Click on the Grant Reference No. to view the abstract

Grant Reference No.	Institution	Principal Investigator	Project Title
PREPARE-CS1-2022-001	Nanyang Technological University	Julien Lescar	Building and validating a modular vaccination platform via multivalent presentation of antigens using protein-based scaffolds
PREPARE-CS1-2022-002	National University of Singapore	Sylvie Alonso	Harnessing the Clec9A targeting strategy to sustain and broaden protective immune responses in SARS-CoV2 mRNA-vaccinated populations
PREPARE-CS1-2022-003	Institute of Materials Research and Engineering (IMRE), A*STAR	Su Xiaodi	Nanomaterial-based Infectious Airborne Virus Sensors for On-site Environmental Surveillance
PREPARE-CS1-2022-004	Institute of High Performance Computing (IHPC), A*STAR	Ooi Chin Chun	Development of Risk Assessment Model for Infectious Respiratory Diseases Considering Human Mobility
PREPARE-CS1-2022-005	Advanced Remanufacturing and Technology Centre (ARTC), A*STAR	Nicholas Tong	Integrated MasterMix and Sample Preparation Automated System
PREPARE-CS1-2022-006	National University of Singapore	Kevin White	RNA-based mucosal platform
PREPARE-CS1-2022-007	Duke-NUS Medical School	Antonio Bertoletti	Development of a rapid assay for the evaluation of pathogen-specific T cells in human samples
PREPARE-CS1-2022-008	Tan Tock Seng Hospital	Kalisvar Marimuthu	Preparedness program for air and environmental sampling for future outbreak from emerging or novel pathogens
PREPARE-CS1-2023-010	Diagnostics Development Hub	Ou Chung-Pei	Development of a PCR based Point of Care Testing (POCT) Device
PREPARE-CS1-2023-011	National University of Singapore	Tan Chee Wah	Next-generation all-in-one multiplex surrogate assay for viruses with pandemic potential
PREPARE-CS1-2024-012	Experimental Drug Development Centre (EDDC)	Kantharaj Ethirajulu	Assessment of 3CL protease inhibitors for broad-spectrum activity to respond to future pandemics caused by coronaviruses
PREPARE-CS1-2024-013	A*STAR Infectious Diseases Labs	Guillaume Carissimo	Cell based viral reporters for future pandemic preparedness
PREPARE-CS1-2024-014	National University of Singapore	Andy Tay Kah Ping	TOPAD: Tonsil Organoid Platform for Antibody Discovery
PREPARE-CS1-2024-015	National University of Singapore	Justin Chu Jang Hann	Augmenting Cardiac Glycosides as Broad-Spectrum Therapeutics for Orthomyxoviruses and Coronaviruses
PREPARE-CS1-2025-016	Experimental Drug Development Centre (EDDC)	Xu Weijun	Computer-aided and Artificial Intelligence-Empowered Drug Discovery

Grant Reference No.	Institution	Principal Investigator	Project Title
			of Best-in-Class/Third-Generation H5N1 Neuraminidase Small Molecule Inhibitors
PREPARE-CS1-2025-017	Duke-NUS Medical School	Adamberage Ruklanthi de Alwis	Original Antigenic Sin in Humanized Mice: A Platform for Broadly Protective Antibody Discovery Against Pandemic Pathogens
PREPARE-CS1-2025-019	Nanyang Technological University	May O. Lwin	Vaccines and vaccinations: A study of evolving attitudes and uptake behaviours
PREPARE-CS1-2025-020	Institute of Policy Studies, Lee Kuan Yew School of Public Policy, National University of Singapore	Mathews Mathew	IPS-PREPARE Panel Study on Sociobehavioral Influences in Public Health: A Multi-Year, Multi-Method Approach
PREPARE-CS1-2025-021	Institute of Mental Health (IMH)	Mythily Subramaniam	Understanding the Needs of Persons with Mental Illness during the COVID-19 Pandemic and Assessing their Preparedness and Willingness for Research Participation during Future Pandemics
PREPARE-CS1-Dx-CORE-2025-001A	Institute of Microelectronics (IME), A*STAR	Doris Ng Keh Ting	Photonics-based Rapid Detection with multiple biomarkers for Respiratory Infectious disease screening
PREPARE-CS1-Dx-CORE-2025-001B	National University of Singapore	Yan Jie	Development of Multiplexed Immunodiagnostic for Respiratory Viral Infections
PREPARE-CS1-Dx-CORE-2025-001C	National University of Singapore	Tedrick Thomas Salim Lew	Antibody-Free Nanosensors for Accelerated Viral Protein Diagnostics (AsAP)
PREPARE-OC-ETM-Dx-2023-002	Singapore-MIT Alliance for Research and Technology	Megan McBee	Wastewater-based assay for epidemic surveillance and population serology
PREPARE-OC-ETM-Dx-2023-005	Genome Institute of Singapore, A*STAR	Chew Wei Leong	Viral detection by direct sample application on functionalised solid state nanopores
PREPARE-OC-ETM-Dx-2023-006	Genome Institute of Singapore, A*STAR	Niranjan Nagarajan	SurVirAR: An end-to-end rapidly deployable on-site platform for sensitive whole-genome surveillance of viruses using AI-based read-until nanopore analytics
PREPARE-OC-ETM-Dx-2023-010	Institute of Materials Research and Engineering (IMRE), A*STAR	Laura Sutarlie	Ultrasensitive nanomaterial-based sensor coupled with multistage filtration Sample treatment for on-site SARS-CoV-2 wastewater surveillance
PREPARE-OC-VT-2022-001	Infectious Diseases Labs, A*STAR	Matthew Zirui Tay	Development of an ACE2 decoy as a universal anti-coronavirus therapeutic

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Grant Reference No.	Institution	Principal Investigator	Project Title
PREPARE-OC-VT-2022-008	National University of Singapore	Herbert Schwarz	Evaluation of CD137 ligand as adjuvant for enhancing vaccine efficacy
PREPARE-OC-VT-2022-010	National University of Singapore	Tan Yee Joo	Establishment and use of robust assays for measuring functional antibodies binding to hemagglutinin (HA) and neuraminidase (NA) of multiple subtypes of influenza A virus in serological analysis of vaccinated and infected people in Singapore
PREPARE-OC-VT-2022-012	National University of Singapore	Tran Thai	One-stop integrative platform for fast-track screening of species and tissue tropism of unknown respiratory disease threats (Disease X), and for emerging respiratory pathogen surveillance
PREPARE-OC-VT-2024-002	Duke-NUS Medical School	Sook Yuin Jessica Ho	Discovery of anti-influenza A therapeutics with a strategy for simultaneous heterosubtypic targeting that hedge against drug resistance
PREPARE-OC-VT-2024-003	Nanyang Technological University	Xiao Tianshu	AI-facilitated development of pan-coronavirus fusion inhibitors
PREPARE-OC-VT-2024-004	Nanyang Technological University	Luo Dahai	Development of new self-amplifying RNA based mRNA vaccines for epidemic preparedness and response
PREPARE-OC-VT-2024-008	Genome Institute of Singapore, A*STAR	Chew Wei Leong	Broad-spectrum antiviral RNA nucleases
PREPARE-OC-Dx-2024-002	Nanyang Technological University	Hou Han Wei	ViroArc: Integrated microfluidics for rapid isolation, enrichment and early detection of live viruses from nasopharyngeal swabs
PREPARE-OC-Dx-2024-004	Nanyang Technological University	Xueming Dong	An Automated Rapid Structural-Based Epitope Mapping Platform Based on Hydrogen-Deuterium Exchange Mass Spectrometry
PREPARE-OC-Dx-2024-005	Institute of Molecular and Cell Biology, A*STAR	Farid Ghadessy	Development of novel robust polymerase enzymes with increased resistance to inhibitors present in clinical samples and higher processivity for rapid pathogen detection
PREPARE-OC-Dx-2024-008	National University of Singapore	Hu Chunyi	VirNASTURE: Virus Nucleic Acid on-Site Testing via Ultra-sensitive Nucleases Escalation
PREPARE-OC-Dx-2024-009	National University of Singapore	Lanry Yung Lin Yue	Isothermally Amplified Antibody-Oligo Probes as Ultrasensitive Rapid Protein Test
PREPARE-OC-Dx-2024-010	National University of Singapore	Justin Chu Jang Hann	Development of a cell-free influenza virus infectivity assay with portable-biochips (FLUVIABLEBiochips)

Grant Reference No.	Institution	Principal Investigator	Project Title
PREPARE-OC-Dx-2024-012	National University of Singapore	Tan Yee Joo	Establishing an efficient workflow for in vitro selection of synthetic nanobody libraries to identify high affinity binders of influenza A viral proteins so as to accelerate the development of antigen detection assay in the event of a flu pandemic
PREPARE-OC-Dx-2025-017	Singapore University of Technology and Design (SUTD)	Ye Ai	Field-deployable quantitative molecular diagnosis by a portable digital polymerase chain reaction (pdPCR) device empowered with integrated optofluidic technology
PREPARE-OC-VT-2025-001	Institute of Molecular and Cell Biology (IMCB), A*STAR	Wong Fong Tian	FAST-TRACK: FDA-approved Antiviral Screening Through Translational Research Accelerated by Computational Knowledge
PREPARE-OC-VT-2025-002	Infectious Diseases Labs, A*STAR	Marco Vignuzzi	A self-amplifying RNA vaccine against Henipaviruses
PREPARE-OC-VT-2025-003	Infectious Diseases Labs, A*STAR	Matthew Zirui Tay	Single-cell sVNT screening platform for rapid discovery of neutralizing monoclonal antibodies
PREPARE-OC-VT-2025-004	Infectious Diseases Labs, A*STAR	Ning Li	Developing multisulfonated copper iodide clusters as broad-spectrum intranasal prophylaxis and treatment for viral infections with high outbreak potential
PREPARE-OC-VT-2025-008	Nanyang Technological University	Nam-Joon Cho	Development of Broad-Spectrum Peptide-Based Antivirals
PREPARE-OC-VT-2025-011	Duke-NUS Medical School	Nina Le Bert	Development of a disease-protective vaccine to control zoonotic paramyxovirus infections through the identification of broadly cross-reactive T cell epitopes
PREPARE-OC-VT-2025-015	National University of Singapore	Ge Ruowen	ISM1 as a novel host anti-influenza factor
PREPARE-OC-VT-2025-019	National University of Singapore	Chen Kaiwen	Targeting and rewiring of host cell death pathways to fight severe viral infection
PREPARE-OC-ETM-2025-001	Institute of High Performance Computing (IHPC), A*STAR	Ooi Chin Chun	Development of a **S**ystematic **A**ssessment **F**ramework for **E**valuating **A**ir-Purification **I**nterventions in the **R**eal-world (SAFE-AIR): A First Demonstration on Worker Dormitories
PREPARE-OC-ETM-2025-002	National University of Singapore	Soh Siow Ling	Charged Spray for Rapid and Targeted Clearance of Airborne Virus-loaded Aerosols
PREPARE-OC-ETM-2025-006	Nanyang Technological University	Li Hongying	**AIM-Mitigation**: **A**dvanced **I**ntegrated **M**ultimodal **Mitigation (AIM-Mitigation)** Strategies for Reducing Respiratory Disease via Experimental Testing and

Grant Reference No.	Institution	Principal Investigator	Project Title
			Data-Assimilated Artificial Intelligence Modelling
PREPARE-SF-2023-003	National Centre for Infectious Diseases	Ng Oon Tek	Metagenomics for emerging infectious disease detection and surveillance
PREPARE-SF-2023-004	Oxford University (Mahosot Hospital), Lao PDR	Audrey Dubot-Pérès	A pilot surveillance system for RSV in children presenting to Hospitals in Lao PDR
PREPARE-SF-2023-005	National Institute of Public Health (NIPH), Cambodia	Chhorvann Chhea	Genomic Surveillance for Strengthening Public Health Response in Cambodia
PREPARE-SF-2023-006	National Institute of Hygiene and Epidemiology (NIHE), Vietnam	Mai Le	Pathogen Genomic Surveillance and Immunology in Vietnam
PREPARE-SF-2024-007	National Centre for Infectious Diseases	Conrad Chan En Zuo	Clinical validation and cost effectiveness study of a self-administered multi-pathogen antigen rapid test (SMART)
PREPARE-SF-2024-013	Duke-NUS Medical School	Gavin Smith	Assessing the zoonotic risk and pandemic potential of highly pathogenic avian influenza (HPAI) H5N1 viruses
PREPARE-UKRI-SF-2025-004	National University of Singapore	Tan Chee Wah	Development and assessment of novel, high-throughput immunological assays to improve surveillance of spillover of viral families of pandemic potential
PREPARE-UKRI-SF-2025-007	National Centre for Infectious Diseases	Ng Oon Tek (Singapore)	Studying Hygiene Interventions to reduce Nosocomial Infections in southeast Asian Intensive Care Units (SHINIA-ICU)

Annex – Abstracts of Awarded Projects

PREPARE-CS1-2022-001: Building and validating a modular vaccination platform via multivalent presentation of antigens using protein-based scaffolds

Vaccination remains key to combat infectious diseases. In the context of covid and future flu pandemics, a timely response requires the development of “plug and play” vaccination platforms that can be readily used to protect the population. In parallel to RNA vaccines that can be rapidly deployed, we propose to validate an alternative, modular, nanoparticle-based vaccination platform, that will help to respond to future viral outbreaks and pandemics. This vaccine platform entails the multivalent presentation of antigens at the surface of a protein-based scaffold to elicit a protective immune response. The presentation of a repetitive array of antigens at a surface of a protein scaffold enables efficient binding and activation of multiple B-cell receptors and hence a much more sustained immune response compared to immunization with isolated protein antigen subunits.

With the genetic addition of small handles, protein-based assemblies such as ferritin or lumazine synthase constitute biocompatible, homogeneous scaffolds that can be stored and readily used to accept various viral antigens overexpressed separately, once the circulating virus/variant has been identified. We propose to (i) display major antigens of SARS-Cov2 and Influenza H5 at the surface of nanoparticle scaffolds (NPs) and (ii) assess whether these NPs can generate both strong and sustained B-cell IgG and T-cell protective responses. For comparison purpose, the presentation of Spike or haemagglutinin antigens to a large scaffold (ferritin or lumazine synthase) will be carried out using three parallel approaches: (i) through genetic fusion (ii) using proprietary ultrafast peptide asparaginyl ligases of Singzyme Pte Ltd, a Singapore company for which the PI is cofounder (iii) using the Spytag/Spycatcher system. Accordingly, yield, homogeneity, and scalability of NPs will be compared.

To validate the platform efficacy, immunogenicity following mice immunization with the best NPs formulations, will be performed as well as pseudovirus infection neutralization assays against variant of concerns.

PREPARE-CS1-2022-002: Harnessing the Clec9A targeting strategy to sustain and broaden protective immune responses in SARS-CoV2 mRNA-vaccinated populations

We have developed a versatile vaccine platform that induces a rapid and exceptionally sustained immune response upon a single shot of a small amount of antigen, thereby featuring the most desired vaccine attributes. The technology consists of fusing the antigen vaccine candidate to a proprietary anti-Clec9A monoclonal antibody (mAb) that targets a specific dendritic cell subset.

We have generated a complete proof-of-concept dataset in mice using the universal influenza vaccine candidate M2e, and more recently, we have applied this technology to COVID19 by fusing the Spike's Receptor Binding Domain (RBD) to Clec9A mAb. We showed that single-shot systemic immunization with Clec9A-RBD construct induced in mice neutralizing antibody titers that kept improving over a 15-month monitoring period, suggesting sustained affinity maturation and generation of long-lived plasma cells. An on-going study in non-human primates (NHP) using fully humanized Clec9A-RBD (huClec9A-RBD) construct indicates that this clinical vaccine candidate is safe and immunogenic.

The goal of this proposal is to evaluate **Clec9A-RBD immunization as a booster vaccine approach** to address the rapid waning of antibody titers in COVID19 mRNA-vaccinated individuals, **thereby reducing the need for frequent booster immunizations**. In addition, we aim at inducing a **broad, pan-sarbecovirus protective immune response** by boosting with a Clec9A construct that harbors SARS-CoV1 RBD sequence or a consensus RBD.

In the first phase of the project, we will generate the Clec9A-RBD constructs. In a second phase, they will be evaluated as a heterologous booster in COVID19 mRNA-vaccinated mice. Both the systemic and nasal route of immunization will be assessed. Humoral and cellular immune responses will be evaluated against SARS-CoV2 Variants of Concern (VoC) and other members of the Sarbecovirus family, and will be compared to mRNA-vaccinated mice only. In phase 3, fully humanized huClec9A-RBD constructs will be generated, and their immunogenicity will be evaluated in mRNA-vaccinated NHP.

PREPARE-CS1-2022-003: Nanomaterial-based Infectious Airborne Virus Sensors for On-site Environmental Surveillance

Aerosol transmission plays a major role in large-scale infectious disease outbreaks. It is important to monitor and mitigate airborne transmission of respiratory viruses. However, surveillance of environmental airborne viruses remains challenging due to the low viral concentration in air, inaccessibility to surrogate research tools, and limited knowledge on the effective viral load and behavioural patterns of aerosols containing viruses during airborne transmission. Furthermore, rapid, portable virus detection tools compatible with aerosol sampling techniques for on- site detection are not available.

In this project, we aim to develop nanomaterial-based sensors that can couple with commercial air samplers (liquid impinger or membrane-based samplers) for on-site detection of aerosolized virus. We target SARS-related coronaviruses, by exploiting SARS-related CoV spike pseudotyped viruses as surrogates and engineer the nanoparticle surface with specific ligands (e.g. antibodies and ACE2 receptor) that recognize all SARS-related CoV. The nanoreagents will provide visible color readout when the spike proteins interact with the ligands, thus providing a rapid, hassle-free on-site detection. Furthermore, our sensor will be able to differentiate SARS-related CoV from common cold viruses (e.g. OC43). To support the sensor development, experimental chambers will be developed with airflow simulations for effective aerosolization of virus particles mimicking enclosed or air-conditioned environments, optimal placement of air samplers, and understanding virus particle distribution. We will also test the sensor on live SARS-CoV-2 in a BSL-3 environment. Upon successful sensor development, we will demonstrate its on-site detection in an identified hospital hotspot or in the scale-down model for the selected on-site environment.

In the post-pandemic era, on-site air-surveillance sensor helps to monitor indoor air quality and mitigate aerosol transmission risk. Such platform sensor can be quickly customized targeting to new virus during an outbreak to identify contaminated areas for more targeted disinfectant/mitigation steps.

PREPARE-CS1-2022-004: Development of Risk Assessment Model for Infectious Respiratory Diseases Considering Human Mobility

The evolution of COVID-19 virus requires government interventions to devise effective control measures for different stages. Mapping and understanding the risk to individuals in a population under various circumstances is crucial to development of mitigation strategies. This proposal aims to develop a generic model to evaluate both aerosol and large droplet transmission risk for respiratory diseases such as COVID-19.

The model incorporates flow physics of infectious droplets and people's mobilities to study the spread of respiratory diseases. Droplet trajectories will be predicted by computational fluid dynamics (CFD) and generalized by Artificial intelligence (AI). Agent-based models (ABMs) will be developed to study people's mobility across various places. Human mobility datasets and crowd simulation will be utilized to generate time-3D space trajectories to understand the spread of disease in dynamic environments. This is coupled with CFD to capture both spatial and temporal aspects for disease transmission. For large-scale case studies, social contact network and time-space itinerary will be created from available data from LTA DataMall and Household Interview Travel Survey (HITS). Lab experiments will be performed using time resolved Particle Image Velocimetry (PIV), laser diffraction and high-speed camera for droplet distribution to provide critical initial conditions, analysis and validation. Strategies for air sampling using droplet counters at critical locations will be developed.

The developed model can quantify infection risk for both individuals and populations in different places such as malls. The change in risk arising from human behavior and mobility such as, but not limited to, use of PPEs, physical activity, and transport will be predicted. The model serves as a basis for scientific inquiry of dynamic transmission risk of respiratory disease, at the same time practical and accessible for public health specialists. The results will provide insights for government to respond nimbly by designing effective mitigation measures for future pandemic situations.

PREPARE-CS1-2022-005: Integrated MasterMix and Sample Preparation Automated System

The aim of the project is to resolve the remaining gaps in the workflow when operating BRAVE+ equipment. This is achieved by automating laborious and skill dependent work and move towards a fully automated solution that can operated with minimal training and longer walkway. Identified remaining risks like spillage during transfer into liquid handling system, bio contamination and spillage risk during manual pipetting of positive control and sealing of PCR plate would be addressed with improved workflow and automation. The project will deliver a fully verified working equipment and build capability within research institutes and system integrator to enable quick deployment when in need for the next pandemic.

PREPARE-CS1-2022-006: RNA-based mucosal platform

Recent advances in RNA based vaccines have proven their utility. The rapid development and deployment of effective SARS-CoV-2 mRNA prophylactic vaccines is a testament to the power of human scientific innovation in the face of crisis. However, the mRNA vaccines in current use are still first-generation products with much room for improvement. Gaps to be filled with future innovations include stability (elimination of ultra-cold chain), toxicity/immunoreactivity, and long-term efficacy. Both innovations involving the RNA substance and the delivery vehicle that carries it to cells are required to tackle these gaps. Biologically, it is likely that a mucosal active vaccine would constitute an important step toward improved efficacy. Current vaccines are administered through intramuscular injection and most immediately elicit strong IgM and IgG responses. However, eliciting a mucosal response including IgA maturation would add another protective dimension to SARS-CoV-2 and many other types of diseases. Delivery through oral or nasopharyngeal routes may also prove desirable compared to intramuscular injection. Here we propose to systematically test two different RNA approaches and three different delivery approaches toward the development of an RNA-based mucosal vaccine platform. In the first nine months we intend to narrow the candidates from a matrix of at least 8 combinations to 3-4 combinations. In the second six months we intend to test those 3-4 combinations for efficacy in eliciting an immune response in mice.

PREPARE-CS1-2022-007: Development of a rapid assay for the evaluation of pathogen-specific T cells in human samples

Coordinated activation of humoral and cellular immune response is necessary for rapid elimination of different pathogens. However, while measurement of pathogen-specific antibodies is a routine test in different infections, pathogen-specific T cell characterization is performed only in selected infections (Tuberculosis and HCMV) and not evaluated for clinical or epidemiological purposes due to the technical complexity and the length of the assay protocol required to evaluate pathogen-specific T cells.

Here, we aim to setup a testing pipeline that can be rapidly deployed to analyze the T cell responses against known or emerging infectious diseases of concern.

We recently applied a rapid and simple assay to robustly quantify SARS-CoV-2-specific T cells from the whole blood based on cytokine secretion and/or gene expression changes after stimulation with SARS-CoV-2 peptides. This rapid T cell assay was used extensively to evaluate the SARS-CoV-2 specific T cell response in infected and/or vaccinated individuals during the current COVID-19 pandemic.

We propose to adapt this SARS-CoV-2 specific T cell assay to analyze T cell responses against other infectious pathogens only by changing the stimulatory antigens (with peptides or proteins). The robustness of the assay will be evaluated to determine whether the approach is readily generalizable across different infections, mainly viruses (i.e. seasonal coronaviruses, influenza, dengue, or other viruses of concern) but also bacteria. We would also determine whether the protocol could be further simplified. In addition, due to the increased likelihood of transmission of respiratory viruses and its primary site of infection (respiratory tract), we would develop and apply the rapid T cell assay to detect the virus-specific T cell responses directly in the nasal cavity.

This T cell testing pipeline will be an important complement to existing antibody assays for the holistic immunological monitoring of newly emerging or known infections that are actively circulating in the population.

PREPARE-CS1-2022-008: Preparedness program for air and environmental sampling for future outbreak from emerging or novel pathogens

Aim

The aim of the proposal is to bring together a team of experts who, during peacetime, will establish the necessary infrastructure, work processes, methods and the research networks in infectious diseases transmission dynamic field in order to better prepare for future outbreak/pandemic. The team also aims to develop research credentials in transmission dynamics investigations in order to strengthen national preparedness program. Lastly, collected data on transmission dynamics will help form the basis of modelling studies for future outbreak projection.

Hypothesis

1. Expiratory emissions from patients may contain infectious micro-droplets.

2. Asymptomatic and minimally symptomatic cases may emit infectious aerosols through aerosolising activities.

3. Air and surface contamination pose significant transmission risks and may propagate outbreak, both in the hospital and community settings.

4. Duration of viral shedding and degree of environmental contamination by emerging or novel pathogens can be inferred by longitudinal observation of their genetic material and fluorescent properties detected in air and surfaces sample.

Methodology

The proposed project is divided into two parts depending on the national/global situations. Preparedness plan will encompass peace time work, which include air sampling investigation to study respiratory viral particles using various air samplers, real-time bioaerosols monitoring using fluorescent based equipment, method development for collection of exhaled breath using G-II machine and Perspex box and environmental sampling of MDROs to understand its transmission dynamic. Outbreak/ pandemic response works will include investigational work of emerging/novel pathogens, to evaluate the extent of air and environmental contamination and assess infectivity potential of new variants.

Clinical significance

Understanding transmission data is crucial in developing safe infection control protocols to reduce the risk of health care-associated transmission of emerging/novel pathogens. From public health point of view, understanding the kinetics of viral shedding in relation to transmission dynamic is important to tailor appropriate outbreak mitigation measures.

PREPARE-CS1-2023-010: Development of a PCR based Point of Care Testing (POCT) Device

Our project aims to develop a point-of-care test (POCT) device with Polymerase Chain Reaction (PCR) grade capabilities that can be produced locally at scale to prepare for future pandemics. This portable device will provide rapid, accurate, and reliable results, bringing diagnostic capabilities closer to the patient's location for enhanced diagnostics and promptness. By leveraging scalable and established technologies, the device will deliver precise diagnoses at the testing point.

With its intuitive interface and user-friendly operation, the device will be accessible to individuals without prior training requirements. Its rapid and accurate diagnostic capabilities will empower healthcare professionals to make informed decisions and initiate timely interventions, ultimately improving patient outcomes, reducing transmission risks, and optimizing resource allocation.

Our project will focus on developing a minimal viable product (MVP) through the creation of PCR based POCT diagnostic device with interchangeable modules. These modules can be easily modified or incorporated for different assays or sample types, providing flexibility and adaptability to the multiple potential technologies identified within this project to accommodate future needs. This approach allows for the improvement of one module while keeping the other modules unchanged. The approach avoids a complete redesign of the whole system resulting in reductions in development time and cost.

By prioritizing local design and sourcing, we aim to foster self-sufficiency and reduce dependence on external supply chains. This approach will enable us to create a robust and reliable testing solution that can be rapidly deployed on a large scale during a pandemic. The device's seamless integration of technologies and its adaptability to evolving diagnostic needs will drive innovation and advance healthcare delivery.

The device would address major functions required in a POCT workflow involving molecular and engineering processes illustrated in Figure 1.

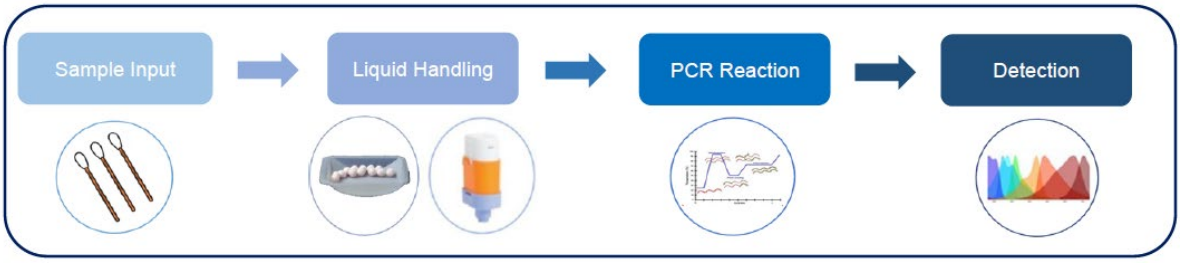

Figure 1: Workflow of operating our POCT Device.

PREPARE-CS1-2023-011: Next-generation all-in-one multiplex surrogate assay for viruses with pandemic potential

The importance of preparedness for public health emergencies is evident in the catastrophic COVID-19 pandemic. The unprecedented loss of human lives, livelihood disruptions, and shuttering of economies during the COVID-19 pandemic highlighted that the inadequacy in pandemic preparedness and the urgency to improve existing public health emergency readiness strategies. Extensive land uses and climate changes have significantly altered the wildlife ecosystem, which is then expected to increase spillover of zoonotic pathogens from wildlife to human populations. Therefore, early detection of spillover events is needed to prevent the progression of outbreaks to pandemics. This proposal aims to develop a lab-based and point-of-care multiplex surrogate virus neutralization test (sVNT) to monitor the spillover of zoonotic viruses with pandemic potential in human-animal interfaces. These include pathogens with the highest priority defined by NIH and WHO, such as coronaviruses, influenza viruses, arenaviruses, filoviruses, and henipaviruses. We have demonstrated the feasibility of multiplex microsphere-based sVNT in SARS-related coronaviruses. We will develop a next-generation, all-in-one multiplex sVNT to detect neutralizing antibodies for viruses with pandemic potential. Preliminary, we have demonstrated that sVNT applies to viruses beyond SARS-CoV-2, including MERS-CoV and Nipah virus, and are multiplexable. To offset the limitations of a lab-based assay, we aim to establish a point-of-care lateral flow assay (LFA) to detect virus-specific neutralizing antibodies. Our data demonstrated that SARS-CoV-2 LFA sVNT detects neutralizing antibodies in nasal swab samples in individuals with COVID-19 breakthrough infection. As part of the effort in pandemic preparedness, we will perform multination active serosurveys of highly pathogenic viruses in this region to better understand the regional infectious disease threats and pre-emptively prepare for the outbreaks through therapeutic and vaccine development. We also aim to identify individuals with broad-spectrum pre-existing immunities to these viruses for therapeutic monoclonal antibody isolation as part of the effort to develop universal antibody vaccines.

PREPARE-CS1-2024-012: Assessment of 3CL protease inhibitors for broad-spectrum activity to respond to future pandemics caused by coronaviruses

In response to pressing unmet need from the COVID-19 pandemic, EDDC discovered a novel and potent class of macrocyclic 3CLpro inhibitors. These inhibitors demonstrated in vitro efficacy against various α and β coronaviruses, including SARS-CoV-2, MERS, and 229E. Given their activity profile, they are promising interventions for current and future outbreaks.

The 3CL protease plays a pivotal role in virus replication and is a critical and clinically validated drug target. The conservation of coronavirus 3CLpro across strains underscores the potential for developing a broad-spectrum inhibitor to address potential future coronavirus outbreaks. Existing Covid-19 treatment options using small molecules face significant challenges, with approved small molecule drugs exhibiting limitations mainly due to their suboptimal safety profile and drug-drug interaction issues because of the need to combine some of them with a CYP3A4 inhibitor. In addition, occasional rebound episodes have been reported after treatment with nirmatrelvir–ritonavir. Mutations in coronavirus 3CLpro have also been described that confer drug resistance to nirmatrelvir. As part of safeguarding and preparation for a future coronavirus induced outbreak, there is a need to develop compounds with potent, broad-spectrum activity against α and β coronaviruses.

Our studies aim to further progress our most promising macrocyclic compounds, and to evaluate their potential towards clinical application. Specific aims include 1. scaling up two most promising compounds, 2. evaluating their antiviral activity against 3CLpro of SARS-CoV-2 mutants and other coronaviruses, and 3. assessing in vivo PK properties of both compounds, and the efficacy of one selected compound in a SARS-CoV-2 and in a MERS mouse model. The results of this project would guide the way for further preclinical development activities leading to Investigational New Drug (IND) submission.

PREPARE-CS1-2024-013: Cell based viral reporters for future pandemic preparedness

Recent emergence of highly pathogenic members of the coronavirus (CoV) family (SARS-CoV-1, -2 and MERS) led to severe outbreaks that gravely impacted human health, and there are recurrent reports of avian influenza transmission to humans with high potential for pandemic. SARS-CoV-2 still remains a persistent threat, especially for the immunocompromised and elderly. Limited therapeutics are available to combat SARS-CoV-2 or influenza infections. Additional therapeutics are needed against these viruses, and a better pipeline for faster discoveries of antiviral compounds are necessary for Singapore to be ready for the next pandemic. To this end, we aim to constructs and optimize cellular reporters allowing screening of compounds using wild type viruses. Reporter will be designed to allow a broad range of utilization.

PREPARE-CS1-2024-014: TOPAD: Tonsil Organoid Platform for Antibody Discovery

Antibodies with high specificity and potency are one of the best treatment options against global scale infectious diseases that threaten human health and the economy. However, existing platforms including *in vitro* B cell culture, phage/ribosome/yeast display technologies and humanized mice suffer from disadvantages including poor human immune cell diversity and species differences.

Organoid technology is a promising alternative to existing models for human antibody discovery because of its physiological resemblance to human organs. We have developed tonsil organoids with rich cell diversity and biofunctions by co-culturing heterologous lymphoid cells with their donor-matched stromal fibroblastic reticular cells. Our tonsil organoids contained an unparalleled cell diversity with 21 different immune cell types. Antigenic stimulation with phytohemagglutinin and tetanus toxoid proteins increased CD83+/CXCR4+ expressions, suggesting that the organoids supported the formation of germinal centers. Organoids derived from SARS-CoV-2 vaccinated donors mounted effective adaptive immune response to COVID-receptor binding domain (RBD) stimulation. Using naïve R-phycoerythrin antigen, we also found that dendritic cells in lymphoid organoids were able to uptake, process, and present antigens to B cells.

Here, we propose a tonsil organoid platform for antibodies discovery (TOPAD). Organoids from at least 10 donors (5 vaccinated and uninfected with SARS-CoV-2 and 5 vaccinated and infected with SARS-CoV-2) will be characterized for immune and stromal cell diversity, tissue organization and biofunctions against previously exposed antigens. We will also assess the ability of the organoids to recognise naïve antigens and mount adaptive humoral and cellular response to them. We believe that our technology will be a key enabling technology for human antibody discovery to advance human health, generate economic value, protect national interest, and reduce global inequality in infectious disease treatment.

PREPARE-CS1-2024-015: Augmenting Cardiac Glycosides as Broad-Spectrum Therapeutics for Orthomyxoviruses and Coronaviruses

Singapore as a travel hub is inevitably exposed to infectious disease circulating from neighbouring countries, as evidenced by the COVID-19 pandemic. As such, strong pandemic preparedness resources, including a healthy stockpile of **broad-spectrum antivirals**, are required. Human viruses from families of ***Coronaviridae*** and ***Orthomyxoviridae*** are medically important as they cause millions of life-threatening diseases annually. These viruses are single-stranded RNA (ssRNA) viruses that hijack host functions to replicate during infections. Therefore, identifying shared host factor mechanisms will provide broad-spectrum treatment against these viruses. We have recently identified potent **plant-derived cardiac glycosides** as broad-spectrum antiviral against these viruses. These compounds work with efficacy at nanomolar concentration with no observable cellular toxicity and their mechanistic action targets host factors are shared by the ssRNA viruses. However, challenges persist including identification of pharmacophore/s responsible for the broad-spectrum antiviral activities, and determination of *in vitro* efficacy. Nonetheless, as these compounds have undergone clinical trials for various diseases such as hypertension, asthma, and congestive heart failure, established pipelines and dosages of these compounds will allow for the **ideal preclinical testing of broad-spectrum antivirals that can be rapidly channeled into future clinical trials**. Henceforth, we envisaged to refine the small molecule cardiac glycosides for rapid development of broad-spectrum antivirals, especially against highly pathogenic ssRNA viruses. We aim to identify and further refine the pharmacophore/s responsible for antiviral activities and synthesise analogues with improved efficacy, and safety profile. To facilitate the enhancement of these analogues for broad-spectrum inhibition across the selected viruses, we will test them in *in vitro* models, to generate relevant data that can improve the antiviral's translation into preclinical settings. Overall, this will set the foundation for future progress of these cardiac glycoside class of compounds into **new broad-spectrum antiviral applications** for preclinical trials.

PREPARE-CS1-2025-016: Computer-aided and Artificial Intelligence-Empowered Drug Discovery of Best-in-Class/Third-Generation H5N1 Neuraminidase Small Molecule Inhibitors

On Friday, 13th-December 2024, a patient was hospitalized with a severe case of avian influenza A(H5N1) virus ("H5N1 bird flu") infection in Louisiana. This marked the first instance of severe illness linked to the virus in the United States. Since April 2024, there have been a total of 61 reported human cases of H5 bird flu reported in the United States. The emergence of drug-resistant strains of influenza has raised significant concerns regarding the effectiveness of current neuraminidase (NA) inhibitors, such as oseltamivir (Tamiflu) and zanamivir (Relenza). Resistance, or low oral-bioavailability, of current drugs significantly limits their use for future pandemic preparedness. Thus, there is a need for the development of new therapeutics that are effective against resistant strains and provide a broader spectrum of action with superior oral bioavailability.

In alignment with Singapore's (MOH) Programme for Research in Epidemic Preparedness and Response's (PREPARE) initiative, we/EDDC, as a national platform for drug discovery, propose to employ cutting-edge computational chemistry tools including virtual screening, generative AI and free energy perturbations to identify inhibitors that could provide a starting point for further optimization and development into pre-clinical broad-spectrum candidates in combating influenza, increasing our preparedness for future pandemics, where new strains caused by mutation within NA could significantly threaten public health.

PREPARE-CS1-2025-017: Original Antigenic Sin in Humanized Mice: A Platform for Broadly Protective Antibody Discovery Against Pandemic Pathogens

The COVID-19 pandemic underscored the urgent need for robust countermeasures against emerging zoonotic viruses, particularly in Asia, a region with heightened spillover risk. Monoclonal antibodies (mAbs) are crucial tools for both therapy and prophylaxis, but their rapid development hinges on access to virus-exposed B cells. This proposal addresses this limitation by establishing an end-to-end pipeline for isolating and characterizing broadly neutralizing mAbs from immunoglobulin-humanized mouse models, thereby enhancing Singapore's pandemic preparedness.

The project leverages the concept of original antigenic sin, employing sequential immunization with heterologous viral antigens to elicit broadly protective mAbs. This approach builds on recent findings demonstrating that prior SARS-CoV infection, followed by SARS-CoV-2 mRNA vaccination, can induce mAbs neutralizing diverse SARS-CoV-2 variants and related sarbecoviruses. The study comprises of three aims, where aim 1 proposes to validate the humanized mouse model's ability to generate broadly neutralizing SARS-CoV-2 antibodies through a prime-boost regimen, comparing immune responses to existing human data. Aim 2 focuses on isolating pan-sarbeco and pan-betacoronavirus neutralizing mAbs by optimizing sequential immunization strategies with heterologous coronavirus spike proteins. Aim 3 extends this approach to henipaviruses, another high-risk viral family, aiming to generate cross-neutralizing mAbs against multiple henipavirus strains. This proposal includes detailed characterization of polyclonal antibody responses, memory B cell profiling, and isolation and functional analysis of neutralizing mAbs. By establishing this pipeline, the project aims to develop a rapid response strategy for future pandemics, providing a library of broadly protective mAbs against critical viral threats.

PREPARE-CS1-2025-019: Vaccines and vaccinations: A study of evolving attitudes and uptake behaviours

The COVID-19 pandemic has altered public discourse on vaccine safety, efficacy, and trust in public health communication, particularly as new vaccine technologies were met with scepticism. The introduction of new vaccine technologies, information overload, floods of medical misinformation and conspiracy theories contributed to an "infodemic" that challenged public health efforts in Singapore and around the world. As attitudes and trust towards vaccines and vaccinations have shifted since the pandemic, this research seeks to explore how vaccine uptake patterns might have evolved since the pandemic and whether acceptance of COVID-19 vaccines has influenced the acceptance of other vaccines. Taking a two-prong approach, this study will first examine sociodemographic differences in post Covid era vaccine attitudes and behaviours, to make sense of why some consistently vaccinate, vaccinate within recommended timeframes, and choose specific vaccine technologies. This will be done through a triangulation of vaccination records, survey and news media data that will be analysed using time-series, cluster analysis, and feature identification. The second prong focuses on tracking Singaporeans' evolving attitudes and beliefs towards vaccines, including factors that may be influencing their beliefs, such trust in government and healthcare systems, as well as cultural and religious factors. These attitudes will be examined through sociodemographic and psychosocial lenses via a nationally representative survey, focus groups, and social media monitoring. The data will be analysed through multivariate regression, cluster analysis, and thematic analysis. Findings of this research will inform public engagement and education strategies for public health, improve immunisation infrastructure, and provide evidence-based support for public health policies. Through a thorough understanding of sociodemographic and psychosocial factors that are influencing vaccine attitudes and vaccination behaviours, the study has the potential to enhance public health resource allocation, support herd immunity, and contribute to longer-term disease management strategies, ensuring greater preparedness for future health crises.

PREPARE-CS1-2025-020: IPS-PREPARE Panel Study on Sociobehavioral Influences in Public Health: A Multi-Year, Multi-Method Approach

How can we effectively plan for future epidemics if we do not fully understand how perceptions and needs have evolved since COVID-19? The pandemic's ripple effects on beliefs, behaviors, and societal norms continue to shape the world we live in, highlighting the importance of examining these societal dynamics over time. Building on lessons learned from the COVID-19 pandemic, the IPS-PREPARE panel study aims to explore how the government can enhance its ability to meet the needs of Singapore's diverse population in future public health crises, with a particular focus on supporting vulnerable communities and ensuring equitable outcomes across all sectors of society. A key question underpinning this study delves into the strategies which can improve the effectiveness of government measures and ensure clear dissemination of information during future health emergencies.

To address these research aims, the study will evaluate the general population's perceptions of Singapore's pandemic response, assess its effectiveness across various sectors of society, and identify areas for improvement, while leveraging on the IPS COVID-19 Panel. The study will employ a comprehensive mixed-methods approach over three years to capture nuanced and longitudinal insights, comprising: (i) a 12-wave online panel (n=1000), (ii) three online survey experiments (n=2000), (iii) message testing via eye-tracking (n=200), and (iv) qualitative research through expert interviews (n=20) and focus groups (n=60).By examining existing systems and testing innovative, forward-thinking approaches, the study aims to optimize the government's public health measures and the populace's pandemic readiness for future crises.

This research is structured to be both reflective and forward-looking, aiming to derive insights from past experiences while developing comprehensive and inclusive frameworks for future responses. These findings will better equip Singapore to navigate future public health challenges and foster better outcomes through a society that is more resilient and adaptable to uncertainties.

PREPARE-CS1-2025-021: Understanding the Needs of Persons with Mental Illness during the COVID-19 Pandemic and Assessing their Preparedness and Willingness for Research Participation during Future Pandemics

The proposed study employs a mixed-method approach to understand pandemic preparedness among and challenges faced by people with mental illness (PMI) during pandemics. This mixed-method study will pilot a cohort of PMIs and structured methods of data collection that can be tapped on in future pandemics, enabling more rapid policy response.

The qualitative study will explore psychological and social factors affecting access to healthcare, as well as unmet needs experienced by PMI during the COVID-19 pandemic, while the survey will collect a non-pandemic baseline measures for this population's answers to the pilot questionnaire. The development of this cohort and questionnaire is critical as no PMI cohort or PMI-specific questionnaire currently exists to be leveraged in crises, and we hypothesise that PMI will have unique needs during pandemics, which have not been identified before in the Singapore's population-based studies.

Semi-structured interviews will be conducted with those with depression, schizophrenia spectrum disorder, and anxiety disorders (n=15-18 per group up to 45-54 total) to obtain detailed and meaningful insights while the quantitative study will identify the pandemic preparedness of different PMI populations, and their preferred sources of information. Furthermore, participants will also be asked about their willingness to participate in research during pandemics and the preferred modes of survey administration.

In all 225 patients with MDD, schizophrenia spectrum and anxiety disorders will be surveyed on their pandemic preparedness and perceived challenges and facilitators for coping with a pandemic.

This study will contribute to

- Development of an enhanced Pandemic Preparedness Questionnaire and a pilot PMI survey cohort.
- Identification of critical gaps and needs in pandemic preparedness among PMI.
- Providing suggestions for improving pandemic preparedness and survey administration to PMIs.
- Generating a recommended list of tracking indicators to monitor the ability of PMI to cope during a pandemic.

PREPARE-CS1-Dx-CORE-2025-001A: Photonics-based Rapid Detection with multiple biomarkers for Respiratory Infectious disease screening

Respiratory viruses are transmitted via multiple channels (Fig. 1). They exhibit variability in structure, susceptibility, disease severity, and transmissibility, complicating clinical differentiation. Influenza such as influenza A and B, causes significant morbidity and mortality, with 3-5 million severe cases annually. SARS-CoV-2/COVID-19 pandemic altered respiratory virus epidemiology, with non-pharmaceutical interventions mitigating spread. Respiratory viruses can cause epidemics, with influenza infecting 10‑15% globally each year and attack rates up to 50% during major outbreaks. It is therefore important to be able to do quick quantifiable screening at triage, hospital isolation wards, clinics, airport and seaport to sieve out infected patients, isolate them and provide required treatment before the spread become uncontrollable. This will lessen hospitalization burden, load at triage area and empower healthcare providers to make informed decisions. The current clinical practice is by performing reverse transcription polymerase chain reaction (RT-PCR) or an antigen rapid test (ART) from nasal swab biospecimen. RTPCR requires 24 hours of processing time whereas ART, which is a qualitative output, requires 15-30 mins (after obtaining the nasal swab). In this proposal, 2 teams A*STAR (Institute of Microelectronics and Singapore Institute of Manufacturing Technology) with different expertise (Sensors and MedTech respectively), clinicians from Singapore General Hospital and a photonics Professor from UCLA come together to develop a fast response optical-based biosensor to rapidly detect and quantify multiple respiratory viruses. The sensor chip will leverage on high performance Si-based photonics wafer-level technology developed by IME. This biosensor promises response in less than 30s and with ability to quantify the specific biomarkers for respiratory infectious disease screening. Deliverable will be an optical breadboard proof-of-concept biosensor that does not require extensive sample preparation and with ability to detect and quantify Influenza A and SARS-CoV-2 as a first demonstration. This can further expand to more than 2 respiratory viruses beyond Year 1.

PREPARE-CS1-Dx-CORE-2025-001B: Development of Multiplexed Immunodiagnostic for Respiratory Viral Infections

This proposal aims to enhance epidemic preparedness by developing two cutting-edge Point-of-Care (POC) diagnostic platforms tailored for detecting pandemic-potential viruses, with a focus on Flu A and SARS-CoV-2 as the standard targets for cross-platform benchmarking. Using our patented immunodiagnostic technology—leveraging mechanical force to dissociate nonspecific biomolecular complexes—these platforms provide highly specific and sensitive, quantitative or qualitative, detection with significantly shorter turnaround times, substantially simplified workflows, and improved cost-effectiveness compared to traditional ELISA. This research will advance these technologies to Technology Readiness Level (TRL) 4, validating them in relevant environments and strengthening Singapore's capability to monitor transmission of respiratory viruses.

PREPARE-CS1-Dx-CORE-2025-001C: Antibody-Free Nanosensors for Accelerated Viral Protein Diagnostics (AsAP)

The COVID-19 pandemic exposed critical delays in the development of diagnostics for emerging infectious diseases, driven by dependence on antibodies or aptamers, which require lengthy production workflows. This proposal aims to address this gap with an antibody-free nanosensor platform for rapid, multiplexed viral protein detection. The sensing platform utilizes single-walled carbon nanotubes (CNTs) wrapped with single-stranded DNA to create synthetic molecular recognition motifs, eliminating reliance on biological receptors to detect specific viral antigens. These DNA-functionalized nanosensors leverage CNTs' stable fluorescence and DNA's programmable binding to detect viral proteins with high sensitivity and adaptability. With these sensors, we will develop real-time detection platforms for clinical diagnostics. The system can be rapidly configured for new or mutated pathogens, enabling deployment within days or weeks, significantly accelerating diagnostic readiness during outbreaks. Its quick customizability allows for swift validation and deployment in early stages of an infectious disease outbreak, overcoming delays associated with antibody or aptamer production.

We propose two specific aims to demonstrate the platform's utility. Specific Aim 1 will establish proof-of-concept by engineering CNT-DNA nanosensors to detect the N protein of SARS-CoV-2, demonstrating binding sensitivity of the sensors for viral protein detection. Specific Aim 2 will expand this platform for detection of Flu A Hemagglutinin, providing design principles for broader adaptability. Performance of the nanosensors will be evaluated by quantifying the limit of detection (LoD), reproducibility, as well as an assessment of the usability and manufacturability in the Singapore context. Overall, this project aims to showcase the promise of antibody-free nanosensors to distinguish between negative samples and spiked SARS-CoV-2 or Flu A Hemagglutinin samples, thereby accelerating diagnostics for future unknown viruses. By enabling rapid and highly adaptable protein diagnostic solutions, this antibody-free nanosensor platform addresses critical unmet needs in outbreak preparedness and represents a significant advancement in medical diagnostics for clinical applications.

PREPARE-OC-ETM-Dx-2023-002: Wastewater-based assay for epidemic surveillance and population serology

This proposal aims to develop a wastewater monitoring workflow for a **quantitative population serology** method to determine antibody responses against viral antigens. As a complementary platform to current nucleic acid-based wastewater surveillance systems, the human response (antibodies) provides a longer-scale and differential surveillance timeline of the population. Wastewater is a complex matrix from which specific recovery of human viruses remains a challenge, albeit significant progress has been made during the COVID19 pandemic. We aim to develop a quantitative method to profile antiviral immune responses of a community via virus-specific antibodies in wastewater using digital ELISAs. The average human excretes 2-3g of antibodies a day into feces and urine. However, in part due to the chemical complexity and proteolytic degradation in wastewater, conventional ELISAs have insufficient sensitivity to detect antigen-specific antibodies in wastewater. To overcome this, we will use the rcSso7d binder technology coupled with digital ELISA to achieve quantitative detection of antiviral antibodies at femtomolar sensitivity. The workflow, initially for SARS-CoV-2, is quickly adaptable to survey other viruses. This proposal combines key competencies of multiple labs – McBee's immunology, Preiser's infectious diseases, Alm's waste water surveillance and genomics, and Sikes's rcSso7d binder and diagnostic technologies. This work utilizes our ability to generate high-affinity binders, trained personnel, and infrastructure that are available at SMART and NTU.

PREPARE-OC-ETM-Dx-2023-005: Viral detection by direct sample application on functionalised solid state nanopores

We propose an innovative technology for real-time surveillance of viral proteins from environmental samples, advancing two specific aims in a unique integration of technologies. Aim 1 focuses on highly sensitive and specific viral protein detection by coupling solid-state nanopores with viral-specific affinity probes, specifically aptamers and/or antibodies. These nanopores contain individually addressable and sensitive electric sensors that provide real-time and quantitative readouts for the presence and concentration of bound viral proteins based on disruptions in ionic current. Aim 2 focuses on streamlining sample processing by incorporating a denaturation reagent and simple filter for rapid fragmentation and filtration of environmental samples spiked with viral antigens. The processed samples will be applied onto the functionalized solid state nanopores for evaluating quantitative detection of viral antigens. Both Aims 1 and 2 are supported by proof-of-concept data. The direct application of environmental samples, in conjunction with the novel detection and processing technologies, will bring the targeted solution closer towards a sensitive, rapid, and on-site solution for viral surveillance and outbreak monitoring.

PREPARE-OC-ETM-Dx-2023-006: SurVirAR: An end-to-end rapidly deployable on-site platform for sensitive whole-genome surveillance of viruses using AI-based read-until nanopore analytics

Acquisition and analysis of viral genomes at scale is critical for effective pandemic preparedness. Viral genomes derived from readily accessible surveillance samples hold essential data which can be exploited to delineate disease transmission dynamics and pinpoint infection hotspots. However, current metagenomic surveillance methodologies face a significant limitation in obtaining whole viral genomes, particularly when viral titers are low, thus curtailing the full potential of metagenomic surveillance. Our proposal seeks to overcome this gap by developing a novel end-to-end platform, to facilitate comprehensive environmental Surveillance of Viral genomes integrating Artificial Intelligence (AI) and Read-until nanopore sequencing (SurVirAR). We hypothesize that a real-time AI-based read-filtering approach can sensitively identify viral reads, providing a 10-100 fold enrichment of viral sequences obtained via metagenomics, while addressing limitations of state-of-the-art experimental viral enrichment protocols (Aim #1). We then employ machine learning models that can enrich viral reads and assign the reads into different "bins"‑ each representing sequences that originate from the same viral genome (Aim #2). Our models thus enable the sensitive tracing of novel viruses and facilitate precise genome reconstruction. Using a targeted genome assembly algorithm, we anticipate the retrieval of hundreds of viral genomes directly from complex environmental samples (Aim #3). We envisage that this rapidly deployable onsite platform will transform our ability to perform and scale viral surveillance from environmental samples. This AI-augmented capability to acquire whole viral genomes from surveillance samples will enable comparative genomic analysis with viruses identified in patient samples, thereby rapidly identifying and linking any potential public health threats. Our proposed platform, therefore, seeks to address the limitations inherent in existing metagenomic surveillance technologies, thereby significantly enhancing our capacity to prepare for future pandemics.

PREPARE-OC-ETM-Dx-2023-010: Ultrasensitive nanomaterial-based sensor coupled with multistage filtration Sample treatment for on-site SARS-CoV-2 wastewater surveillance

Infectious pathogens shed from symptomatic and asymptomatic individuals can be carried into wastewater. As seen in the COVID-19 pandemic, wastewater surveillance can provide early warning, situational and transmission risks monitoring of pathogens of pandemic potential. Currently wastewater surveillance relies on laboratory-based sample treatment and analysis, upon samples collection and transportation to laboratories. On-site sample treatment and detection of infectious pathogens from wastewater are in demand. The elimination of sample transportation and tedious laboratory-based detection can provide faster information for real-time assessment, particularly in regional context where laboratories are far from sample collection areas.

We aim to develop an ultrasensitive nanomaterial-based sensor coupled with multistage filtration sample treatment for on-site wastewater surveillance with SARS-CoV-2 as the use case, which has been actively monitored in Singapore. A rapid sensor targeting the S and N proteins of SARS-CoV-2 will be developed by exploiting catalytic/ luminescence nanoparticles functionalized with specific affinity-ligands (antibodies, cell receptors, or aptamers with high selectivity to S/ N proteins) on an affinity-modified column which concentrates the S/ N proteins through filtration of high sample volume. The sensor will be coupled with a portable multi-stage wastewater filtration to eliminate wastewater background interference on-site. The whole system will be designed as a platform technology that can be automated in the future and customizable for monitoring of different infectious pathogens. We will test the sensor with wastewater samples from three on-site locations in Singapore. The ultrasensitive sensor coupled with the portable multistage filtration will increase on-site wastewater surveillance capabilities and enhance public health monitoring for future pandemic preparedness.

PREPARE-OC-VT-2022-001: Development of an ACE2 decoy as a universal anti-coronavirus therapeutic

ACE2 decoys have great potential to treat or prevent all current and potential ACE2 mediated coronavirus infection. In this study, we aim to create an optimized recombinant human soluble ACE2. We have previously generated initial candidates by performing *in silico* SSM using a native human ACE2‑SARS-CoV-1/2 complex homology model, in tandem with high-throughput screening. In particular, we identified a candidate, ACE2-YHA, which exhibits high neutralizing potency across a wide range of COVID-19 viral mutations, as well as SARS-CoV-1. However, this candidate displays a tendency toward aggregation, hindering its developability.

Here, we propose to develop alternative ACE2 decoys with good developability properties. We will attempt two approaches. Firstly, leveraging our past findings, we will identify the landscape of ACE2 decoy candidates with similar or even broader activity against diverse coronaviruses, and identify whether any of these candidates have reduced aggregation issues. Second, we will use bioinformatic prediction on problematic areas within our existing lead candidate, ACE2-YHA, and test if mutations in these areas will reduce aggregation. The top leads will be tested for aggregation propensity and thermal stability. They will also characterized for preclinical efficacy against live SARS-CoV-2 *in vivo* and preliminary toxicology studies. A final lead ACE2 decoy with low aggregation propensity and potent efficacy is selected.

Together, these will bring the molecule through TRL4, ready for scale-up and additional toxicology studies, followed by GMP-compliant pilot lot production and human clinical trials. When further brought through clinical trials for human safety, an ACE2 decoy will be highly useful for Singapore's pandemic defense, as a ready-to-use therapeutic against ACE2-dependent coronaviruses.

PREPARE-OC-VT-2022-008: Evaluation of CD137 ligand as adjuvant for enhancing vaccine efficacy

Vaccination is one of the most effective strategies against emerging infectious diseases and pandemics. Vaccination can also be a potent defence against bioterrorism. Adjuvants are necessary for most vaccines to enable them to induce protective immunity. Adjuvants are often inorganic molecules such as aluminium hydroxide derivatives that cause a limited tissue damage to induce immune activation. During a natural immune response to a pathogen, expression of costimulatory molecules is induced on antigen presenting cells that potently enhance T cell activation, and steer the immune response to the polarization that is most effective for the elimination of that particular pathogen. Learning from nature, we hypothesize that inclusion of the T cell costimulatory molecule CD137 ligand (CD137L) into a vaccine will boost its efficacy and steer the resulting immune response towards a cellular Th1 / Tc1 response that is most appropriate for eliminating virus-infected cells. We propose to test this hypothesis on three different vaccine types, recombinant Adenovirus (AdV), synthetic mRNA and a protein subunit vaccine. We will focus on two respiratory viruses namely SARS-CoV2 and influenza A that cause a significant disease burden on societies and are of pandemic potential. We will use established vaccine antigen candidates, namely the receptor binding domain (RBD) from SARS-CoV-2 spike protein, and the M2 protein and the nucleoprotein (NP) of Influenza A. CD137L will be added either as a separate molecule or expressed as a fusion protein with these antigens. Protective cellular and humoral immune responses will be evaluated in murine models. This multidisciplinary endeavour will help identifying effective vaccine prototypes against prevalent viruses.

PREPARE-OC-VT-2022-010: Establishment and use of robust assays for measuring functional antibodies binding to hemagglutinin (HA) and neuraminidase (NA) of multiple subtypes of influenza A virus in serological analysis of vaccinated and infected people in Singapore

Influenza A virus (IAV) has caused 4 global influenza pandemics. Several IAV subtypes have infected humans and there are many avian subtypes that can cause zoonotic transmission. Our objective is to establish robust assays for measuring functional antibodies binding to hemagglutinin (HA) and neuraminidase (NA) of multiple subtypes of IAV. Due to historical infection and vaccination, most people in Singapore have anti-IAV antibodies targeting both HA and NA and we hypothesize that people who are vaccinated or infected with IAV have different levels of anti-N1 and anti-N2 inhibition antibodies which bind to homologous and intra-subtypic heterologous NA.

As there are multiple HA subtypes circulating in animals, we will set up pseudo-typed virus for all 18 HA subtypes and a novel cell-free hemagglutination inhibition assay to measure anti-HA neutralizing antibodies which protect against infection. These surrogate assays will be designed to allow high throughput capacity. Recombinant proteins of N1 to N9 subtypes will also be purified and used in enzyme-linked lectin assay for NA inhibition measurement. For NA inhibition antibodies, it is important to determine the stimulation by vaccination versus infection in different age groups and cross-reactivity of these antibodies to heterologous virus of avian IAV because anti-NA antibody is an independent correlate of protection against disease and could be crucial when there is a lack of strain-matching anti-HA neutralizing antibodies due to antigenic shift or drift in HA. Using our assays, which do not require high biosafety contamination and can be modified to match any newly emerged IAV, we will optimize the workflow for deployment in the event of an influenza pandemic. To validate the performance of these assays, they will be used to measure antibodies titers in human sera and compared side-by-side with current assays using live virus of H3N2 and H1N1 subtypes that are circulating in human population.

PREPARE-OC-VT-2022-012: One-stop integrative platform for fast-track screening of species and tissue tropism of unknown respiratory disease threats (Disease X), and for emerging respiratory pathogen surveillance

Respiratory infections remain the world's most deadly communicable disease (WHO)1. The COVID-19 pandemic highlighted the need to fast-track our understanding of the virus functions on the host and to also consider long-term changes in lung function (breathing difficulties) among patients surviving COVID-19 pneumonia (termed post-COVID or long-COVID)2, long after the viral load becomes undetectable by test kits. Many virological-based studies lack an assessment of virus interactions on lung function mechanics, which is essential to preventing and treating long-term respiratory deterioration. We have established a 3D-lung slice model that closely recapitulates the complexity of the lung's native environment with immune cells, which is not possible with other lung model systems (air-liquid interface cell cultures). For a novel pathogen, early determination of which tissues are susceptible to infection (tissue tropism) is critical in informing host cell surface receptors and therapeutic strategies. As part of pandemic preparedness, our specific aims are: (1) To establish a tissue bank consisting of live and cryopreserved precision-cut 3D lung slices from various species (mice, bats, ferrets, chickens, pigs, and humans), which are natural hosts and/or reservoirs for respiratory pathogens. (2) To streamline workflow for using 3D-lung slices from different species for functional bioassays (immune trafficking screening, cytokine evaluation, and spatial transcriptomics for identification of tissue tropism dynamics screening) to mimic clinical scenarios of disease pathogenesis closely. (3) To use the above platform to assess the risk of emerging zoonotic viruses with pandemic potential by evaluating their readiness to infect humans. Our overarching theme is to establish a one-stop integrative platform for fast-track screening of species and tissue tropism of unknown respiratory disease threats (disease X), and for emerging respiratory pathogen surveillance, which will be the first-of-its-kind in Singapore.

PREPARE-OC-VT-2024-002: Discovery of anti-influenza A therapeutics with a strategy for simultaneous heterosubtypic targeting that hedge against drug resistance

Through analysing 123,060 segment sequences from 35,938 strains of the most prevalent subtypes also infecting humans – H1N1, H1N1pdm09, H3N2, H5N1 and H7N9, we discovered 30,125 unique pairs of vRNA sequence (≥15- mer) each achieving 100% coverage in simultaneous heterosubtypic targeting. By utilizing such multiple pairs in different combinations, we showed the possibility to hedge against antiviral resistance at propensities defined by our hedge- factor (Wee et al. *PLoS Computational Biology* 12:e1004663). The proposed study aims to discover anti-viral therapeutics based on this strategy.

(1) Identification of antisense oligonucleotides (ASOs) that engage the target sequences

ASOs (GAPmers that target RNAs for RNaseH-mediated degradation, and steric blockers) will be designed rationally and optimized chemically, using our validated nuclei-acid-therapeutics platform technology, to engage vRNA, cRNA and mRNA.

(2) Validation of simultaneous heterosubtypic targeting coverage using pairs of ASO

Pairs of validated ASO shall each be co-transfected on cells expressing the respective viral segments, where the target sites reside, and their vRNA, cRNA and mRNA abundance quantified to demonstrate simultaneous target engagements. Subsequently, each validated pairs of ASO will be co-transfected on host cells infected with specific viral strains and subtypes, upon which the virus titre, overall viral genome replication, and/or assembly into infectious virion quantified.

(3) Validation and characterization of propensity to hedge against antiviral resistance using combinatorial pairs of ASO Selective multiple ASO pairs versus single ASO pair (validated under Aim 2) shall be co-transfected at low effective concentrations on infected host cells, and maintain in cultures while monitoring for viral titre. RNA-Seq to be performed to characterize evolutionary genetic changes.

The discovery of next-generation antivirals possessing heterosubtypic effectiveness and prolonged durability against influenza can forthwith prepare for upcoming influenza pandemics when subsequently progress to pre-clinical studies.

PREPARE-OC-VT-2024-003: AI-facilitated development of pan-coronavirus fusion inhibitors

Coronavirus pandemics are unsolved global healthcare threats. Current coronavirus therapeutics are almost exclusively SARS-CoV-2 specific and have limited efficacy against emerging variants with adaptive mutations. There is no effective therapy against other coronavirus strains in the clinic. The need for an effective pan-coronavirus inhibitor is critical and dire. Spike protein (S) is essential for coronavirus entry into host cells to initiate viral infection. S is structurally and functionally conserved across coronaviruses. In contrast to the highly mutable receptor binding domain and N-terminal domain of the S1 subunit, S2 of S is highly conserved across genera, but not an ideal target for traditional drug screening platforms due to the nature of the transient conformation and difficulties in establishing a screening assay. Leveraging on rational AI-facilitated drug discovery approaches, we will comprehensively and rapidly interrogate conserved regions on S2 including the fusion peptide, stem helix, and recently identified S1/S2 interface cryptic pockets to design targeted peptidic and small molecule inhibitors. Further, we hypothesize that combinatorial approach targeting multiple regions of S will potentiate therapy responses. Using cutting-edge biochemistry, protein-based, structural, and *in vitro* approaches, we will systemically dissect underlying mechanisms/structures to develop a pan-coronavirus inhibitor cocktail with high efficacy, tolerance to future mutation, with reduced likelihood to develop resistance to therapy. In sum, our work will provide a critically important pan-coronavirus inhibitor cocktail that is strategically aligned and important to the national healthcare goals of pandemic preparedness. More importantly, we will establish an AI-guided drug discovery platform that is widely applicable to other diseases, and complementary to work carried out by other AI-drug discovery groups locally.

PREPARE-OC-VT-2024-004: Development of new self-amplifying RNA based mRNA vaccines for epidemic preparedness and response

The development of effective mRNA vaccines against COVID-19 extends far beyond the current pandemic. It opens new avenues for vaccine development against various diseases, from influenza to Zika, heralding a new era in medical science where responses to pandemics are swift, and vaccines are more readily adaptable. Among the emerging RNA based therapeutic technologies, saRNA vaccines offer a promising and flexible platform for vaccine development with the potential for rapid response to emerging health threats. We described a novel saRNA platform that is based on the RA27/3 vaccine strain of the Rubella virus (RuV). This strain of RuV is formulated in a widely used MMR (Measles, mumps, rubella) vaccine. We foresee that such a saRNA vector (hereafter named RuV-saRNA) will likely be effective, safer, and cause fewer side effects when compared to other saRNA replicons such as Venezuelan equine encephalitis virus (VEEV) and Sindbis virus (SINV). The specific aims include designing and producing a RuV-saRNA-based COVID-19 vaccine candidate, examining the immune response in a SARS-CoV-2 mouse model, and assessing the vaccine's immunogenicity, protective efficacy, and safety profile. Preliminary studies involve the optimization of the RuV-saRNA vector design, measuring innate immune responses in dendritic cells, and initial testing in mouse models. The experimental plans for Aim 1 focus on antigen expression and cytokine profiling, while Aim 2 aims to characterize the immune response, assess antibody levels, and evaluate protection against a lethal challenge with SARS-CoV-2. The future steps include transitioning to Phase I clinical trials and addressing manufacturing processes and intellectual property (IP) position. Our original and innovative proposal aligns with the national interest in epidemic preparedness and RNA biotechnology research and development.

PREPARE-OC-VT-2024-008: Broad-spectrum antiviral RNA nucleases

The objective of this project is to expedite ongoing development of broad-spectrum RNA nucleases that directly eliminate RNA viruses of pandemic potential in vivo. This project builds on our ongoing inter-institutional collaboration in developing antiviral RNA nucleases, which we recently showed reduce viral titers by >99.9% in infected cells and mice. Importantly, the technological pipeline is the same for other RNA viruses, which are amenable to direct viral elimination. Unlike current small-molecule or antibody drug modalities that typically target specific viral proteins, RNA nucleases target RNA and directly eliminate viral RNA genomes and transcripts, representing a new mechanism of action. Our hypothesis is that these RNA nucleases cleave viral RNA genomes and transcripts in avian influenza H5N1, MERS-CoV, and human parainfluenza virus infection models, thereby reducing the viral titers and associated pathology. Our approach is twofold:

Specific Aim 1: Development of RNA nucleases against viral RNA sequences.

Specific Aim 2: Development of viral infection models and evaluation of RNA nucleases for antiviral efficiencies.

The deliverables of this project are: (i) the RNA nuclease compositions of matter against pathogenic viruses, (ii) primary human ALI culture for MERS and avian influenza H5N1, and (iii) know-hows in therapeutic development for rapid response against future viruses of pandemic potential. If successful, this work will prepare us against pathogens of pandemic potential with a new class of therapeutics.

PREPARE-OC-Dx-2024-002: ViroArc: Integrated microfluidics for rapid isolation, enrichment and early detection of live viruses from nasopharyngeal swabs

Background

Efficient virus surveillance in respiratory infections is crucial for virus prediction and outbreak preparedness. Culture-based methods remain the gold standard for determination of live virus to assess transmissibility and pathogenicity, but they are labor intensive and have a long turnaround time to detect cytopathic effects in infected cells by microscopy imaging.

Specific aims

We will develop a novel automated microfluidic platform technology (ViroArc) to sort virus from nasopharyngeal swabs directly and concentrate sorted viruses using vacuum-induced evaporation. We will develop a sensitive microfluidic impedance cell-based assay as a follow-on assay for earlier and quantitative detection of cytopathic effects to shorten overall live virus detection window from 2-3 days to less than 12 hours.

Hypothesis

We hypothesize that a centrifuge-free, closed and single-step microfluidic sample processing will increase virus recovery with minimal user operation. Microfluidic impedance viral detection is more sensitive than imaging due to enhanced virus-cell interactions in confined small volumes.

Methodology

Using clinical isolates of SARS-CoV-2, we will develop 1) a label-free microfluidic device (ViroArc) for high throughput size-based fractionation of virus (~100 nm), bacteria (~1 to 3 µm) and nasal cells (> 10 µm) based on Dean-induced lateral migration, 2) a benchtop prototype setup with temperature and pressure control to concentrate viruses by evaporation under negative pressure, and 3) a microfluidic device for on-chip cell culture (Vero E6) to detect live/dead coronavirus using a portable impedance analyzer (Palmsens4).

Clinical significance

A user-friendly sample testing workflow and cost-efficient (scalable) device manufacturing will improve clinical adoption of developed technologies. Microfluidic closed processing of infectious samples (without centrifugation) will reduce the risk of exposure to healthcare workers and provide a safer working environment. We envision that the rapid quantitative detection of live viral materials will be complementary to RT-PCR for in-patient care to guide treatment and infection control measures.

PREPARE-OC-Dx-2024-004: An Automated Rapid Structural-Based Epitope Mapping Platform Based on Hydrogen-Deuterium Exchange Mass Spectrometry

During the latest COVID-19 pandemic, rapid and simple-to-use disease detection kits such as lateral flow assays (LFA) served as one of the most effective means to counter the disease outbreaks. The timely development of LFA heavily depends on streamlined antibody engineering, which in turn relies on rapid antibody epitope mapping. Conventional epitope mapping techniques lack either comprehensive structural resolution, or speed and cost-effectiveness. In this regard, hydrogendeuterium exchange mass spectrometry (HDX-MS) has recently been recognized as a powerful general-purpose platform for rapid, cost-effective, and structural-based epitope mapping, thus holding great promise in accelerating antibody engineering for LFA development and other antibody-oriented applications. HDX-MS relies on measuring deuterium incorporation rates on peptide amide hydrogens, which are decreased at epitopes. However, HDX-MS's availability is limited as it requires specialized instrumentation and tedious experimental processes. Here, we aim to address these two problems with a new fluidics design that enables an automated HDX-MS experimental platform. Compared to the current industrial standard HDX-MS automation solution, our design is specialized for epitope mapping, featuring cost-effectiveness, distributability, and ease of operation. A local large molecule therapeutic company that is interested in this platform will also join us as collaborator to develop the platforms commercial applications. In addition to hardware development, we will also develop a standard operating procedure (SOP) for Batchelor-level operators and a distribution procedure for the rapid expansion of the platform in times of need. The distribution procedure will be tested by implementing and validating the platform at an additional site. Overall, we believe the HDX-MS platform will offer significant advantages for the rapid development of diagnostic assays and for the local large molecule therapeutic/diagnostic industry.

PREPARE-OC-Dx-2024-005: Development of novel robust polymerase enzymes with increased resistance to inhibitors present in clinical samples and higher processivity for rapid pathogen detection

The specificity and sensitivity of molecular diagnostic protocols are compromised by inhibitory agents present in clinical samples such as immunoglobulins, proteases and hematin. These bind either pathogen-derived nucleic acids (RNA, DNA) or assay enzyme components (e.g. Taq polymerase) to disrupt efficient target recognition and molecular amplification. Many inhibitors can be removed by sample preparation. However, the additional cost, time and handling steps are not desirable. Therefore, there is a need for robust inhibitor-resistant enzymes capable of detecting pathogen nucleic acids in minimally processed clinical samples. To this end, fusion of a DNA-binding domain to polymerases can confer resistance to inhibitors. However, only two domains have thus far been employed, Sso7d and the helix-hairpin-helix motif of topoisomerase V. Given the high diversity of DNA-binding protein folds used by nature, it is very likely that many other promising candidates have been overlooked. We propose to employ Compartmentalised Self Replication (CSR), a well-established directed evolution platform to select for novel peptides and protein domains derived from randomly fragmented proprietary metagenomes that confer inhibitor resistance when fused to Taq polymerase. In parallel, machine learning will be used to extract potentially novel nucleic acid binding domains from sequenced thermophilic metagenomes. This focused collection will then be interrogated using CSR to identify novel enhancers of polymerase activity. Accessory domains have also been shown to increase enzyme processivity. CSR will therefore be used to screen for both novel accessory domains and polymerase mutants that increase processivity to enable significantly faster diagnostic reaction times. The output from this proposal will be novel accessory domains that confer resistance to inhibitors that confound molecular diagnostics assays and/or increase enzyme processivity. These will enable direct amplification of pathogen nucleic acids with minimal sample processing and shorter reaction time. Diagnostics workflows will be simplified, assay times expedited, and cost to patients reduced.

PREPARE-OC-Dx-2024-008: VirNASTURE: Virus Nucleic Acid on-Site Testing via Ultra-sensitive Nucleases Escalation

Dengue, an acute infectious disease caused by the dengue virus and transmitted primarily by mosquitoes, is prevalent in tropical and subtropical regions. In 2020, Singapore experienced its worst dengue outbreak with over 35,000 cases and 28 deaths. By May 12, 2024, there were already 6,815 cases and 7 deaths reported for the year. Given Singapore's status as a major international trading hub with high population mobility, the risk of a dengue-related public health crisis remains substantial. Effective diagnostic tools are crucial for disease management and public health. Current rapid diagnostic methods, such as colloidal gold platforms, have limited sensitivity, while more sensitive laboratory-based methods like virus culture and fluorescent PCR lack on-site applicability and promptness. To address these challenges, we introduce VirNASTURE (Virus Nucleic Acid on-Site Testing via Ultra-sensitive Nucleases Escalation), a novel amplification-free method for detecting nucleic acids within 30 minutes using a tandem cascade of endonucleases. VirNASTURE is highly sensitive and specific, making it ideal for detecting low viral loads in urine and saliva, which are easier to obtain compared to serum samples. The development of a portable Point-of-Care (POC) testing instrument streamlines the diagnostic process, especially in resource-limited settings. This project aims to facilitate rapid and accurate identification of dengue fever and demonstrates versatility by potentially detecting other pathogens like the influenza virus with slight modifications to DNA probes. This enhances precision medicine capabilities and prevents the misuse of antibiotics. VirNASTURE has broad industrialization prospects and significant economic potential, driving advancements in the diagnostic reagent sector and related industries. In summary, VirNASTURE represents a promising tool for biosensing and point-of-care diagnostics, offering substantial benefits for public health and industry.

PREPARE-OC-Dx-2024-009: Isothermally Amplified Antibody-Oligo Probes as Ultrasensitive Rapid Protein Test

Protein represents a robust class of biomarker for routine screening due to its accessibility (sample extraction not required) and well-established immuno-recognition chemistry. However, protein cannot be directly copied for exponential amplification unlike its nucleic acid counterpart, and thus is perceived as the fast but “less sensitive” biomarker class despite that they both originate from the same viral particle in the context of infectious disease. By improving the sensitivity of protein rapid test to rival that of lab test accuracy while retaining its speed and ease-of-use, its utility as a first line screening tool can now be elevated as a confirmatory test for more effective disease management especially during a pandemic surge.

We propose to develop an oligo-mediated isothermally amplified immunoassay as an ultrasensitive rapid protein test for the diagnosis of flu, which will be demonstrated using influenza A nucleoprotein as the target antigen in this project. We hypothesize that the inclusion of a barcoded isothermal amplification chemistry will significantly improve the detection sensitivity of rapid influenza diagnostics test from the current 50 – 70% to rival that of molecular assays (ca. >95%) and enable test confirmation at the point-of-need. Our approach is to use a proximity-activated antibody-oligo probes to trigger a built-in isothermal amplification mechanism to achieve PCR-like sensitivity while preserving the simplicity (add-mix-incubate) and speed (< 30 min) expected of a protein rapid test. In this project, we aim to establish a robust workflow to prepare the critical antibody-oligo probe reagent (from antibody screening, probe optimization to scaled-up production) and verify the performance (both analytical and pre-clinical) of the as-proposed assay.

PREPARE-OC-Dx-2024-010: Development of a cell-free influenza virus infectivity assay with portable-biochips (FLUVIABLEBiochips)

Influenza, also known as flu, is a respiratory illness caused by influenza virus which belongs to the *Orthomyxoviridae* family. The flu has been associated with relatively high morbidity and mortality rates annually, especially during the seasonal outbreaks in falls and winters. Effective surveillance programs which provide early detection of infected individuals will enable successful containment of virus spread, hence limiting the coverage and severity of imminent outbreaks. Diagnostic techniques including virus isolation, nucleic acid amplification tests as well as immunochromatography-based rapid diagnostic tests have been used for influenza virus detection within clinical settings. However, there is a crucial need for a diagnostic strategy that provides distinct identification of actively infectious individuals as this will facilitate the implementation of efficient disease management approaches by attending physicians towards respective patients, which are crucial to prevent further human-to-human transmission within the community. In this proposed study, we will develop a biochip prototype which enables simultaneous detection of multiple viral markers indicative of the presence of actively replicating virus particles within tested specimens. Our diagnostic approach will cover the bandwidth of three crucial stages of the virus life cycle namely virus uncoating, viral genomic replication as well as viral protein production and release. Firstly, structurally intact virus particles will be determined via a virus viability molecular assay based on metal compound-mediated suppression of RNA elongation during real-time polymerase chain reaction. Furthermore, viral RNA intermediates generated during virus replication will be detected via a colorimetric pH-driven biochemistry approach, whereas viral NS1 protein released from infected cells will be recognized with a gold colloid immunochromatography method. Ultimately, these biological assays will be optimized and validated for their respective sensitivity and specificity against specific influenza virus strains of interest, followed by integration into a biochip prototype for future development and incorporation for routine use within clinical settings.

PREPARE-OC-Dx-2024-012: Establishing an efficient workflow for in vitro selection of synthetic nanobody libraries to identify high affinity binders of influenza A viral proteins so as to accelerate the development of antigen detection assay in the event of a flu pandemic

If an avian influenza A virus (IAV) spreads among human, antigen-detection tests are important for controlling the outbreak. However, the development of such assays depend on the availability of suitable affinity reagent to bind to selected viral protein(s). Since we cannot predict the strain and subtype of IAV that may cause the next pandemic, it is impossible to know if existing reagents, like monoclonal antibodies, can bind to the newly emerged virus and distinguishes it from seasonal influenza. After the new virus is sequenced, time is needed to generate new affinity binders to it. Nanobodies have been shown to be good diagnostic reagents due to its smaller size and ease of production, and nanobodies can be isolated from pre-made nanobody libraries once a newly emerged IAV is sequenced. We hypothesize that *in vitro* selection of synthetic nanobody libraries will allow rapid identification of high affinity binders of viral proteins which can be used to accelerate the development of antigen detection assay. As a proof-of-concept, we will establish an efficient workflow that is suitable for generating new nanobodies with high affinity and ability to bind to diverse H5Nx viruses which pose a high pandemic threat. We will develop strategies to screen home-made nanobody libraries, which are either generic or hemagglutinin-focused, and identify nanobodies binding to the HA proteins of different clades of H5Nx by targeting the subtype-specific domain(s) so as to differentiate H5Nx from the circulating H3N2 and H1N1 subtypes. We will characterize these newly isolated nanobodies in terms of their binding kinetics, thermal stability and propensity to aggregate. We will also set up sandwich ELISA to determine sensitivity and specificity. The outcome of this study is a workflow using unique libraries for rapid isolation of new nanobodies with high binding affinity to any newly emerged IAV whenever the need arises.

PREPARE-OC-Dx-2025-017: Field-deployable quantitative molecular diagnosis by a portable digital polymerase chain reaction (pdPCR) device empowered with integrated optofluidic technology

This proposal aims to develop a **p**ortable **d**igital **p**olymerase **c**hain **r**eaction (pdPCR) device that will enable field-deployable quantitative molecular diagnosis in places like airport, maritime port and resource-limited sites. Since the emergence of COVID-19 pandemic, PCR test has become the gold standard for infectious diseases diagnosis as it can detect a small amount of target pathogenic DNAs in human bodies. However, the current PCR test can only be performed in centralized biomedical laboratories by skilled technicians. Another major issue of current real-time PCR is that it is a semi-quantitative molecular detection based on the cycle threshold (Ct) values.

Digital PCR (dPCR) has emerged as the next-generation PCR that can absolutely quantify the target DNA without calibrations, which significantly minimizes errors in sample handling and cross-platform comparisons. Especially, dPCR has shown significantly enhanced sensitivities in detecting extremely low viral load, which allows more reliable early diagnosis of viral infections. However, commercial dPCR devices are still bulky, labour-intensive, and complicated to operate, which have become the significant barriers for them to be adopted in the field for point-of-care testing.

This proposal will apply an integrated optofluidic technology to further miniaturize the dPCR system and develop a new generation of portable dPCR. Optical fluorescence detection is one of the key technologies in dPCR that accounts for the major cost, device footprint and sample processing throughput. The ability to embed key optical components into microfluidics using our integrated optofluidic technology can significantly reduce the device footprint and cost. In addition, the integrated optofluidic technology will allow multiplexed dPCR to process multiple samples simultaneously. This on-chip level multiplexing can significantly increase the throughput of molecular diagnosis. This proposal aims to increase the TRL of the integrated optofluidic technology from level 3 to level 5 to enable the broad adoption of pdPCR device in field-deployable applications.

PREPARE-OC-VT-2025-001: FAST-TRACK: FDA-approved Antiviral Screening Through Translational Research Accelerated by Computational Knowledge

Traditional antiviral discovery faces prolonged timelines, high costs, and scalability challenges, hindering rapid responses to influenza. We propose an AI-driven platform integrating computational modelling, high-throughput screening, and 3D airway organoids to accelerate drug repurposing and de novo design. Targeting conserved influenza proteins (hemagglutinin stem, polymerase) and host dependency factors, AI models predict binders from FDA-approved drugs and novel scaffolds. Predicted hits undergo validation via protein-binding assays, virological screens, and efficacy testing in 3D tissue models mimicking human respiratory physiology. Iterative machine learning optimizes candidates for potency and resistance-proof mechanisms. The platform will be benchmarked against three influenza strains (H1N1, H3N2, H5N1) to confirm broad-spectrum utility, with adaptability to other viruses for delivering clinical candidates within 24 months of pathogen emergence. Future efforts focus on converting hits into bifunctional therapeutics (e.g., PROTACs) using FDA-approved scaffolds for rapid translation. This strategy targets conserved viral-host interactions to counter mutational resistance, reduces development timelines by >50%, and establishes a scalable framework for pandemic preparedness. Successful implementation will yield mutation-resistant treatments and a modular AI-experimental pipeline adaptable to diverse viral threats, transforming public health responses to outbreaks.

PREPARE-OC-VT-2025-002: A self-amplifying RNA vaccine against Henipaviruses

Self-amplifying RNA (saRNA) vaccines offer a promising platform for combating emerging infectious diseases. Our project aims to develop next-generation saRNA vaccines targeting Henipaviruses, including Nipah, Hendra, Ghana, and Cedar viruses, using an optimized Ross River virus (RRV)-based replicon system. By designing and validating both monocistronic and bicistronic saRNA constructs encoding the F and G glycoproteins, we will assess their replication efficiency and antigen expression in neural and epithelial cell lines. Subsequently, we will evaluate the immunogenicity of these constructs in BALB/c mice, analyzing neutralizing antibody responses over six months. In a final proof-of-concept study, IFNAR KO mice will be immunized and challenged with recombinant Cedar virus to assess protective efficacy. Our approach leverages the self-amplifying nature of saRNA to enhance antigen expression and immune responses while reducing the required RNA dose. This project aim to contribute to the advancement of scalable and effective saRNA vaccine technologies, addressing the urgent need for Henipavirus countermeasures.

PREPARE-OC-VT-2025-003: Single-cell sVNT screening platform for rapid discovery of neutralizing monoclonal antibodies

Pandemic preparedness includes the pre-development of a suite of broadly acting therapeutics that can be readily deployed upon outbreak. While pan-sarbecovirus mAbs have been achieved, no broadly neutralizing mAbs exist for other coronavirus subgenera, such as Merbecovirus. This project aims to establish a high-throughput platform for functional mAb discovery, using pan-merbecovirus mAbs as a proof of concept. Traditional high-throughput screening primarily relies on binding assays, which do not guarantee neutralization. To directly screen mAb candidates for neutralization function at high throughput, we propose here a novel platform, the single-cell surrogate virus neutralization test (sc-sVNT). This platform utilizes picodroplets as miniature reaction chambers, within which independent sVNT assays can be carried out on single antibody-secreting cell candidates. This enables the screening of >1,000,000 antibody candidates for their neutralization function per run. This platform will be first applied to identify potent pan-DPP4-using-merbecovirus mAbs with clinical applications against MERS and future merbecovirus outbreaks. Given the proven versatility of sVNT, the sc-sVNT platform can be extended to other viral families, including paramyxoviruses, arenaviruses, and additional coronavirus subgroups. Collectively, these form a broadly protective mAb repertoire for pandemic response.

PREPARE-OC-VT-2025-004: Developing multisulfonated copper iodide clusters as broad-spectrum intranasal prophylaxis and treatment for viral infections with high outbreak potential

In this proposal, we aim to develop a novel class of multisulfonated copper iodide clusters (MSCIC) as broad-spectrum antivirals for intranasal prophylaxis and treatment of viral infections with high outbreak potential. Most current/pipeline vaccines and antiviral drugs are strain-specific with a narrow action spectrum, making them inadequate to combat the upcoming 'Disease X'. The proposed MSCIC integrate two proven broad-spectrum antiviral modalities into a single core-shell molecular design: (1) a multisulfonated shell that mimics heparan sulfate proteoglycans (HSPG) on host cell surface to block viral attachment and (2) a cuprous iodide cluster core that inactivates viral particles upon contact. This multivalent design enables high-affinity binding with viral spike proteins, preventing virus-host interaction while simultaneously bringing the copper clusters into the virus' vicinity, thereby producing a synergistic virucidal effect with low toxicity. The proposed project will involve the design and synthesis of MSCIC, in vitro evaluation of antiviral activity and cytotoxicity, mechanistic understanding, and in vivo assessment of the antiviral efficacy against model viruses via intranasal administration. Success of this project will reveal new knowledge to guide advanced antiviral designs and, more importantly, create non-invasive broad-spectrum antiviral intranasal sprays that can be rapidly deployed to safeguard public health during future endemics/pandemics.

PREPARE-OC-VT-2025-008: Development of Broad-Spectrum Peptide-Based Antivirals

Viral epidemics pose a major global health threat, with rapidly evolving pathogens causing severe disease and overwhelming healthcare systems. Current antiviral treatments are limited, and viral mutation rates hinder the development of long-lasting therapies. This project aims to develop broad-spectrum peptide-based antivirals by leveraging advanced peptide engineering. Our hypothesis is that alpha-helical (AH) peptides can effectively target viral envelopes by recognizing high membrane curvature, disrupting viral integrity, and inhibiting infection. We will refine a 16-amino acid AH peptide sequence, optimizing its antiviral activity against multiple viral pathogens. Chemical modifications, including capping strategies, will be explored to enhance peptide stability, membrane interaction, and therapeutic potential. The library of peptide candidates will be directly screened against live ancestral SARS-CoV-2 and H1N1 influenza virus, facilitating translation to physiologically relevant validation. Preclinical models will assess peptide efficacy, blood-brain barrier permeability, and the reduction of viral loads and inflammation. By targeting fundamental biophysical properties of virus-infected cells, this approach circumvents virus-specific resistance mechanisms, making it adaptable to emerging viral threats. The clinical significance lies in its potential to provide an effective therapeutic platform for treating viral infections, reducing disease burden, and strengthening pandemic preparedness. This research will advance peptide-based therapeutics and contribute to the development of next-generation antiviral strategies with broad applicability in medical science.

PREPARE-OC-VT-2025-011: Development of a disease-protective vaccine to control zoonotic paramyxovirus infections through the identification of broadly cross-reactive T cell epitopes

Traditionally, vaccines are designed to induce humoral immunity to protect against infection. However, their effectiveness can be rapidly compromised by the ability of pathogens to mutate and the challenge of maintaining high titers of neutralizing antibodies. This was evident in infections like SARS-CoV-2. However, viral variants can still be recognized by T cells, which target diverse epitopes across multiple viral proteins. These SARS-CoV-2-specific T cells do not prevent infection, but they can abort it or significantly reduce disease severity by lowering viral load through direct lysis of infected cells. Building on this concept, we propose developing a research platform to design a T cell-based vaccine against zoonotic paramyxoviruses such as Hendra virus and Nipah virus. Our approach involves studying individuals previously vaccinated against or infected with human *Paramyxoviridae* (e.g., measles, mumps, RSV, parainfluenza) to assess the presence and diversity of T cells recognizing conserved epitopes in the non-structural proteins shared between human and zoonotic *Paramyxoviridae*. Identifying these cross-reactive T cell epitopes and demonstrating their ability to induce responses against cells mimicking Nipah and Hendra virus infections will lay the foundation for a T cell-based vaccine designed to rapidly enhance immunity against zoonotic paramyxoviruses.

PREPARE-OC-VT-2025-015: ISM1 as a novel host anti-influenza factor

Highly pathogenic influenza A virus (IAV) can cause devastating pandemics and high mortality. High mutation rate combined with genome fragment recombination led to rapid emergence of new drug-resistant IAV strains, making it challenging to develop broadly protective flu vaccines. Hence, approaches that enhance host anti-influenza arsenal are highly desirable.

Interferons (IFNs) are antiviral cytokines that play a key role in the innate immune response to viral infections. Type III interferons (IFNλs) are the predominant IFNs induced by IAV infection in vivo in respiratory epithelia. Notably, IFNλ lowered viral load and protected mice from IAV-induced disease without triggering inflammatory cytokine production. Thus IFNλ represents a potent anti-influenza therapeutic without the inflammatory side effects of IFNα.

We discovered an anti-inflammatory protein highly expressed in respiratory epithelia. In cultured human pulmonary epithelial cells, this protein potently induces IFNλ1 production and suppressed IAV infection without impacting IFNα. We therefore hypothesize that this protein could be a novel anti-influenza host factor by inducing the antiviral IFNλ response while mitigating the pro-inflammatory response.

In this pilot project, we aim to study the anti-influenza efficacy of this protein in mouse models. This work will facilitate the development of novel anti-influenza therapeutics.

PREPARE-OC-VT-2025-019: Targeting and rewiring of host cell death pathways to fight severe viral infection

Programmed cell death pathways including apoptosis and necroptosis are key innate immune defence mechanisms that removes the replicative niche of viral pathogens, however, dysregulated cell death contributes to tissue damage and lethality during severe viral infection. The aim of this study is to reduce viral-induced cell death and associated tissue damage, whilst rewiring the cell death pathway to promote anti-viral type I interferon response.

PREPARE-OC-ETM-2025-001: Development of a **S**ystematic **A**ssessment **F**ramework for **E**valuating **A**ir-Purification **I**nterventions in the **R**eal-world (SAFE-AIR): A First Demonstration on Worker Dormitories

Airborne transmission of respiratory pathogens poses a significant public health risk, particularly in high-density settings like worker dormitories, yet the real-world effectiveness of air purification technologies remains a matter of scientific debate. This study aims to develop a Systematic Assessment Framework for Evaluating Air-purification Interventions in the Real-world (SAFE-AIR) that can be used for rigorous evaluation of the efficacy of air purification strategies in mitigating respiratory infection within an environment. We seek to show that optimized deployment of air purification technology will significantly decrease airborne pathogen loads and lead to a measurable reduction in infections among residents compared to control conditions in a worker dormitory.

Our approach involves four interconnected Work Packages (WPs). WP1 will establish and validate a tri-modal suite of metrics, encompassing clinical health outcomes (infection incidence via healthcare records and surveys), biological proxies (pathogen load in air/surface samples via qPCR), and physical indicators (particulate matter, CO2). WP2 will experimentally evaluate selected commercial air purification technologies and utilize validated computational fluid dynamics (CFD) simulations to determine an optimal deployment strategy (number, type, placement, operation) considering efficacy, energy use, and safety (by-product monitoring). WP3 will quantify the impact of baseline natural ventilation differences across dormitory rooms on health outcomes and environmental metrics, thereby verifying its impact as a potential passive mitigation measure. WP4, the capstone, implements a prospective, cluster-allocated control-intervention field trial that compares rooms with an optimized air purification strategy (from WP2) against matched control rooms using the validated metric suite (from WP1), while accounting for ventilation effects (from WP3).

This research is clinically significant as it will provide crucial, real-world evidence to inform public health guidelines on using air purification to reduce infectious disease transmission in high-density living environments. The developed systematic framework will also offer a transferable methodology for evaluating future air purification and mitigation technologies.

PREPARE-OC-ETM-2025-002: Charged Spray for Rapid and Targeted Clearance of Airborne Virus-loaded Aerosols

This proposal aims to develop a system that effectively and rapidly removes airborne aerosols with viruses from the atmosphere in practical settings to prevent the spread of infectious diseases. The system is based on a fundamentally novel physical phenomenon discovered by the group at the National University of Singapore (NUS). We have tested that the system clears airborne aerosols effectively and rapidly in laboratory settings. The system involves a mechanism and operation that are fundamentally superior to current air-purifying systems (e.g., with HEPA filters). In this proposal, we plan to implement the system in practical settings. We will first develop a small-scale system followed by a larger-scale system for covering a wider area in enclosed spaces (e.g., rooms at NUS and/or NTU). The main task of this proposal is to design, construct, and test the system for clearing airborne aerosols in practical settings. We will evaluate the effectiveness of clearance by monitoring the levels of atmospheric aerosols. We will enhance fundamental understanding and optimize the system. Periodic activation of the system will maintain very low levels of aerosols in the atmosphere to prevent transmission. A handheld system will be developed to be used by individual personnel for personal protection. These systems will provide a line of defense for people to carry out their necessary activities (e.g., using public transportation such as buses or MRT or working in offices) during any dire situations (e.g., a pandemic) that was not available during our recent COVID-19 pandemic.

PREPARE-OC-ETM-2025-006: **AIM-Mitigation: A**dvanced **I**ntegrated **M**ultimodal **Mitigation (AIM-Mitigation)** Strategies for Reducing Respiratory Disease via Experimental Testing and Data-Assimilated Artificial Intelligence Modelling

This project proposes a multimodal framework integrating experiments and artificial intelligence (AI)-driven modelling to assess indoor air quality (IAQ) mitigation strategies for reducing respiratory disease transmission. These strategies span from conventional approaches to the novel CPI-FUN (Cold Plasma Ionizer, Fast-flow, Ultra-efficient, Novel), which targets both airborne and fomite transmission, a key innovation to be developed and validated in this study. A sustainable IAQ monitoring system, specifically tailored for this framework, will be developed to capture both physicochemical indicators (temperature, relative humidity, CO2, PM2.5) and biological agents, including airborne respiratory pathogens with pandemic potential.

We employ a phased, randomized controlled trial design, progressing from controlled laboratory test in a virus-inoculated chamber without human exposure, to a room-scale laboratory trials with human participants. Mitigation strategies, including natural ventilation, fans, electrostatic precipitators, CPI, and CPI-FUN purifiers, are evaluated under progressively realistic conditions.

Beyond experiments, we develop an AI platform that integrates AI for pathogen flow with real-time sensor data through data assimilation to simulate airflow and pathogen dispersion. This AI-driven approach enables fast and accurate predictions of the IAQ experienced by occupants. It is complemented by the DA-FlowNet surrogate model developed in this work, which delivers near-instantaneous predictions of mitigation efficacy, supporting demand-based control and energy-efficient operation.

We propose an integrated, three-pronged assessment to evaluate various mitigation strategies based on environmental performance, health outcomes, and pandemic resilience. Top-performing solutions will be potentially deployed in indoor room enabling the quantification of IAQ improvements and respiratory-infection reduction. The findings will provide robust, evidence-based insights into the relationship among mitigation strategies, IAQ parameters, and respiratory health. These insights will inform air quality management for daily routine and enhance pandemic preparedness in various indoor environments such as community care centres, offices, to name a few, enabling effective deployment of developed interventions in this proposal.

PREPARE-SF-2023-003: Metagenomics for emerging infectious disease detection and surveillance

Background: Singapore, being a travel hub, is constantly exposed to emerging and imported infectious diseases. The need for an effective disease surveillance system for pandemic preparedness is pressing. A key feature of pandemic preparedness is the ability to detect the pandemic threat early and prevent its spread. Most surveillance and clinical testing currently focus on known pathogens and rely on targeted detection methods, limiting the detection of novel or emerging pathogens. Metagenomic sequencing overcomes these limitations by allowing the detection of all microbes in a sample. The application of metagenomic sequencing has been demonstrated in investigations of diseases with unknown aetiology and detection of novel pathogens from human or environmental samples.

Aim: The aim of this study is to establish and validate the protocols for metagenomics sequencing and analysis for identification of viral genomes from clinical samples, with initial focus on human respiratory samples from the Respiratory Infections Research and Outcome (RESPIRO) cohort and the PREPARE-funded Aetiologic Agents of Community Acquired Pneumonia in South-East Asia study. We envisage that further sample sources to test would include wastewater and human blood samples.

Methodology: High-risk human and environmental samples will be obtained from ongoing surveillance programs. Microbiological processing protocols and bioinformatics data analysis will be adapted from published literature, optimized and validated.

Clinical significance: Once established, metagenomics testing would be able to be conducted on high-risk patient samples, including patients with pneumonia of unknown aetiology or undiagnosed fever, as well as sentinel wastewater sampling sites. It is envisioned that the capabilities built will enable rapid identification of emerging infectious diseases from local human and environmental samples, as well as regional samples.

PREPARE-SF-2023-004: A pilot surveillance system for RSV in children presenting to Hospitals in Lao PDR

We propose to set up a pilot system for genomic RSV surveillance in children presenting to hospitals in Lao PDR (two in capital and four in provinces) including the use of rapid diagnostic tests as the source of RSV RNA for genome sequencing which would improve the access to samples from remote areas. RSV epidemiological and genomic data will be displayed in a DHIS2 dashboard held by the National Center for Laboratory Epidemiology in Laos. This approach will be scalable to national surveillance system to provide data on the true burden of RSV and to help understanding RSV genetic evolution.

PREPARE-SF-2023-005: Genomic Surveillance for Strengthening Public Health Response in Cambodia

With global resurgence of non-COVID viral pneumonia, there is a clear role for building upon pandemic sequencing infrastructure to combat other scourges. The Cambodian CDC and National Institute of Public Health are responsible for identifying pathogens from the Severe Acute Respiratory Infection (SARI) surveillance network, but currently are limited to multiplex PCR of influenza A/B and SARS-CoV-2 sequencing. Hence, the majority (~89%) of SARI cases go uncharacterized. In collaboration with NIAID ICER Cambodia, we propose to integrate metagenomic pathogen surveillance to the SARI network of Cambodia, to describe disease epidemiology in a known epicenter of respiratory pathogen diversity in Asia.

PREPARE-SF-2023-006: Pathogen Genomic Surveillance and Immunology in Vietnam

We plan to build on the established capacity during the COVID-19 pandemic by continuing support for the sentinel surveillance system for ILI (and include both influenza and coronaviruses) and re-establishing the SARI surveillance network, including agnostic sequencing. Systematic surveillance and sequencing of priority potential pandemic pathogens as influenza and coronaviruses is an important public health responsibility. Resulting pathogen data and agnostic sequencing of influenza, coronaviruses as well as undiagnosed pathogen will contribute to the global databases and may potential inform national vaccination policy.

PREPARE-SF-2024-007: Clinical validation and cost effectiveness study of a self-administered multi-pathogen antigen rapid test (SMART)

The introduction of self-administered Antigen Rapid Tests (ART) during the COVID pandemic significantly enhanced surveillance and diagnosis with minimal additional burden to the national laboratory testing system. Having local capability to rapidly roll out ART testing during the emergence of a novel respiratory pathogen is therefore critical. This project aims to build this capacity by developing a prototype ART in collaboration with a local MedTech company. The ART cartridge will also target multiple pathogens to enable rule-out of common acute respiratory-tract infections (ARIs) during an emerging pandemic, with the flexibility to substitute in new antibodies against novel pathogens when the need arises. For validation, the ART will have to target existing common respiratory pathogens of interest such as Influenza A & B, SARSCoV-2 and RSV. Selection of these pathogens were based on their high population incidence, availability of means for clinical intervention and technical feasibility for testing by ART.

This validation will be accomplished through recruitment of patients presenting with ARI symptoms at primary care clinics to self-conduct the ART and complete a short usability survey. The clinics will also collect a reference swab for determination of ART sensitivity and specificity by RT-PCR. In addition, a prospective cohort demographically representative of Singapore residents will be recruited for quality-of-life studies to determine the cost-effectiveness of ART use. The study will recruit from healthcare workers (HCWs) and their families who are familiar with using ART and who are more likely to participate actively. This can be expanded to the community subsequently if there are interested participants. Medical, vaccination and illness history data will be collected via online survey for cost-effectiveness and epidemiological studies. Serum will also be collected on recruitment, end-of-study and during convalescence for immunological characterization. In addition, positive swab samples will be used to generate reference isolates for biorepository purposes.

PREPARE-SF-2025-013: Assessing the zoonotic risk and pandemic potential of highly pathogenic avian influenza (HPAI) H5N1 viruses

This project aims to assess the zoonotic risk and pandemic potential of highly pathogenic avian influenza (HPAI) H5N1 viruses, leveraging advanced genetic, experimental, and immunological techniques. We will conduct a detailed genetic characterization of H5N1 variants to identify mutations that influence viral fitness across different hosts. By mapping amino acid changes on phylogenies, we will explore intra- and inter-gene interactions and generate recombinant viruses with specific mutations to evaluate host susceptibility. To enhance pandemic preparedness, we will develop organoid models derived from relevant animal hosts and use these to measure H5N1 replication dynamics and tissue tropism, creating an organoid-based platform for rapid risk assessment. Additionally, we will implement a novel rapid whole-blood assay to detect T cells specific to H5 influenza, building upon an established pipeline used during the SARS-CoV-2 pandemic. This assay, combined with serological tools developed with PREPARE finding, will enable us to assess cellular and humoral immune responses in humans and animals, thereby providing a comprehensive understanding of H5 exposure. By quantifying virus-specific T cells and antibodies, this study will inform surveillance efforts, especially regarding emerging strains with zoonotic and pandemic potential. Our multidisciplinary approach integrates genetic, immunological, and organoid technologies to address critical gaps in avian influenza A virus research, aiming to strengthen pandemic preparedness and public health responses.

PREPARE-UKRI-SF-2025-004: Development and assessment of novel, high-throughput immunological assays to improve surveillance of spillover of viral families of pandemic potential

The importance of public health readiness to an outbreak is evident in the catastrophic COVID-19 pandemic. The unprecedented loss of human lives, livelihood disruptions, and shuttering of economies during the COVID-19 pandemic highlighted the inadequacy of pandemic preparedness and the urgency to improve existing public health emergency readiness strategies. Constant spillover of zoonotic viruses, for example, Nipah virus, avian infuenza virus, and MERS-CoV in Bangladesh/India, Vietnam/Cambodia and Saudi Arabia, respectively, indicates the need for early detection of spillover events to existing “known-known” and “Known-unknown” diseases, especially in people with constant close contact with wild-animal in the Southeast Asia countries. In this proposal, we will establish high-throughput, high-resolution antibody and T-cell assays to detect possible past exposure to zoonotic viruses in high-risk cohorts collected in Vietnam and Cambodia. The humoral data obtained will be used to develop a suite of within-host models of the adaptive and cellular immune response, which will be applied to inform sampling strategies and broader routine surveillance to improve the likelihood of earlier detection of spillover events. Individuals with positive hits will be recalled, and the B-cells will be collected to discover potent, broadly neutralizing antibodies. We aim to build regional research capacity in Vietnam and Cambodia in the aspect of early detection of spillover events for timely public health response and monoclonal antibody discovery, and more importantly, to build a strong collaborative, apolitical environment with our regional partners in infectious disease research.

PREPARE-UKRI-SF-2025-007: Studying Hygiene Interventions to reduce Nosocomial Infections in southeast Asian Intensive CareUnits (SHINIA-ICU)

Background: Hospital environment hygiene is fundamental to breaking the vicious cycle of antimicrobial overuse and the resultant high burden of multidrug-resistant healthcare-associated infections. Contaminated surfaces harbouring these pathogens sustain the spread of antimicrobial resistance bacterial clones and mobile genetic elements (e.g. plasmids) in both outbreak and non-outbreak settings. Despite an emphasis on environmental hygiene and availability of international guidelines, there is insufficient evidence in the current literature which identifies the most effective cleaning strategy to prevent transmission of multidrug-resistant organisms, especially in low and middle-income countries (LMICs). This contributes to the low awareness and prioritization of environmental cleaning in these resource-limited settings.

Aim: The overall aim of this study is to determine cost-effective and locally-appropriate environmental cleaning interventions for low-resource settings.

Methodology: The study is a stepped wedge cluster randomised trial in ICUs across hospitals in Southeast Asia. The UK team will lead the development and implementation of locally-optimized multimodal cleaning bundles (study intervention) through a phased approach. Environmental and patient (both colonization and clinical) multidrug-resistant isolates will be collected and sent to Singapore for whole genome sequencing and analysis to determine clonal and plasmid-mediated transmission. Evaluation of outcomes will be holistically assessed using incidence of multidrug-resistant hospital-acquired bloodstream infections and ventilator-associated pneumonia (clinical outcome), adherence to cleaning bundles (process measures), estimation of the impact of the intervention on transmission quantification of the reservoirs and transmission pathways of multidrug-resistant organisms informed by genomic sequencing and data-driven mathematical modelling, and a cost-effective evaluation.

Clinical significance: This work will raise awareness and prioritization of environmental cleaning in LMICs, and support global and regional efforts to reduce multidrug-resistant hospital-acquired infections and health-economic burden. Our robust genomic-epidemiology pipeline overcomes the limitations of traditional single nucleotide variant-based phylogenetic methods, enabling thorough analysis of the transmission dynamics of MDROs and identification of hidden resistance gene reservoirs.