Multidisciplinary Research Program in Medicine Project*: Wastewater-based surveillance of infectious diseases*

Hypothesis or Research Question(s): Can nanotechnology bead-based capture of SARS-CoV-2 generate high quality RNA extracts that suitable for detecting cryptic SARS-CoV-2 lineages in wastewater using long-read sequencing technology?

Can this approach be used for other viral pathogens as well?

PROJECT BACKGROUND & SUMMARY

Wastewater based epidemiology (WBE) is a growing field that utilizes molecular information from the engineered urban water system to infer community spread of disease. WBE has been applied in the past to identify outbreaks of poliovirus in urban areas, and more recently has been expanded in a global effort to monitor the transmission of the SARS-CoV-2 virus that causes COVID-19. Sequencing every clinical patient sample in a highly populous area is a difficult feat, and thus sequencing SARS-CoV-2 RNA in municipal wastewater offers great promise to augment genomic surveillance by characterizing a pooled population sample matrix. In the past two years, our interdisciplinary team of microbiologists, bioinformaticians, epidemiologists, and environmental engineers has developed approaches to quantify SARS-CoV-2 in wastewater, as well as methods to generate whole genome sequence data of SARS-CoV-2. While our methods have been useful for detecting the emergence of variants of concern in BC wastewater, our approach currently relies on existing knowledge of SARS-CoV-2 genomic sequences, which may become less available as clinical testing wanes globally. Thus, there is a critical need to detect emerging variants, or potentially novel pathogens, in wastewater without relying on genomic information from clinical samples. This information can be critical to detect potential immune or vaccine escape variants as we potentially head into a `post-pandemic` era.

This interdisciplinary project will leverage recent advances in environmental engineering based on metagenomic sequencing of wastewater using long-read sequence technologies combined with public health and clinical microbiology to develop new tools to identify cryptic SARS-CoV-2 lineages in wastewater. One limitation of existing approaches is that the method to concentrate viruses from wastewater (centrifugal filtration) generates RNA of inadequate quality and quantity for de novo detection of variants. Student #1 will validate and optimize an engineered bead-based nanotechnology to capture and enrich viral particles from Metro Vancouver influent wastewater samples and perform RNA extraction. The two students will then develop and apply new approaches to amplify and sequence full RNA transcripts on the Oxford Nanopore Technologies platform. Student #2 will perform bioinformatic analysis of the RNA sequence data to identify genomic segments of novel or rare variants of SARS-CoV-2, as well as evidence of other pathogens of concern (RSV, norovirus, influenza, etc). They will also be responsible for analyzing the data in the context of wastewater metadata, including wastewater flow, viral load, and sewer catchment. This project thus combines principles from the engineered and built environment, environmental genomics, clinical microbiology, and public health. The outcomes of this project will be tools to identify and monitoring emerging variants and pathogens in wastewater, for use in public health epidemiology and policy.

BENEFIT TO THE STUDENTS

This project seeks to involve two students in high-impact and interdisciplinary research on pathogen surveillance in the engineered and built environment. The project will offer opportunities to learn and conduct rigorous and quality-driven research by our existing collaborative team of BCCDC microbiologists and UBC engineers. The students will be exposed to the fields of civil engineering,

environmental sampling, molecular biology and genomics, bioinformatics, clinical microbiology, and public health. They will gain experience working in an interdisciplinary project team; refine their written and spoken communication skills; learn to prioritize and manage multiple tasks; and gain practical hands-on experience in molecular biology/genomics techniques and analysis.

Both students will gain wet-lab experience in working with RNA and next-generation sequencing (Oxford Nanopore). Student #1 will gain additional experience in validating a novel technology for concentrating viruses and will work closely with the project mentors to develop and carry out validation experiments. Student #2 will gain experience with bioinformatics, including command-line coding in languages like python, bash, and/or R, as well as data visualization. They will also analyze the sequencing data in context of the wastewater metadata. Both students will gain experience designing and carrying out experiments, as well as documenting work and sharing findings with the team. These activities will enable the students to gain experience in communicating the data analysis and conclusions in a concise scientific manner. All of these duties and responsibilities will be performed under the direct supervision of the principal investigators and an engineering PhD student that has experience in wastewater-based epidemiology and genomics.

The students will experience working in an academic research environment and an applied public health organization. They will rotate through both the Ziels laboratory on UBC campus and the Prystajecky laboratory at the BCCDC Public Health Laboratory. Their research endeavours will be supported by a large multidisciplinary team at both sites. They will also engage with project stakeholders and end-users. For example, ¬the students will work with Metro Vancouver to coordinate sample collection from an engineered water system, and with BCCDC epidemiologists to understand how the wastewater data is integrated into public health surveillance products. This is a unique opportunity for the students to build a diverse network of contacts, which will be meaningful in their academic careers. Furthermore, the students have the opportunity to work on a fast-paced COVID-19 project, where research methods are quickly implemented for routine use. They will observe the rigour that is needed in developing new molecular and genomics-based tools, and the documentation that is needed prior to implementation. The student will contribute to a significant and ongoing disease outbreak by providing meaningful data for public health decision making.

The two students will attend weekly joint-lab meetings with UBC/BCCDC team members to ensure effective communication of the results. They will also be invited to attend meetings with internal and external collaborators. At both laboratories, there are many undergraduate students, graduate students and other trainees, so that they students can build a rapport with other students working on similar topics. This provides an opportunity for peer-to-peer support and building relationships with like-minded individuals. Between the PIs, PhD student mentor and laboratory staff at both sites, there will always be someone to assist the students and answer questions. Frequent feedback and open communication will ensure the projects are on track and that the students are receiving the support they need to complete the projects.

Lastly, the principal investigators have a strong record of mentoring students. The PIs will ensure that the students are well trained, supported and have all the tools needed to carry out their work. They will introduce them to a diverse network of scientist and engineers that will be beneficial for their career endeavours. The PIs will routinely check-in with the students to support their personal and academic growth.