24ng)	Positive detection ~25 minutes after MinION run start
	BtK spores (sporulated bacteria)	1e8 CFU on air filter	Positive detection ~25 minutes after MinION run start
Air-gapped system, with *manual* reverse transcription/ amplification during air gap	VEEV (RNA virus)	Culture (9ng RNA; 25ng cDNA)	Positive detection ~11 minutes after MinION run start
	BtK spores *and* VEEV	5e4 CFU/PFU on air filter	Positive detection ~10 minutes after MinION run start for BtK; ~20 minutes for VEEV

Table 2. Approximate Sample-to-Answer Time for DNA Targets

Process	Component	Time (minutes)
Automated Nucleic Acid Extraction	Biomeme ISP	15
Automated Library Prep & Flow Cell Loading	MRIGlobal ALLPS	10
Initiation of the Sequencing Run	ONT MinION	5
Real-Time, Subspecies-level Detection of BtK	MRIGlobal PanGIA	15-25
Total turnaround time from sample insertion to answer:		**45-55**

Conclusions & Next Steps

Initial feasibility testing using the air-gapped workflow demonstrated feasibility for rapid (<1 hour) and specific (to the subspecies level) detection from high biomass, low complexity samples. After completing 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 the nucleic acid extraction, sequencing library preparation, sequencing, data analysis, curation, and reporting into a portable, ruggedized, and battery-operated device. Importantly, this device will 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.

In addition to developing an initial fully integrated prototype for DNA, we are also performing R&D to expand the device's capability to include lower biomass and higher complexity samples, as well as RNA targets.

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