INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Nationwide Bioaerosol Metagenomic Repository For Environmental Biodetection

Kamil Khanipov University of Texas Medical Branch   **Vivian Tat** University of Texas Medical Branch   **Kostiantyn Botnar** University of Texas Medical Branch   **Shanna Ratnesar-Shumate** U.S. Environmental Protection Agency   **Sean Kinahan** United States Department of Homeland Security Science and Technology Directorate   **Madison Farnsworth** University of Texas Medical Branch   **Sarah Alnemrat** University of Texas Medical Branch   **Justin Nguyen** University of Texas Medical Branch   **David Kimmel** United States Department of Homeland Security Science and Technology Directorate   **Hema Narra** University of Texas Medical Branch   **Christine Tomlinson** United States Department of Homeland Security Science and Technology Directorate   **George Golovko** University of Texas Medical Branch

Background information: Biological threat agents (bioagents) are pathogens that can pose substantial harm to human health. We have a fundamental understanding of most existing bioagents and the ongoing development of novel therapeutics has substantially enabled effective interventions over time. However, these bioagents continue to present significant public health challenges. Early detection is critical in effectively containing, mitigating, and treating a biothreat. However, commonly utilized detection methods rely on a priori knowledge of the potential biothreat vectors. Sequencing-based approaches have the capability to detect any biothreats in a single assay agnostically. Previously, the wide utilization of sequencing technologies was limited by cost and infrastructure requirements. Currently, sequencing data poses challenges in discerning endemic pathogens from intentional releases. These challenges can be addressed by understanding the background bioaerosol makeup and developing novel algorithms to identify novel and unknown pathogens in complex backgrounds. Background bioaerosol data would serve as a common repository to enable the development of computational approaches to discriminate between background aerosols and hazardous aerosols.

Purpose: The Hazard Awareness & Characterization Technology Center within the Department of Homeland Security Science and Technology Directorate is supporting the development and standardization of approaches to isolate nucleic acids and perform field-capable metagenomic sequencing of air filters. Developed standardized approaches have been used to sequence and characterize the microbial composition of over 800 high-volume air filters from over 140 locations throughout the continental United States. Background bioaerosol characterization across the United States will enable the development of computational algorithms to identify hazardous aerosols.

Methods: Starting in January 2024, 3-micron fluoropore filters from environmental aerosol sample collectors located in major metropolitan areas and at select special events throughout the United States were gathered during each of the four seasons. A logistic operational protocol has been developed to preserve the microbial content of the air filters during transport. Commercially available DNA/RNA isolation kits have been evaluated. Additional optimization was implemented in utilizing the Oxford Nanopore Technologies sequencers. Captured bioaerosols from over 800 filters across the United States have been characterized by metagenomic and metatranscriptomic sequencing.

Results: Environmental bioaerosol metagenomic data has been generated, analyzed, and will be made accessible to the community. The identified naturally occurring background aerosol composition will enable the development of computational approaches to detect/define potential anomalies and novel outbreaks. The developed standard operating procedures will allow the community to continually source additional background bioaerosol data.

Conclusions: The optimized methodology and publicly available databases describing the bioaerosol composition around the nation will provide rich spatial and temporal bioaerosol data. This information will enable the development of technologies to identify anomalies and potential bioagents in aerosols.

Impact to the Defense Threat Reduction Agency Joint Science and Technology Office mission and Joint Force: This activity impacts the defense community by providing the first comprehensive baseline environmental microbial Next-Generation Sequencing database generated from aerosol sample collectors throughout the United States. The publicly available database may be leveraged to enable the maturation of technology that can detect hazardous bioaerosols.