PRoposal to assess s**E**rum neutralization against **ci**rculating A(H3N2) strains in the 2025-2026 **SE**ason (PRECISE-Immunogenicity)
V1.1 8Dec2025

Objective:
We aim to
(i) characterize neutralization activity of sera from persons vaccinated against influenza in the fall of 2025 against recently emerging sub-clade K H3N2 influenza strains. This activity will be compared to that of serum from persons who have not been exposed to the vaccine. We will evaluate vaccine efficacy in eliciting neutralizing sera, with a comparison of high- and low-dose vaccinee sera for antibody titers, potency, and breadth of neutralization against authentic H3N2 viruses and pseudoparticles.
(ii) investigate the antiviral activity of these sera against newly emerging H3N2 isolates bearing potential escape or virulence mutations.
(iii) if a vaccinated participant develops influenza due to subclade K during the 2025-2026 winter season, we will collect convalescent serum to permit Structural mapping via Electron Microscopy Polyclonal Epitope Mapping (EMPEM) to support studies of how epitope usage shifts over time and which binding patterns are associated with broader neutralization and reduced susceptibility to infection.

Rationale:

As of the 2024-2025 winter season, influenza has once again become the infection that is the most common cause of hospitalization and death in Canadian adults (surpassing COVID-19 for the first time since the onset of the pandemic). (1) The 2025-2026 season is predicted to be severe, with most disease due to a new variant of A(H3N2) influenza (called subclade K, or J.2.4.1). (1,2)

Subclade K first appeared early during the southern hemisphere influenza season in 2025. Very rapid growth of the strain was seen during the latter half of the southern hemisphere season, and early and intense influenza activity has been reported from at least Japan, Malaysia and the UK, with Hong Kong also reporting a recent influenza wave (3-7). It has become clear that subclade K of A(H3N2) will dominate in this season in Canada (1,8).

Subclade K has evolved substantially away from the influenza strains used for 2025 northern hemisphere vaccines with numerous mutations in antigenic sites(2,3,9). Early evidence suggests that antibodies generated by the 2025 fall influenza vaccines react substantially less well against subclade K as compared to previous A(H3N2) strains (2,3,9). There has also been very rapid evolution of subclades of A(H3N2) in recent months (9), such that it is difficult to predict how this virus will evolve over the 2025-2026 northern hemisphere influenza season and raises concerns about reduced immune recognition The existence of the PRECISE network provides an opportunity to assess differences in the effectiveness of different vaccines against this strain, and to study immune responses to different variants of clade K and related strains as they evolve (9).

Post-vaccine human sera have been successfully used to evaluate antibody responses against seasonal H3N2 influenza strains and related mutations in previous studies. For instance, Wu et al. used sera from influenza vaccine recipients in 2015-16 to show differential neutralization of H3N2 pseudoviruses exhibiting an HA mutation in comparison to the wild-type strain (10). Huang et al. demonstrated that vaccinated human sera could neutralize H3N2 pseudoviruses in MDCK cells, validating vaccine efficacy (11). Cheng et al. collected pre- and post-vaccine sera to perform an ELISA-based microneutralization assay, showing that these samples exhibit differential vaccine efficacy across seasonal influenza strains H1N1, H3N2, and Influenza B (12).

In addition, structural mapping via Electron Microscopy Polyclonal Epitope Mapping (EMPEM) can provide insight into exactly where polyclonal antibodies bind on viral antigens, and when combined with neutralization assays the functional and mechanistic relevance of binding specificity can be determined. EMPEM requires that plasma or serum be obtained before (or very early during) and after infection. Thus, the EMPEM project will largely recruit participants from other studies, but if a participant in this study develops influenza and consents, we will be able to contribute samples.

Methods:

We will advertise for the study using posters in the hospital and via email news communications from Sinai, using a study-generated QR code to link to the online study request, protocol,and consent form. The wording will be: "A researchstudy led by Dr. Christopher Kandel and Dr. Allison McGeer is looking for participants who are in good health, have received the 2025 influenza vaccine more than 2 weeks ago and are willing to donate a 20 ml (4 teaspoons) sample of blood. This purpose of this study is to assess different methods for measuring the ability of antibodies produced after vaccination to kill influenza viruses. For more information, please use the QR code, or email or call shiva.barati@sinaihealth.ca, or 416-586-4800 ext. 3122. Please note that email is not a secure form of communication and should not be used for conveying information that is considered sensitive."

We require 20 serum samples from individuals who are not immunocompromised, have not had a recent (<6 months) serious influenza infection, who received seasonal influenza vaccine in 2025 in Canada ≥2 weeks prior to participating, and who can provide proof of vaccination which includes the date of vaccination and (for those over 65 years of age) the brand/type of vaccine received. Ten samples will be collected from standard dose, unadjuvanted, vaccine recipients (those who received Fluzone, Fluviral®, Flucelvax®), and 10 from high-dose vaccine recipients (those who received Fluzone - high dose).

Participants will be asked to share their age, health status (as immunocompromised Y/N), sex at birth, and date and type of influenza vaccine received in 2025. Participants will receive at $25 gift card as partial re-imbursement for their time and travel costs.

Participants will also be asked to consider agreeing to report episodes of acute respiratory illness during the 2025/26 influenza season, to have a test to determine if the illness is due to influenza, and to submit a convalescent serum sample 3-7 weeks after an influenza illness. If participants agree, they will be given a kit for obtaining a mid-turbinate swab and instructions for what to do if they develop illness. They will receive 2 $50 gift cards if they have to visit the hospital either to be tested for influenza or to have convalescent serum drawn.

To obtain serum from persons who have not received the 2025 influenza vaccine, we will use leftover aliquots of serum specimens from the COVID-19 Cohort study (REB# 20-0088E) from 15 participants who agreed to permit use of their specimens for other studies.

With REB approval at receiving sites, and appropriate collaboration agreements, specimens will be shared with PRECISE WP2 members (Dr. Christina Guzzo (UT), Dr. Samira Mubareka (UT), Dr. Michael Norris (UT), Mario Ostrowski (UT), Dr. Jimmy Dikeakos (Western U). We will evaluate vaccine efficacy in eliciting neutralizing sera, with a comparison of high- and low-dose vaccinee sera for antibody titers, potency, and breadth of neutralization against authentic H3N2 viruses and pseudoparticles. We will also investigate the antiviral activity of these sera against newly emerging H3N2 isolates bearing potential escape or virulence mutations as they arise.

The sample size is determined by the need to compare neutralizing capacity of serum post vaccination versus without vaccination. We estimate that 10% of persons not exposed to the 2025 vaccine will have neutralizing antibodies while 60% of those who have been vaccinated will. With 80% power, the sample size needed is 20 vaccinated person and 15 unvaccinated persons.

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