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PROTOCOL

CTN 328: Immunogenicity outcomes in people living with HIV in Canada following vaccination for COVID-19 (HIV-COV)-Protocol for an observational cohort study

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ABSTRACT

Introduction: Most existing vaccines require higher or additional doses or adjuvants to provide similar protection for people living with HIV (PLWH) compared to HIV-uninfected individuals. Additional research is necessary to inform COVID-19 vaccine use in PLWH.

Methods and analysis: This multi-centred observational Canadian cohort study will enroll 400 PLWH aged ≥16 years from Montreal, Ottawa, Toronto and Vancouver. Subpopulations of PLWH of interest will include: 1) >55 years of age 2) CD4 counts <350 cells/mm 3) multimorbidity (≥2 comorbidities) and 4) "stable" or "reference" PLWH (CD4 T cells ≥350 cells/mm3, suppressed viral load for≥ 6 months and ≤1 comorbidity). Data for 1000 HIV-negative controls will be obtained via a parallel cohort study (Stop the Spread Ottawa) using similar time points and methods. Participants receiving ≥1 COVID-19 vaccine will attend 5 visits: pre-vaccination; 1 month following the first vaccine dose; and at 3, 6 and 12 months following the second vaccine dose. The primary endpoint will be the percentage of PLWH with COVID-19-specific antibodies at 6 months following the second vaccine dose. Humoral and cell-mediated immune responses, and the interplay between T cell phenotypes and inflammatory markers, will be described. Regression techniques will be used to compare COVID-19-specific immune responses to determine whether there are differences between the "unstable" (CD4<350) PLWH group, the stable PLWH cohort and the HIV-negative controls, controlling for factors that are believed to be associated with immune response. Unadjusted analyses will reveal whether there are differences driving factors associated with group membership.

Ethics and Dissemination: Written informed consent will be obtained from all study participants prior to enrolment. These findings will inform the design of future COVID-19 clinical trials, dosing strategies aimed to improve immune responses and guideline development for PLWH.

Trial registration: clinicaltrials.gov NCT04894448

Keywords: HIV; COVID-19; observational study; COVID-19 vaccines; vaccine immunogenicity

Strengths and Limitations of this study

- The largest and most comprehensive immunogenicity study in people living with HIV in Canada receiving COVID-19 vaccination
- Emphasis on recruiting participants frequently excluded from pharmaceutical company-sponsored trials and those most likely to have poor outcomes following COVID-19 infection (including individuals of older age, immune non-responders and persons with multimorbidity)
- Assays used will enable differentiation between individuals with immunity from natural COVID-19 infection *vs.* vaccine-induced immunity, in addition to detection of immunity towards key variants of concern (VOCs)
- Involvement of community members from study conception to protocol development and study implementation
- Limitations include relatively late study start, recruitment restricted to major urban centres and variations in timing between vaccine doses amongst participants

BACKGROUND

In Canada today, an estimated 67,000 people are living with HIV (PLWH), 30% of whom are immune non-responders,[1] defined as achieving undetectable HIV viral levels without robust CD4 T cell count recovery (<350 cells/mm^3). Even with fully suppressed viral load on antiretroviral therapy (ART), chronic HIV infection is characterized by a low-grade elevation in pro-inflammatory and procoagulant biomarkers linked with higher mortality[2-4]. Poor immunogenicity to common vaccines, including influenza[5], pneumococcal[6,7], meningococcal[6,7] and hepatitis A[8-10] and B vaccines,[11-13] is well-documented in PLWH with low CD4 T cell counts (<200 cells/mm^3) and unsuppressed viral loads[14-16]. PLWH face other intersecting vulnerabilities that increase their risk of SARS-CoV-2 acquisition and symptomatic/severe COVID-19; they commonly belong to low socioeconomic or racialized groups disproportionately affected by COVID-19 and have higher rates of risk factors for severe COVID-19 disease (e.g., multiple chronic comorbidities).[17-19] Yet, this priority population has been understudied in COVID-19 vaccine clinical trials.[3, 20] Most HIV seropositive participants enrolled in COVID-19 vaccine trials had normal CD4 T cell counts (>500

cells/mm^3) and few comorbidities.[21,22] As such, the immunogenicity results may not represent the wide spectrum of PLWH who are followed in Canadian centres today.

For the AstraZeneca/Oxford COVID-19 vaccine trial (ChAdOx1, n=160 PLWH) inclusion criteria specified younger age (<55 years old) and high CD4 T cell count (>350 cells/mm3) while excluding medical comorbidities (e.g., heart, kidney, liver, respiratory diseases etc.).[21] The data obtained from PLWH were not included in the primary publication.[21] The Moderna trial included HIV-positive participants (n=176 PLWH) with CD4 T cell count ≥350 cells/mm^3 and an undetectable HIV viral load within the past year.[23] COVID-19 infection developed in 11 PLWH who received placebo but none who received the Moderna vaccine. The Johnson and Johnson trial (n=1218 PLWH) included participants with "stable/well-controlled HIV infection" (defined as CD4 T cell counts ≥300 cells/μL within 6 months prior to screening and documented HIV viral load <50 copies/mL within 6 months prior to screening) but excluded participants with ongoing and progressive comorbidities associated with HIV infection.[24] COVID-19 infection developed in 2 vaccinated PLWH and 4 PLWH given placebo. The Novavax trial, conducted in South Africa, excluded PLWH with chronic cardiovascular disease, gastrointestinal disease, liver disease, renal

disease, endocrine disorder and neurological illness, as well as participants with very high body mass index (≥40 Kg/m2).[22] As reported by Vivek *et al.,* efficacy of the NVX-CoV2373 Covid-19 vaccine against the B.1.351 variant was examined in 1,857 individuals in South Africa, of whom 30% (500 individuals) had HIV infection[25]. The vaccine efficacy estimate in baseline seronegative HIV-negative participants was 52.2% (95% confidence interval: −24.8 to 81.7). During the first 60 days of follow-up, the incidence of Covid-19 in HIV-negative placebo participants (5.3% [95% CI: 4.3 to 6.6) was comparable to the incidence in PLWH placebo participants with HIV (5.2% [95% CI: 3.6 to 7.2])[26]. Among HIV-negative participants, there were four and two cases of symptomatic Covid-19 among NVX-CoV2373 and placebo recipients, respectively (N<109 in each group)[26]. No cases were observed in the baseline HIV-positive population (N<33 in each group)[26]. Among 94% of participants without HIV, vaccine efficacy was 60.1%. The study was not powered to detect efficacy in the small population of PLWH[26].

In the ongoing CanSino study in Argentina, which includes approximately 900 PLWH, preliminary findings are not available to date. While several other trials included PLWH, they excluded their data from primary publication[20,21,27]. In a recent report by Ruddy *et al.* which examined COVID-19 antibody response in 12 PLWH a median of 21 days (interquartile range 17-27) following the first dose of mRNA vaccine (50% Moderna and 50% Pfizer), antibodies were

detected in all participants although lower levels were observed in persons with lower CD4 T cell counts[26]. In this study, all 12 individuals were male, 8% were non-white, all had been on ART ≥6 months and 92% had an undetectable HIV viral load. Two individuals had a CD4 T cell count below 200 cell/mm^3.[26]

We lack robust data on vaccine immunogenicity and immune response durability in subpopulations of PLWH. No evidence is available on the durability of immunogenicity in PLWH beyond 3 months following vaccination. Since the hallmark of HIV infection is a reduced number and function of CD4 T cells, and cell-mediated immunity has emerged as a critical aspect of the COVID-19 immune response,[28,29,30] it is critical to characterize cellular immune and cytotoxic T cell responses to COVID-19 vaccination.[31-33].

Given that inflammatory markers may influence immune cell activation status and shift cell profiles towards either Th1 or Th2 responses, impacting vaccine-elicited immune response[34], understanding the interplay between immune activation and dysfunction is also important. To address this need, we are establishing a pan-Canadian prospective cohort of PLWH receiving COVID-19 vaccines to assess humoral and cellular immunogenicity and to describe the inflammatory milieu in this context. Safety and tolerability of COVID-19 vaccines in this cohort of PLWH will also be captured. Of note, COVID-19 vaccines currently approved for use in Canada include those manufactured by Pfizer, Moderna, AstraZeneca and Janssen (Johnston &

Johnston) (https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/drugs-vaccines-treatments/authorization/list-drugs.html). Since the beginning of the vaccine roll-out Pfizer and Moderna vaccines were administered most often as they were the first to gain approval by Health Canada. Furthermore, approved, due to concerns associated with cerebral venous sinus thrombosis and vaccine-induced immune thrombotic thrombocytopenia, use of the AstraZeneca COVID-19 vaccine has been restricted in some provinces. Furthermore, the COVID-19 vaccine manufactured by Novavax is not licensed in Canada.

Study Objectives:

Primary objective:

To evaluate the immunogenicity of COVID-19 vaccination in PLWH, as assessed by COVID-19-specific immunoglobulin G antibody (IgG) enzyme-linked immunosorbent assay (ELISA), at 6 months following 2nd vaccine dose.

Secondary objectives:

1) To assess neutralization capacity of COVID-19-specific IgG at 6 months following 2nd vaccine dose.

2) To assess the durability of COVID-19-specific IgG response in PLWH at 12 months following vaccination.

3) To examine changes in the proportion and activation phenotype of CD4 T cells, CD8 T cells, B cells, natural killer cells and monocytes, including gene expression and cytokine production, pre- and post-COVID-19 vaccination at 6 months following 2nd vaccine dose.

4) To determine safety and tolerability of COVID-19 vaccines in PLWH, based on local or systemic adverse events following first or second injections.

Exploratory objectives:

1) To determine if subpopulations of PLWH respond differently to COVID-19 vaccination. Subpopulations of interest include, 1) PLWH >55 years of age; 2) immune non-responders (ART treated, and fully suppressed HIV RNA (<40 copies/mL), but CD4 T cell counts below 350 cells/mm^3 and CD4/CD8 T cell ratio <0.75); 3) PLWH with multimorbidity (2 or more chronic diseases) and 4) PLWH "reference" participants (with CD4 T cells >350 cells/mm^3, suppressed viral load for at least 6 months, and have at most 1 comorbidity) *(Note: groups will not be mutually exclusive but will likely have overlapping characteristics)*

(2) To investigate if current COVID-19 vaccines elicit IgG that cross-recognize key COVID-19 variants of concern (VoC), and if this differs in PLWH compared to individuals without HIV

(3) To compare virus-specific T cell responses generated by COVID-19 vaccines in PLWH and compare results with HIV-negative populations

METHODS AND ANALYSIS

Study design

This study is a multi-centre prospective observational cohort study. Approximately four hundred (400) PLWH aged ≥16 years will be recruited from 4 sites in 3 Canadian provinces including 1) McGill University Health Centre/Jewish General Hospital (Montreal), 2) Ottawa Hospital Research Institute, 3) The University Health Network (Toronto), and 4) St. Paul's Hospital (Vancouver). Many sites provide HIV care for many clients who are visible minorities and

multiple morbidities. This will enable our sites to recruit a study population representative of PLWH most likely to be impacted by detrimental COVID-19-related outcomes.[19] Data for HIV-negative individuals will be obtained from the Stop the Spread Ottawa (SSO) cohort. Since we will not perform additional analyses on the samples of the SSO cohort who we are going to include in our study, then we can include all the participants in the SSO study and matching of HIV-negative and HIV-positive participants will not be required.

Determination of which vaccine is administered at which time point and to which individuals is dictated by Canada's provincial governments, with input from the National Advisory Committee on Immunization (NACI), and is not influenced by study investigators or staff. We will include participants irrespective of the specific type of COVID-19 vaccine. Furthermore, the duration of the interval between 1st and 2nd doses time from when the vaccine was administered will not influence eligibility, since Canada has decided to extend dose intervals for all 2 dose vaccines to 4 months. However, the duration of interval between vaccine doses will included as an outcome variable.

Methods: Participants, intervention and comparator and outcomes

Inclusion Criteria*:* 1) Age ≥16 years; 2) HIV-positive for HIV group, immunocompetent and generally in good health for the HIV-negative group; and 3) Receiving ≥1 dose of COVID-19 vaccine. Persons are still eligible to participate if they have already received one or two vaccine doses.

Exclusion Criteria: 1) Receipt of any blood product or immunoglobulin preparation within_1 month of vaccine administration and until study completion; 2) signs/symptoms of active COVID-19 at time of enrollment; 3) for the HIV-negative group: immune-compromising conditions or on immunosuppressant medications. Prior receipt of other vaccines ≤12 months or past COVID-19 infection are not exclusion criteria but will be recorded. Detectable HIV viral load on ART is not exclusionary.

The following groups of PLWH will be prioritized for study enrolment:

1. Older age (55 years and over). Older age is associated with immunosenescence and results in lower vaccine efficacy.[35-37] We have selected 55 as the specific age cut-off since PLWH tend to develop comorbidities at an earlier age.
2. Immune non-responders (CD4 T cell count <350 cells/mm^3, CD4/CD8 <0.75 with undetectable viral load for 1+ year). Immune non-responders may be at risk of more adverse COVID-19 related outcomes than HIV immune responders[38,39,40,41].
3. Multi-morbidity (defined as having ≥2 comorbidities). Comorbidities may include cardiovascular disease (CVD) or CVD equivalents such as tobacco smoking. Other comorbidities include CMV co-infection, hypertension, dyslipidemia, diabetes, chronic obstructive pulmonary disease and obesity, as these are all common comorbidities among PLWH[2,42-45] and factors that contribute to worse outcomes with COVID-19.[46]
4. HIV-positive "stable" or "reference" group. These persons will have undetectable HIV viral load for >6 months, CD4 T cell counts >350 cells/mm^3, and a maximum of 1 comorbidity. To capture the full spectrum of individuals in the HIV-negative group, we will include this HIV-positive "stable" group so that we can determine whether there are

particular characteristics within PLWH which impact on immune response. In comparing the stable and unstable groups, we will be able to determine whether participants with low CD4 counts (<350cells/mm^3) differ in their response to vaccine from those with normal CD4 T cell counts while controlling for other characteristics. We aim to enroll HIV-negative and HIV-positive "stable" individuals with overlapping characteristics (i.e., some should have multiple comorbidities) so that the groups are comparable.

General Methodology and participant timeline

Participants will attend 5 visits over 12 months: pre-vaccination; 1 month following first vaccine dose; and at 3, 6 and 12 months following the second vaccine dose **(Table 1**). Each visit will last between 20-60 minutes.

Primary Endpoint: Percentage of PLWH with COVID-19-specific antibodies at 6 months following 2nd vaccine dose.

Secondary Endpoints: Percentage of individuals with 1) COVID-19 neutralization capacity at 6 months following 2nd vaccine dose and 2) COVID-19-specific antibodies at 12 months following 2nd vaccine dose. 3) Proportion and activation status of CD4 T cells, CD8 T cells, B cells, natural killer cells and monocytes, pre-vaccination and 6 months post 2nd vaccine dose.

Exploratory Endpoints: 1) COVID-19-specific antibodies at 6 months following 2nd vaccine dose, stratified by subpopulations. Critically, we will also assess the ability of vaccine-elicited antibodies to cross-recognize SARS-CoV-2 S protein variants, including N501Y and/or E484K, using in-house assays.

Sample size

Our primary outcome is the proportion of individuals in each group who mount a satisfactory immune response, although the best marker of what constitutes a satisfactory immune response is unclear at the moment and the science is rapidly evolving. We initially defined a successful immune response as a 4-fold relative rise in IgG production at 6 months.[47] We anticipated that 90% of HIV-negative individuals would mount an adequate IgG response at this timepoint[27,34], versus 70% of PLWH.[12,48] If 20% of the sample were to have the characteristic of interest or predictor variable (e.g., 20% with multimorbidities), enrolling 200 PLWH and 50 HIV-negative participants would provide sufficient statistical power (>80%) to detect a 20% difference in outcomes between groups whatever the exact proportions. In addition, we calculated that we would have >80% power to detect a 20% difference in outcome between those with suppressed CD4 count or unsuppressed viral load and the HIV- group. Previous studies of temporal differences in humoral and cellular responses to COVID-19 have shown differences between individuals when sample sizes included 100 participants or fewer.[49-53] We would also have >80% power to detect a 20% difference in outcome between the higher risk PLWH (ie, with CD4 counts <200 cells/mm^3 and/or unsuppressed HIV viral load) and the HIV-negative group. Such recruitment targets would also also provide a sufficient buffer to account for potential drop-outs of 5-10%. However, to increase our ability to detect differences in our primary outcome between the four sub-populations of interest (individuals of older age; immune non-responders; persons with multimorbidity; and an HIV-positive "stable" or "reference" group), we plan to recruit 400 PLWH and use data from the *entire* cohort of HIV-negative individuals in the SSO study (approximately 1000 individuals) to increase power. Inevitably, the higher risk groups will be overlapping, so we will recruit a

minimum of 20% of the 400 PLWH per category. By using the entire cohort of HIV-negative individuals in the SSO study, we avoid the need to match PLWH and HIV-negative participants.

Recruitment

Participants will be informed about the study through a recruitment flyer during routine physician visits to their HIV clinic and via established recruitment strategies through our community partners and the CIHR Canadian HIV Trials Network (CTN) via webpages, email, and social media platforms. Individuals followed for routine HIV care at clinics other than the 4 enrolment sites are eligible to participate if they can come to the enrolment site for study visits. Participants will be compensated $40 per study visit to help offset the time commitment and parking fees. We will make a concerted effort, through the use of recruitment quotas, to ensure the HIV-negative and HIV-positive "stable" groups have overlapping characteristics (eg, age >55 years, CD4 count <350 cells/mm^3, comorbidities) so that the groups will be comparable.

Data Collection

Medical history and HIV history will be gathered from both patient interviews and clinic chart reviews following written informed consent. Information extracted will include comorbidities, year of HIV diagnosis, CD4 T cell nadir (if known) in addition to tobacco smoking and cannabis use history. Medications will be recorded in addition to the ART regimen. History of COVID-19 infection and date of confirmatory test will be recorded.

Sample Collection: At each visit, blood will be collected to isolate serum, plasma and peripheral blood mononuclear cells (PBMCs).

Humoral Immunity (SARS-CoV-2 Binding Antibodies): We will evaluate levels of immunoglobulins M and A (IgM, IgA) and IgG targeting the SARS-CoV-2 Spike (S) protein receptor-binding domain (RBD) and nucleocapsid protein using a high-throughput automated ELISA co-developed and validated by Dr Marc-Andre Langlois,[54] thereby distinguishing vaccine-induced (S only) from infection-induced (S and nucleocapsid (N)) responses. We will also evaluate samples for IgM and IgG antibody cross-recognition of RBD VoC, including those harbouring *N501Y and/or E484K* (e.g., United Kingdom and South African strains, respectively) using a multiplex ELISA assay developed by Dr Mark Brockman.[55] This assay can be rapidly adapted to accommodate emerging variants. We will test plasma samples for their capacity to block viral entry using a well-established neutralization assay based on retroviruses pseudotyped with the SARS-CoV-2 S protein.[49,51]

Cellular Immunity*:* Flow cytometry will be performed to enumerate CD4 T cells (including helper and regulatory subsets), CD8 T cells and other T cell subsets (including naïve, central memory, transitional memory, effector memory and terminally differentiated cells), B cells (including naïve, memory and antibody-secreting B cells), Natural Killer cells and monocytes (classical, inflammatory and non-classical). We will also evaluate markers of cellular immune activation, senescence and exhaustion. Following high-resolution Human Leukocyte Antigen class I/II typing, we will examine COVID-19-specific T cell responses using an activation-induced marker (AIM) assay. Briefly, PBMCs will be stimulated overnight with pools of SARS-CoV-2 S peptides. Activated CD4 and CD8 T cells will be quantified by flow cytometry-based expression of CD137, OX40 and/or CD69. Gene expression will be assessed by single-cell RNA sequencing of PBMC as described previously.[56] T and B cell epitope specificity will be confirmed using virus-derived antigens (peptide/HLA or RBD dextramers, respectively). Plasma levels of inflammatory markers

including interferon (IFN)-y, interleukin (IL)-1β, IL-2, IL-4 IL-6, IL-8, IL-10, IL-13, IL-17, transforming growth factor (TGF)-β, IFN-y-induced protein-10 (IP-10), IL-12p70 and tumour necrosis factor (TNF)-α will be measured using multiplex Luminex assays, D-dimer, C-reactive protein, and markers of microbial translocation lipopolysaccharide (LPS), beta-d-glucan (ßdG) and soluble CD14 will be evaluated by ELISA.[57]

Exploratory Safety and Tolerability of COVID-19 Vaccines in PLWH

In the prospective cohort, we will explore vaccine safety and efficacy to inform subsequent studies. Reactogenicity: ***Symptoms Diary****:* Participants will be asked to document specific local and systemic reactions in a diary for 1 week and 1 month following each injection, as was done in Pfizer-BioNTech Phase 3 studies.[47] We will report the proportion of participants developing local (redness, pain or swelling at the injection site) or systemic effects (fatigue, headache, muscle pain, fever, joint pain, diarrhea) within 7-30 days following each vaccine dose with 95% confidence intervals (Supplementary information).

COVID-19 Questionnaires: We will administer the COVID-19 System Questionnaire to participants who develop a flu-like illness to confirm the illness, along with PCR-based tests for COVID-19. Participants will complete the COVID-19 Immunity Task Force (CITF) Standardized Core Survey Data Elements questionnaire prior to vaccination and a modified CITF questionnaire (minus the demographic information) at follow-up visits (Supplementary information).

If participants develop COVID-19 symptoms 14 days+ after vaccination, we will also collect CITF system questionnaire (length of illness and symptomatology, which vaccine was administered, number of doses vaccine received) and saliva specimens to enable study of COVID-19 variants of

concern. (https://www.covid19immunitytaskforce.ca/covid-19-immunity-task-force-releases-standardized-core-survey-data-elements/)

Data management

The study sponsor, the CTN, will be responsible for national project management, database development, data management and data analysis. To facilitate data sharing, we will: 1) use standard encodings for CITF-defined Core Data Elements; 1) request immunogenicity study participants consent to data sharing as guided by the CITF, including collecting survey elements and saliva 14+ days after vaccination on symptomatic participants to determine whether the infecting strain is a COVID-19 VoC and 3) rapidly share interim data and all requested study metadata for cataloguing.

Confidentiality

All participant-related information, including Case Report Forms, laboratory specimens, evaluation forms and reports, will be kept strictly confidential. All records will be held in a secure, locked location and only accessible to research staff. Participants will be identified using a coded number specific to each participant. All computerized databases will identify participants by numeric codes only and will be password protected. Upon request, and in the presence of the investigator or his/her representative, participant data will be made available to the study sponsor, monitoring groups representative of the study sponsor, representatives of funding groups, and applicable regulatory agencies to verify clinical study procedures and/or data, as is permissible by local regulations.

Statistical Analyses

We will use regression techniques to compare COVID-19-specific immune responses applying data transformation where necessary to conform with distributional assumptions in order to determine whether there are differences between the "unstable" (CD4<350) PLWH group, the stable PLWH cohort and the HIV- controls, controlling for factors that are believed to be associated with immune response. We will also perform unadjusted analyses to determine whether there are differences which may or not be driven by factors associated with group membership. We will report data from exploratory analyses with descriptive statistics and data for vaccines from different manufacturers separately and combined. We will stratify results by the number of doses received and the time interval between the two doses in case these factors drive response and differ between groups. We will stratify immunogenicity data by sex as females and males have differences in both vaccine-elicited immune responses[58] and adverse effects from vaccines.[59] Furthermore, we will stratify analyses by individuals who are naive to COVID-19 versus those with pre-existing antibodies as a result of prior COVID-19 infection. This will be important since antibody responses (particularly after the 1st dose) will be much higher in convalescent individuals,[60,61] so it is not appropriate to include them with individuals who do not have pre-existing antibodies to COVID-19.

ETHICS AND DISSEMINATION

Ethics approval and consent to participate

Written informed consent will be obtained from all study participants. The study will be conducted in accordance with the Declaration of Helsinki. At the time of manuscript preparation, a very closely related protocol has been approved by the University of British Columbia/Providence Health Care Research Institute and Simon Fraser University Research Ethics Boards. The present protocol is under review by the Research Institute of the McGill University Health Centre (2nd review), the Ottawa Hospital Research Ethics Board, the University of Toronto Research Ethics Board. Patient enrollment for this trial is anticipated to begin June 2021.

Availability of data and materials

De-identified participant data will be stored on a secure password-protected RedCap database. Access to the database will be controlled by the CTN. Access to the final study database will be provided upon written reasonable request to the corresponding author/principal investigator following publication and CTN and CITF approval.

We will standardize reagents and analysis strategies, where possible, working with the Immune Sciences Network and Testing Working Party recommendations to enable data sharing and avoid duplication in consultation with other CIHR and CITF-funded vaccine surveillance projects. We will also contribute results to SeroTracker, a knowledge hub that tracks and synthesizes findings from SARS- CoV-2 serosurveillance efforts worldwide (https://www.covid19immunitytaskforce.ca/serotracker/). To inform COVID-19 immunization guidelines and future interventions for PLWH in Canada and internationally, we are committed to

sharing results with all stakeholders and will adhere to Wellcome's *Sharing research data and findings relevant to the novel coronavirus (COVID-19) outbreak* statement.[62]

Knowledge Translation (KT) & Dissemination Plan

Serology results will be provided to individual study participants at the end of the study, along with a summary of study results and their implication in lay language. We prioritize meaningful community engagement and our Community Advisory Committee includes PLWH and representation from the Canadian Aboriginal AIDS Network. The Community Advisory Committee reviews protocols and informed consent forms and advises on community priorities. Our well-established relationship with CATIE, Canada's source for HIV information, will enhance KT. We will leverage our KT staff to share lay language updates via press releases, media coverage, website, e-newsletters and social media and undertake targeted KT activities to mobilize knowledge across our network and report results to senior policymakers, via research summaries and policy briefs, and community groups and participants, via factsheets. Our team will publish manuscripts, contribute to guidelines, and present to stakeholders.

Patient and Public Involvement

The CTN Community Advisory Committee (CAC) was involved in the peer review process of this study proposal and deemed that the research questions addressed were of very high priority to PLWH. The CAC's critiques of the initial proposal were taken into account in the revised proposal. Two members of the CAC (SM and EM) were involved in finalizing the study design, inclusion/exclusion criteria, outcome measures and monitoring plans and are formal study investigators and co-authors. Community consultants will receive financial compensation to recognize their time commitment and expertise.

DISCUSSION

Herein, we present the protocol for an observational cohort study to evaluate COVID-19 vaccine-elicited immunogenicity in PLWH, with a priority of determining immunogenicity in PLWH who are of older age, immune non-responders and those with multimorbidity. These 3 groups were selected since they represent subpopulations most likely to experience poor outcomes following COVID-19 infection and have a weaker immune response to vaccination.

PLWH immune non-responders are at risk of more adverse COVID-19 related outcomes than HIV immune responders. In the study performed by Braunstein *et al.,* PLWH with COVID-19 infection had a higher proportion of hospital admissions, intensive care unit (ICU) admission and death. Those who experienced these COVID-19-related outcomes had CD4 T cell counts below 500

cells/mm.[3,38] Similarly, the study by Dandachi *et al*. found that having CD4 T cell counts below 200 cells/mm^3 was associated with severe outcomes such as ICU admission, intubation and death.[39] Furthermore, a multicentre cohort study by Hoffman *et al.* showed that CD4 T cell counts <350 cells/mm^3 were associated with severe COVID-19 (adjusted OR 2.85, 95% CI 1.26-6.44).[40] However, in a group of patients with inborn errors of immunity, Kinoshita *et al.* they demonstrated robust T cell activity and humoral immunity against COVID-19 structural proteins in some patients with antibody deficiency,[63] underscoring the heterogeneity and complexity of immune response.

A major challenge with planning this study is the unprecedented, rapidly-changing nature of the COVID-19 pandemic and evolving scientific information. As a result, data on the optimal time points to assess immune responses post COVID-19 vaccination administration are rapidly changing, resulting in multiple adjustments in our protocol plans. Within Canada, the vaccination schedule is determined by the provinces with input from NACI. However, differences exist between provinces regarding to vaccine supply, eligibility criteria, type of vaccine administered and time period between vaccine doses. We are mitigating these challenges by holding monthly meetings with teams to discuss these issues over the previous month, troubleshoot and adjust recruitment priorities accordingly for the upcoming month. Ideally, and under non-pandemic circumstances, we would establish, a priori, methods for analyzing data from single vs. two-dose vaccines. However, in the current context with uncertainty of vaccine supply and distribution, such detailed plans are impractical and will depend on the methods of vaccination used in the participants enrolling in this study. This statement holds true for other variables we will likely encounter, such as different dosing intervals amongst persons receiving two-dose vaccines.

The importance of advanced age in impaired vaccine-induced immunogenicity is well-documented. Due to a combination of disrupted in posttranscriptional regulation, T cell receptor signalling, and metabolic function, older individuals demonstrate reduced quantity and functionality of T-cells[64,65]. When this balance is disrupted, T-cells exhibit shorter-lived effector phenotypes rather than memory or follicular helper T cells and vaccine-induced antibodies are less protective than in younger persons[64,65]. As the elderly are considered a priority vaccination group, many of them were eligible for vaccination in early 2021 in Canada, before this current study began, meaning that baseline plasma, serum and PBMCs will not be available for participants in this subpopulation of interest. Another drawback with starting this study in May 2021 is that we may miss other important groups of PLWH, including Indigenous persons who were prioritized as high-risk populations for immunization and were eligible to receive COVID-19 vaccines at a younger age than the general population.

Another major challenge with the planning of this study was the need to ensure an adequate sample size to meet the primary objective in the group as a whole, but also in important subpopulations of PLWH. Enrollment of such a large number of individuals, with follow-up until 12 months following the second vaccine dose, is very resource-intensive and requires dedicated study participants. We will provide individualized antibody results to participants at study completion to

increase study engagement and prevent drop-outs. Furthermore, due to the need to rapidly enroll participants to match the pace of the COVID-19 vaccine rollout, we decided to use CTN study sites with large clinic volumes and proven capacity to recruit. However, these clinics are based in urban centres and, therefore, may follow individuals who differ systematically from PLWH followed outside of the cities. PLWH who live in cities may be of higher socioeconomic status and consist of more men who have sex with men (MSM). Therefore, each site will need to make a concerted effort to recruit sufficient participants with other profiles. For this reason, we will employ a flexible recruitment strategy, whereby sites that can recruit the required participants with characteristics of interest more easily than other sites can help to make up for lower recruitment flexibility at other sites. As with many studies, recruitment of women living with HIV may be challenging. Due to our connection with other studies within the CTN and the help of our community advisory board, we are encouraging clinics with predominantly female clients to ensure they inform their female patients living with HIV about this study.

Currently, the immune correlate of protection against COVID-19 is undefined. T cell and B cell responses are usually used as surrogates of protection.[66] We decided to use a 4-fold rise from baseline in IgG production following second vaccine dose as the criteria for a successful vaccine response, as was indicated in the Pfizer study[47], in order to calculate our sample size. Data from the Pfizer study submitted to the Food and Drug Administration and published by Walsh *et al.,* report geometric mean titers that were compared to those from a human SARS-CoV-2 convalescent serum panel as a benchmark. Increased titers were expressed in logarithmic fold increases.[67] There are currently no national standards for presenting the serology data. Some groups prefer to report the raw signal values, whereas others normalize data as fold increases.

Since individual baseline values will not be available for participants who have already received their first vaccine dose, one option is to use the cohort baseline data derived from non-vaccinated participants. Alternatively, we may opt to examine relative titers, or fold-changes, and therefore baseline data will not actually be required. Since science is continuously evolving, we will use the definition of a successful vaccine response which is most widely accepted at the time of publication.

The best methodology to match PLWH with the HIV-negative group remains unclear. As discussed by Wong *et al.,* the ideal comparison group would be individuals who are identical to HIV-negative adults in all aspects except HIV status[68]. As PLWH have distinct characteristics, traditional risk factors, lifestyle factors and socioeconomic factors compared to the general population, the general population may not be the ideal comparison group[68]. Differences include increased tobacco smoking[69], substance use[70] and comorbidities[71] amongst PLWH compared to the general population. However, PLWH also undergo more screening for age-related comorbidities due to frequent contact with health care providers[68]. We plan to match PLWH participants with HIV-negative individuals from the SSO study. As previously mentioned, as long as we ensure there are 20% of individuals with the characteristics of interest in the HIV-negative and HIV-positive "stable" cohorts, we can compare groups while controlling for these characteristics via regression.

Taken together, the findings from this study will provide valuable insight into COVID-19-vaccine-induced immunogenicity in important sub-populations of PLWH. HIV-positive persons of older age, with CD4 counts <350 cells/mm^3 and multimorbidity were not included in the early clinical trials, yet are most likely to suffer from poor outcomes if infected with COVID-19. These findings

will inform clinical guideline recommendations for PLWH and, in turn, reduce COVID-19-induced morbidity and mortality.

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Authors' contributions

Co-principal investigators of the study are CTC, CLC and AA. CC and CLC conceived the study, led the proposal and protocol development. CC wrote the first draft of the manuscript. JS is the biostatistician who provided methodological expertise and performed sample size calculations. All other authors contributed to protocol development, study design and development of the proposal. CTC, MAL, MAJ, MO, MB, and ZB designed the laboratory evaluations. MAL will be responsible for studies on humoral immunity. MAJ will be responsible for flow cytometric studies to define proportions of immune cells and their subsets, while CTC will be responsible for cytokine assessment. MAB and ZLB will be responsible for RNA profiling. MAL and MAB will perform the analysis of antibodies to COVID-19 variants. MO and MAB will perform analyses of T cell responses to vaccine immunogens. Markers of gut barrier damage, microbial translocation and CMV IgG titers will be performed by JPR. HLA typing will be performed as necessary for participants evaluated for T cell responses by MO, ZLB and MB. JS will oversee data analysis between groups and subgroup analyses. All authors critically reviewed and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

Table 1: Visits and Procedure Schedule

Visit number	**1**[1] **(Screen)**	**Vaccine**	**2**	**Vaccine**	**3**[6]	**4**[6]	**5**[6]
Week Number	-12 to 0 weeeks	0	4 weeks		3 mo after dose 2	6 mo after dose 2	12 mo after dose 2
Window	-3 mo						
Inclusion/Exclusion	X		X				
Informed Consent	X						
Medical History	X						

Blood Draw: Immunology[2]	X		X		X	X	X
Blood Draw: CD4/Viral Load[3]	X		X		X	X	X
Vaccination[4]		X		X			
Participant Diary[5]		X	X	X	X		
CITF [7] Questionnaire	X		X		X	X	X
Adverse Events[8]			X		X		
Concomitant Meds	X		X		X	X	X

[1]Screening assessment may be performed same day as vaccination but will be completed prior to vaccination.

[2]Immunoglobulin levels flow cytometry and cytokine secretion (immunogenicity measures); Will be collected at each visit. For participants who have already received a vaccine dose prior to study enrolment, "baseline" immunoglobulin levels (i.e., pre-vaccination) may not be available.

[3]Blood work such as CD4 and Viral load can be collected as part of standard of care

[4]Participants will receive the COVID-19 vaccine outside of the study per standard of care as part of their provincial immunization program.

[5]Participants will maintain a diary after vaccination during which they will record their vaccine reactions, oral temperature and any febrile respiratory tract symptoms as well as general changes to health and medications. The diary will be evaluated up to 30 days following each injection.

[6]Visits 3, 4, 5 will be conducted at 3, 6, 12 months after dose 1 respectively, for COVID-19 vaccines administered as a one-dose schedule.

[7]The full CITF questionnaire will be completed at visit 1 and the modified CITF questionnaire will be completed at subsequent visits.

[8]Adverse events will only be collected at 7 and 30 days post vaccination and for participants who receive a vaccine while currently enrolled in the study (i.e. adverse events will not be collected retrospectively)

For participants who develop COVID19-symptoms 14+ days following vaccination.

Participants will be asked to complete the COVID-19 Symptom Survey (Supplementary materials) and to go for a COVID-19 test at their nearest test centre and notify the study staff of their test result. If positive for COVID-19, the study staff will mail the participant 6 saliva collection kits by courier in order to collect information on SARS-CoV-2 variants

Participants who have already received 1 vaccine dose: These individuals may be enrolled in the study, at any duration of time post first dose, as long as the baseline blood draw is before the second booster. Visits 1 and 2 will be combined.

Participants who have already received 2 vaccine doses: These individuals must be enrolled in the study within 3 months of their 2nd dose. Visits 1 and 3 will be combined and Visit 2 will not be required. Participants will follow-up at visits 4 and 5.

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DATA COLLECTION WORKSHEET

Participant ID | | | | | | |

☐ **Vaccine 1**

☐ **Vaccine 2**

COVID-19 SYMPTOMS QUESTIONNAIRE

Have you experienced any of the following COVID-19 signs or symptoms?

Sign	Yes	No	If yes, provide date, time
Fever, chills			
Cough			
Shortness of breath			
Acute loss of smell or taste			
Fatigue			
Headache			
Muscle aches			
Nausea/Vomiting/Diarrhea			
General weakness			
Nasal congestion			
Sore throat			

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DATA COLLECTION WORKSHEET

Participant ID | | | | | | |

☐ **Vaccine 1**

☐ **Vaccine 2**

PARTICIPANT DIARY

Following each injection of COVID-19 vaccine, please indicate whether you experienced any of the following, within 7 days and within 30 days, and indicate the severity. See the Other Signs or Symptoms worksheet for a guide to the severity levels.

	Date:	**Date:**	**Date:**	**Date:**	**Date:**	**Date:**	**Date:**	**Date:**	
Sign or Symptom	Day 0*	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Within 30 days
Pain at injection site									
Redness									
Swelling									
Lymphadenopathy/Axillary swelling and tenderness									
Fatigue									
Headache									
Muscle Pain									
Chills									
Joint pain									
Fever									
Diarrhea									
Nausea and/or Vomiting									

* Day 0 refers to day vaccine received, Day 1 is th**e *following day,*** and so forth

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DATA COLLECTION WORKSHEET

Participant ID | | | | | | |

☐ **Vaccine 1**

☐ **Vaccine 2**

PARTICIPANT DIARY – Other Signs or Symptoms

Please use this form to record the intensity of signs or symptoms experienced on Days 8 -30, and any other signs or symptoms that are not listed on the chart.

For any sign or symptom, please record the intensity based on the following:

- **Grade 1 = Mild** = does not interfere with participant's usual daily function
- **Grade 2 = Moderate** = interferes to some extent with participant's usual function
- **Grade 3 = Severe** = interferes significantly with participant's usual function
- **Grade 4 = Life-threatening** = life-threatening consequences; urgent intervention indicated

Sign or Symptom	**Date**	**Intensity Grade (1-4)**	**Additional notes**-please describe as much as possible (i.e., duration of time the sign or symptom lasted, if you took any medications for it etc.)

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CTN 328
HIV-COV

DATA COLLECTION WORKSHEET

Participant ID | | | | | | |

☐ **Vaccine 1**

☐ **Vaccine 2**

PARTICIPANT DIARY – Other Signs or Symptoms (continued)

Sign or Symptom	**Date**	**Intensity Grade (1-4)**	**Additional notes**-please describe as much as possible (i.e., duration of time the sign or symptom lasted, if you took any medications for it etc.)

the CTN
CIHR Canadian
HIV Trials Network

le Réseau
Réseau canadien
pour les essais VIH des IRSC

CTN 328: HIV-COV

CITF CDE BASELINE QUESTIONNAIRE

This version is only administered at Screening

Please answer all questions unless otherwise indicated

Participant ID: ___ ___ ___ ___ ___ ___

Section 1: Demographics

1. Date (DD-MMM-YYYY):

___ ___ / ___ ___ / ___ ___ ___ ___

2. What is your age?

___ ___ ___ YRS ___ ___ MO OR ☐ Prefer not to answer

3. What was your assigned sex at birth?

- ○ Male
- ○ Female
- ○ Prefer to self-describe (specify) _____
- ○ Prefer not to answer

4. What is your sex now?

- ○ Male
- ○ Female
- ○ Prefer to self-describe (specify) _____
- ○ Prefer not to answer

5. What is your gender (how do you currently self-identify)?

- ○ Male
- ○ Female
- ○ Non-binary, genderqueer, agender or a similar identity
- ○ Two-spirit
- ○ Prefer to self-describe (specify) _____
- ○ Prefer not to answer

6. Are you an Indigenous person originating from North America?

If NO or Prefer not to answer, please proceed to Q9

- ○ No
- ○ Yes
- ○ Prefer not to answer

7. Which of the following groups do you belong to? Please select all that apply.

Only answer if Q6 = YES

- ☐ First Nations
- ☐ Inuit
- ☐ Metis
- ☐ Non-status First Nations
- ☐ Other Indigenous (specify) _____
- ☐ Prefer not to answer

8. Do you live on reserve?

Only answer if Q7 = First Nations

- ○ Yes
- ○ No
- ○ Prefer not to answer

9. How would you describe your ethnicity or race? Please select all that apply.

If you are an Indigenous person and answered YES to Q6, select any other that apply.

- ☐ White
- ☐ South Asian
- ☐ Chinese
- ☐ Black
- ☐ Filipino
- ☐ Latin American
- ☐ Arab
- ☐ Southeast Asian
- ☐ West Asian
- ☐ Korean
- ☐ Japanese
- ☐ Prefer to self-describe (specify)

_____

- ☐ Prefer not to answer

10. What are the first three digits of your postal code?

___ ___ ___ OR ☐ Prefer not to answer

11. What is the highest level of education you have completed?

- ○ Less than high school graduation
- ○ High school graduation
- ○ Trade certificate, vocational school, or apprenticeship training
- ○ Non-university certificate or diploma from a community college, CEGEP
- ○ University Bachelor's degree
- ○ University graduate degree (Master's, Doctorate, etc.)
- ○ Prefer not to answer

12. How many people live in your household, including yourself?

_____ OR ☐ Prefer not to answer

13. How many bedrooms are in your household?

_____ OR ☐ Prefer not to answer

14. How many bathrooms are in your household?

_____ OR ☐ Prefer not to answer

Section 2: COVID-19

15. Do you think you have had COVID-19?

If NO or Prefer not to answer, please proceed to Q18

- ○ No
- ○ Yes
- ○ Prefer not to answer

16. Why do you think you have had COVID-19? Please select all that apply.

Only answer if Q15 = YES

- ☐ Symptom review online
- ☐ Symptom profile
- ☐ Nasal/throat test result
- ☐ Health care provider
- ☐ Contact with case
- ☐ Other (specify) _____
- ☐ Prefer not to answer

17. Were you hospitalized due to COVID-19?

Only answer if Q15 = YES

- ○ No
- ○ Yes
- ○ Prefer not to answer

18. Have you ever been tested for an active COVID-19 infection (using nasopharyngeal/throat swab, saliva, or gargle test)?

If NO or Prefer not to answer, please proceed to Q21

- ○ No
- ○ Yes
- ○ Prefer not to answer

19. If yes, how may times have you been tested?

Only answer if Q18 = YES

_____ OR ☐ Prefer not to answer

20.1 Answer the following questions about the **first COVID-19 test**, if applicable.

20.1.a What was the date of the **first** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

20.1.b What was the result of the **first** test?

- ○ Negative
- ○ Positive
- ○ Don't know

20.1.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

20.1.d If yes, what symptoms did you have?

Only answer if Q20.1.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

20.2 Answer the following questions about the **second COVID-19 test**, if applicable.

20.2.a What was the date of the **second** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

20.2.b What was the result of the **second** test?

- ○ Negative
- ○ Positive
- ○ Don't know

20.2.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

20.2.d If yes, what symptoms did you have?

Only answer if Q20.2.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

20.3 Answer the following questions about the **third COVID-19 test**, if applicable.

20.3.a What was the date of the **third** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

20.3.b What was the result of the **third** test?

- ○ Negative
- ○ Positive
- ○ Don't know

20.3.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

20.3.d If yes, what symptoms did you have?

Only answer if Q20.3.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

20.4.a Have you **tested positive** for COVID-19 (using nasopharyngeal, throat swab, saliva or gargle test) on a test that wasn't included the questions above (that is, on the **4th or later test**)?

If NO, please proceed to Q21

- ○ No
- ○ Yes

20.4.b If yes, what was the date the first time you tested positive?

Only answer if Q20.4.a = YES

___ ___ DD / ___ ___ MO ___ ___ ___ ___ YR

Section 3: Exposure

21.a Have you traveled outside of your home province since **January 2020**?

If NO, please proceed to Q23

- ○ No
- ○ Yes
- ○ Prefer not to answer

21.b If you think you had COVID, did you travel in the 6 months before your symptoms began?

Only answer if Q15 = YES

- ○ No
- ○ Yes
- ○ Prefer not to answer

22. What province(s)/territory(ies) or country(ies) did you travel to? Select all that apply.

Only answer if Q21.a or Q21.b = YES

- ☐ Alberta
- ☐ British Columbia
- ☐ Manitoba
- ☐ New Brunswick
- ☐ Newfoundland and Labrador
- ☐ Northwest Territories
- ☐ Nova Scotia
- ☐ Nunavut
- ☐ Ontario
- ☐ Prince Edward Island
- ☐ Quebec
- ☐ Saskatchewan
- ☐ Yukon

OR ☐ Prefer not to answer

List countries you travelled to (separated by a comma):

_____

_____

_____

_____

_____

23.a Do you do either paid or unpaid work in an environment where you work in close proximity to other people?

If NO or Prefer not to answer, please proceed to Q24

- ○ No
- ○ Yes
- ○ Prefer not to answer

23.b If yes, have you been working in any of the following occupations or worksites in the past year? Please select all that apply.

Only answer if Q23.a = YES

- ☐ Hospital or health care facility
- ☐ First responder (paramedic/firefighter/police officer)
- ☐ Childcare worker
- ☐ Correctional officer
- ☐ Teacher or other school staff
- ☐ Transit driver
- ☐ Food service industry
- ☐ Grocery store
- ☐ Pharmacy
- ☐ Hairdresser or barber
- ☐ Aesthetician
- ☐ Flight attendant
- ☐ Factor worker
- ☐ Other (specify)

_____

- ☐ Prefer not to answer

24.a How many times have you been in a gathering of 10 or more since **March 2020**?

_____ OR ☐ Prefer not to answer

24.b If you think you have had COVID, how many times were you in gatherings of more than 10 people in the 6 months before your symptoms began?

Only answer if Q15 = YES

_____ OR ☐ Prefer not to answer

Section 4: Health and Health Behaviours

25. Do you currently smoke tobacco?

- ○ No
- ○ Yes
- ○ Prefer not to answer

26. If yes, how often do you smoke tobacco?

Only answer if Q25 = YES

- ○ Less than daily
- ○ Daily

27. Do you currently use e-cigarettes (vape)?

- ○ No
- ○ Yes
- ○ Prefer not to answer

28. If yes, how often do you use e-cigarettes (vape)?

Only answer if Q27 = YES

- ○ Less than daily
- ○ Daily

29. Have you been diagnosed by a physician with any of the following chronic medical conditions? Please provide an answer for each condition.

		Yes	No	Don't Know	Prefer not to answer
a.	Hypertension				
b.	Diabetes				
c.	Asthma				
d.	Chronic Lung Disease				
e.	Chronic Heart Disease				
f.	Chronic Kidney Disease				
g.	Liver Disease				
h.	Cancer				
i.	Chronic Blood Disorder				
j.	Immune Suppressed				
k.	Chronic Neurological Disorder				

30. What is your current weight (circle units)?

_____ kg / lbs OR ☐ Prefer not to answer

31. What is your current height?

____ . ____ ____ m OR ____ ft ____ ____ in OR ☐ Prefer not to answer

32. Do you have a family physician/primary care provider?

- ○ No
- ○ Yes
- ○ Don't know
- ○ Prefer not to answer

33. Do you usually get a flu shot?

- ○ No
- ○ Yes
- ○ Prefer not to answer

34. Indicate if, or how often you have done the following since **March 2020**?

		Never	Rarely	Occasionally	Often	Always	Prefer not to answer
a.	Worn a mask in public places						
b.	Practiced physical distancing in public places						
c.	Avoided crowded places/gatherings						
d.	Avoided common greetings (such as a handshake or hug)						

		Never	Rarely	Occasionally	Often	Always	N/A	Prefer not to answer
e.	Limited contact with people at higher risk (e.g., an elderly relative)							

		No	Yes	N/A	Prefer not to answer
f.	Self-isolated because you thought you were infected with COVID-19				
g.	Self-quarantined because you may have been exposed to COVID-19, but did not show symptoms				

35. **If you think you have had COVID**, have you done the following in the **6 months before your symptoms began**? (indicate how often).

Only answer if Q15 = YES

		Never	Rarely	Occasionally	Often	Always	N/A	Prefer not to answer
a.	Worn a mask in public places							
b.	Practiced physical distancing in public places							
c.	Avoided crowded places/gatherings							
d.	Avoided common greetings (such as handshake or hug)							
e	Limited contact with people at higher risk (e.g., an elderly relative)							

		No	Yes	N/A	Prefer not to answer
f.	Self-isolated because you thought you were infected with COVID-19				
g.	Self-quarantined because you may have been exposed to COVID-19, but did not show symptoms				

Section 5: Vaccine

36. Have you been vaccinated against COVID-19? Answer YES if you have received at least one dose of the COVID-19 vaccine.

If NO or Prefer not to answer, proceed to Q43

- ○ No
- ○ Yes
- ○ Prefer not to answer

37. How many doses of the COVID-19 vaccine have you received so far?

Only answer if Q36 = YES

- ○ One
- ○ Two
- ○ More than two

38. When did you receive the **first dose** of the COVID-19 vaccine?

Only answer if Q36 = YES

___ ___ DD / ___ ___ MM / ___ ___ ___ ___ YR

39. When did you receive the **second dose** of the COVID-19 vaccine?

Only answer if Q37 = TWO or MORE THAN TWO

___ ___ DD / ___ ___ MM / ___ ___ ___ ___ YR

40. Which vaccine did you receive?

Only answer if Q36 = YES

- ○ Pfizer and BioNTech mRNA vaccine
- ○ Moderna mRNA vaccine
- ○ AstraZeneca Oxford vaccine
- ○ Other (specify) _____
- ○ Janssen (Johnson & Johnson) vaccine
- ○ Don't know
- ○ Prefer not to answer

41. Were you pregnant when you received the vaccine?

If NO or N/A, proceed to Q43

- ○ No
- ○ Yes
- ○ N/A

42. If yes, what trimester were you in when you received the vaccine?

Only answer if Q41 =YES

- ○ First
- ○ Second
- ○ Third

Section 6: Cannabis Use

43. Have you used cannabis (even once) in the past 12 months?

If NO, proceed to the end of this questionnaire

- ○ No
- ○ Yes

43.a If yes, how do you use cannabis? Please check all that apply.

Only answer if Q43 = YES

	Yes	No
Smoked dried plant		
Vaporized		
Oil		
Pills		
Added to baked good or other foods		
Other (specify)		

43.b If you smoke cannabis, please specify how you smoked/took cannabis:

☐ I do not smoke cannabis (proceed to Q44)

	Yes	No
Smoked as joint		
Smoked as joint mixed with tobacco		
Smoked as pipe		
Smoked as water pipe (bong)		
Inhaled using a vaporizer		
Eaten (e.g. as brownies, cake, cookies, etc.)		
Other (specify)		

44. Which response **best** describes how often you **currently** use cannabis?

Only answer if Q43 = YES

- ○ Rarely (2-3 times a year)
- ○ Monthly
- ○ Weekly
- ○ Daily
- ○ More than once a day

45. How many grams per week do you consume?

Only answer if Q43 = YES

- ○ Less than 1 gram
- ○ 1-5 grams
- ○ 6-9 grams
- ○ 10 or more grams
- ○ Unknown

46. If you smoke cannabis, on average how many joints/cigarettes do you smoke per day?

Only answer if Q43 = YES

_____ OR ☐ I do not smoke cannabis

END OF QUESTIONNAIRE

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CTN 328: HIV-COV

CITF CDE FOLLOW-UP QUESTIONNAIRE

This version is administered at Visits 2, 3, 4, 5, and at unscheduled visits

Please answer all questions unless otherwise indicated

Participant ID: ___ ___ ___ ___ ___ ___

1. Date (DD-MMM-YYYY):

___ ___ / ___ ___ / ___ ___ ___ ___

Section 1: COVID-19

2. Do you think you have had COVID-19 since your last visit?

If NO or Prefer not to answer, please proceed to Q5

- ○ No
- ○ Yes
- ○ Prefer not to answer

3. Why do you think you have had COVID-19 since your last visit? Please select all that apply.

Only answer if Q2 = YES

- ☐ Symptom review online
- ☐ Symptom profile
- ☐ Nasal/throat test result
- ☐ Health care provider
- ☐ Contact with case
- ☐ Other (specify) _____
- ☐ Prefer not to answer

4. Since your last visit, have you been hospitalized due to COVID-19?

Only answer if Q2 = YES

- ○ No
- ○ Yes
- ○ Prefer not to answer

5. Since your last visit, have you been tested for an active COVID-19 infection (using nasopharyngeal/throat swab, saliva, or gargle test)?

If NO or Prefer not to answer, please proceed to Q8

- ○ No
- ○ Yes
- ○ Prefer not to answer

6. If yes, how may times have you been tested since your last visit?

Only answer if Q5 = YES

_____ OR ☐ Prefer not to answer

7.1 Answer the following questions about the **first COVID-19 test since your last visit**, if applicable.

7.1.a What was the date of the **first** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

7.1.b What was the result of the **first** test?

- ○ Negative
- ○ Positive
- ○ Don't know

7.1.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

7.1.d If yes, what symptoms did you have?

Only answer if Q7.1.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

7.2 Answer the following questions about the **second COVID-19 test since your last visit**, if applicable.

7.2.a What was the date of the **second** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

7.2.b What was the result of the **second** test?

- ○ Negative
- ○ Positive
- ○ Don't know

7.2.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

7.2.d If yes, what symptoms did you have?

Only answer if Q7.2.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

7.3 Answer the following questions about the **third COVID-19 test since your last visit**, if applicable.

7.3.a What was the date of the **third** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

7.3.b What was the result of the **third** test?

- ○ Negative
- ○ Positive
- ○ Don't know

7.3.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

7.3.d If yes, what symptoms did you have?

Only answer if Q7.3.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

7.4.a Have you **tested positive** for COVID-19 since your last visit on a test that wasn't included the questions above (that is, on the **4th or later test**)?

If NO, please proceed to Q8

- ○ No
- ○ Yes

7.4.b If yes, what was the date the first time you tested positive since your last visit?

Only answer if Q7.4.a = YES

___ ___ DD / ___ ___ MO ___ ___ ___ ___ YR

Section 2: Exposure

8. Have you traveled outside of your home province since your last visit?

- ○ No
- ○ Yes
- ○ Prefer not to answer

9.a Since your last visit, have you worked (either paid or unpaid) in an environment where you work in close proximity to other people?

If NO or Prefer not to answer, please proceed to Q10

- ○ No
- ○ Yes
- ○ Prefer not to answer

9.b If yes, have you been working in any of the following occupations or worksites since your last visit? Please select all that apply.

Only answer if Q9.a = YES

- ☐ Hospital or health care facility
- ☐ First responder (paramedic/firefighter/police officer)
- ☐ Childcare worker
- ☐ Correctional officer
- ☐ Teacher or other school staff
- ☐ Transit driver
- ☐ Food service industry
- ☐ Grocery store
- ☐ Pharmacy
- ☐ Hairdresser or barber
- ☐ Aesthetician
- ☐ Flight attendant
- ☐ Factor worker
- ☐ Other (specify) _____
- ☐ Prefer not to answer

10. How many times have you been in a gathering of 10 or more since your last visit?

_____ OR ☐ Prefer not to answer

Section 3: Vaccine

11. Have you been vaccinated against COVID-19 since your last visit? Answer YES if you have **received at least one dose of the COVID-19 vaccine since your last visit**.

If NO or Prefer not to answer, proceed to the end of this questionnaire

- ○ No
- ○ Yes
- ○ Prefer not to answer

12. How many doses of the COVID-19 vaccine have you received since your last visit?

Only answer if Q11 = YES

- ○ One
- ○ Two
- ○ More than two

13. When did you receive the **first dose** of the COVID-19 vaccine since your last visit?

Only answer if Q11= YES

___ ___ DD / ___ ___ MM / ___ ___ ___ ___ YR

14. Which vaccine did you receive for this **first dose** since your last visit?

Only answer if Q11= YES

- ○ Pfizer and BioNTech mRNA vaccine
- ○ Moderna mRNA vaccine
- ○ AstraZeneca Oxford vaccine
- ○ Other (specify) _____
- ○ Janssen (Johnson & Johnson) vaccine
- ○ Don't know
- ○ Prefer not to answer

15. Were you pregnant when you received this **first dose** since your last visit?

If NO or N/A, proceed to Q17

- ○ No
- ○ Yes
- ○ N/A

16. If yes, what trimester were you in when you received this **first dose** since your last visit?

Only answer if Q15 =YES

- ○ First
- ○ Second
- ○ Third

17. When did you receive the **second dose** of the COVID-19 vaccine since your last visit?

Only answer if Q12 = TWO or MORE THAN TWO

___ ___ DD / ___ ___ MM / ___ ___ ___ ___ YR

18. Which vaccine did you receive for this **second dose** since your last visit?

Only answer if Q12 = TWO or MORE THAN TWO

- ○ Pfizer and BioNTech mRNA vaccine
- ○ Moderna mRNA vaccine
- ○ AstraZeneca Oxford vaccine
- ○ Other (specify) _____
- ○ Janssen (Johnson & Johnson) vaccine
- ○ Don't know
- ○ Prefer not to answer

19. Were you pregnant when you received this **second dose** since your last visit?

If NO or N/A, proceed to the end of this questionnaire

- ○ No
- ○ Yes
- ○ N/A

20. If yes, what trimester were you in when you received this **second dose** since your last visit?

Only answer if Q19 =YES

- ○ First
- ○ Second
- ○ Third

END OF QUESTIONNAIRE

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-9
Objectives	3	State specific objectives, including any prespecified hypotheses	9-10
Methods			
Study design	4	Present key elements of study design early in the paper	10-11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10-15, Table 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	11-12
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A (No matching)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	15-17
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11 (SSO cohort-same time points and methods). 12-13, 15-16,
Bias	9	Describe any efforts to address potential sources of bias	14-15, 22-26 (limitations discussed)
Study size	10	Explain how the study size was arrived at	14
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	19
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	19
		(*b*) Describe any methods used to examine subgroups and interactions	19

		(*c*) Explain how missing data were addressed	19
		(*d*) If applicable, explain how loss to follow-up was addressed	19
		(*e*) Describe any sensitivity analyses	19
Results			Protocol paper thus results not yet available
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	N/A protocol paper results not yet available
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22-26
Generalisability	21	Discuss the generalisability (external validity) of the study results	22-26
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on	32

		which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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CTN 328: Immunogenicity outcomes in people living with HIV in Canada following vaccination for COVID-19 (HIV-COV)-Protocol for an observational cohort study

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Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Immunology (including allergy), Infectious diseases, Patient-centred medicine
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, COVID-19, IMMUNOLOGY, VIROLOGY

PROTOCOL

CTN 328: Immunogenicity outcomes in people living with HIV in Canada following vaccination for COVID-19 (HIV-COV)-Protocol for an observational cohort study

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ABSTRACT

Introduction: Most existing vaccines require higher or additional doses or adjuvants to provide similar protection for people living with HIV (PLWH) compared to HIV-uninfected individuals. Additional research is necessary to inform COVID-19 vaccine use in PLWH.

Methods and analysis: This multi-centred observational Canadian cohort study will enroll 400 PLWH aged ≥16 years from Montreal, Ottawa, Toronto and Vancouver. Subpopulations of PLWH of interest will include: 1) >55 years of age 2) CD4 counts <350 cells/mm 3) multimorbidity (≥2 comorbidities) and 4) "stable" or "reference" PLWH (CD4 T cells ≥350 cells/mm3, suppressed viral load for≥ 6 months and ≤1 comorbidity). Data for 1000 HIV-negative controls will be obtained via a parallel cohort study, (Stop the Spread Ottawa (SSO), using similar time points and methods. Participants receiving ≥1 COVID-19 vaccine will attend 5 visits: pre-vaccination; 1 month following the first vaccine dose; and at 3, 6 and 12 months following the second vaccine dose. The primary endpoint will be the percentage of PLWH with COVID-19-specific antibodies at 6 months following the second vaccine dose. Humoral and cell-mediated immune responses, and the interplay between T cell phenotypes and inflammatory markers, will be described. Regression techniques will be used to compare COVID-19-specific immune responses to determine whether there are differences between the "unstable" (CD4<350) PLWH group, the stable PLWH cohort and the HIV-negative controls, adjusting for factors believed to be associated with immune response. Unadjusted analyses will reveal whether there are differences driving factors associated with group membership.

Ethics and Dissemination: Research ethics boards at all participating institutions have granted ethics approval for this study. Written informed consent will be obtained from all study participants

prior to enrolment. The findings will inform the design of future COVID-19 clinical trials, dosing strategies aimed to improve immune responses and guideline development for PLWH.

Trial registration: clinicaltrials.gov NCT04894448

Keywords: HIV; COVID-19; observational study; COVID-19 vaccines; vaccine immunogenicity

Strengths and Limitations of this study

- The largest and most comprehensive immunogenicity study in people living with HIV in Canada receiving COVID-19 vaccination
- Emphasis on recruiting participants frequently excluded from pharmaceutical company-sponsored trials and those most likely to have poor outcomes following COVID-19 infection (including individuals of older age, immune non-responders and persons with multimorbidity)
- Assays used will enable differentiation between individuals with immunity from natural COVID-19 infection *vs.* vaccine-induced immunity, in addition to detection of immunity towards key variants of concern (VOCs)
- Involvement of community members from study conception to protocol development and study implementation
- Limitations include relatively late study start, recruitment restricted to major urban centres and variations in timing between vaccine doses amongst participants

BACKGROUND

In Canada today, an estimated 67,000 people are living with HIV (PLWH), 30% of whom are immune non-responders,[1] defined as achieving undetectable HIV viral levels without robust CD4 T cell count recovery (<350 cells/mm^3). Even with fully suppressed viral load on antiretroviral therapy (ART), chronic HIV infection is characterized by a low-grade elevation in pro-inflammatory and procoagulant biomarkers linked with higher mortality[2-4]. Poor immunogenicity to common vaccines, including influenza[5], pneumococcal[6,7], meningococcal[6,7] and hepatitis A[8-10] and B vaccines,[11-13] is well-documented in PLWH with low CD4 T cell counts (<200 cells/mm^3) and unsuppressed viral loads[14-16]. PLWH face other intersecting vulnerabilities that increase their risk of SARS-CoV-2 acquisition and symptomatic/severe COVID-19; they commonly belong to low socioeconomic or racialized groups disproportionately affected by COVID-19 and have higher rates of risk factors for severe COVID-19 disease (e.g., multiple chronic comorbidities).[17-19] Yet, this priority population has been understudied in COVID-19 vaccine clinical trials.[3, 20] Most HIV seropositive participants enrolled in COVID-19 vaccine trials had normal CD4 T cell counts (>500

cells/mm^3) and few comorbidities.[21,22] As such, the immunogenicity results may not represent the wide spectrum of PLWH who are followed in Canadian centres today.

For the AstraZeneca/Oxford COVID-19 vaccine trial (ChAdOx1, n=160 PLWH) inclusion criteria specified younger age (<55 years old) and high CD4 T cell count (>350 cells/mm3) while excluding medical comorbidities (e.g., heart, kidney, liver, respiratory diseases etc.).[21] The data obtained from PLWH were not included in the primary publication.[21] The Moderna trial included HIV-positive participants (n=176 PLWH) with CD4 T cell count ≥350 cells/mm^3 and an undetectable HIV viral load within the past year.[23] COVID-19 infection developed in 11 PLWH who received placebo but none who received the Moderna vaccine. The Johnson and Johnson trial (n=1218 PLWH) included participants with "stable/well-controlled HIV infection" (defined as CD4 T cell counts ≥300 cells/μL within 6 months prior to screening and documented HIV viral load <50 copies/mL within 6 months prior to screening) but excluded participants with ongoing and progressive comorbidities associated with HIV infection.[24] COVID-19 infection developed in 2 vaccinated PLWH and 4 PLWH given placebo. The Novavax trial, conducted in South Africa, excluded PLWH with chronic cardiovascular disease, gastrointestinal disease, liver disease, renal

disease, endocrine disorder and neurological illness, as well as participants with very high body mass index (≥40 Kg/m2).[22] As reported by Vivek *et al.* (2021), efficacy of the NVX-CoV2373 COVID-19 vaccine against the B.1.351 variant was examined in 1,857 individuals in South Africa, of whom 30% (500 individuals) had HIV infection[25]. The vaccine efficacy estimate in baseline seronegative HIV-negative participants was 52.2% (95% confidence interval: −24.8 to 81.7). During the first 60 days of follow-up, the incidence of Covid-19 in HIV-negative placebo participants (5.3% [95% CI: 4.3 to 6.6) was comparable to the incidence in PLWH placebo participants with HIV (5.2% [95% CI: 3.6 to 7.2])[26]. Among HIV-negative participants, there were four and two cases of symptomatic Covid-19 among NVX-CoV2373 and placebo recipients, respectively (N<109 in each group)[26]. No cases were observed in the baseline HIV-positive population (N<33 in each group)[26]. Among 94% of participants without HIV, vaccine efficacy was 60.1%. The study was not powered to detect efficacy in the small population of PLWH[26].

While several other trials included PLWH, they excluded their data from primary publication[20,21,27]. In a recent report by Ruddy *et al.*(2021) which examined COVID-19 antibody response in 12 PLWH with a median of 21 days (interquartile range 17-27) following the first dose of mRNA vaccine (50% Moderna and 50% Pfizer), antibodies were detected in all participants although lower levels were observed in persons with lower CD4 T cell counts[26]. In this study, all

12 individuals were male, 8% were non-white, all had been on ART ≥6 months and 92% had an undetectable HIV viral load. Two individuals had a CD4 T cell count below 200 cell/mm^3.[26]

We lack robust data on vaccine immunogenicity and immune response durability in subpopulations of PLWH. No evidence is available on the durability of immunogenicity in PLWH beyond 3 months following vaccination. Since the hallmark of HIV infection is a reduced number and function of CD4 T cells, and cell-mediated immunity has emerged as a critical aspect of the COVID-19 immune response,[28,29,30] it is critical to characterize cellular immune and cytotoxic T cell responses to COVID-19 vaccination.[31-33].

Given that inflammatory markers may influence immune cell activation status and shift cell profiles towards either Th1 or Th2 responses, impacting vaccine-elicited immune response[34], understanding the interplay between immune activation and dysfunction is also important. To address this need, we are establishing a pan-Canadian prospective cohort of PLWH receiving COVID-19 vaccines to assess humoral and cellular immunogenicity and to describe the inflammatory milieu in this context. Safety and tolerability of COVID-19 vaccines in this cohort of PLWH will also be captured. Of note, COVID-19 vaccines currently approved for use in Canada include those manufactured by Pfizer, Moderna, AstraZeneca and Janssen (Johnston & Johnston) (https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/drugs-vaccines-treatments/authorization/list-drugs.html). Since the beginning of the vaccine roll-out Pfizer and Moderna vaccines were administered most often as they were the first

to gain approval by Health Canada. Although approved, due to concerns associated with cerebral venous sinus thrombosis and vaccine-induced immune thrombotic thrombocytopenia, use of the AstraZeneca COVID-19 vaccine has been restricted in some provinces.

Study Objectives:

Primary objective:

To evaluate the immunogenicity of COVID-19 vaccination in PLWH, by specific immunoglobulin G antibody enzyme-linked immunosorbent assay (ELISA), at 6 months following 2nd vaccine dose.

Secondary objectives:

1) To assess neutralization capacity of COVID-19-specific IgG at 6 months following 2nd vaccine dose.

2) To assess the durability of COVID-19-specific IgG response in PLWH at 12 months following vaccination.

3) To examine changes in the proportion and activation phenotype of CD4 T cells, CD8 T cells, B cells, natural killer cells and monocytes, including gene expression and cytokine production, pre- and post-COVID-19 vaccination at 6 months following 2nd vaccine dose.

4) To determine safety and tolerability of COVID-19 vaccines in PLWH, based on local or systemic adverse events following first or second injections.

Exploratory objectives:

1) To determine if subpopulations of PLWH respond differently to COVID-19 vaccination. Subpopulations of interest include, 1) PLWH >55 years of age; 2) immune non-responders (ART

treated, and fully suppressed HIV RNA (<40 copies/mL), but CD4 T cell counts below 350 cells/mm^3 and CD4/CD8 T cell ratio <0.75); 3) PLWH with multimorbidity (2 or more chronic diseases) and 4) PLWH "reference" participants (with CD4 T cells >350 cells/mm^3, suppressed viral load for at least 6 months, and have at most 1 comorbidity) *(Note: groups will not be mutually exclusive but will likely have overlapping characteristics)*

(2) To investigate if current COVID-19 vaccines elicit IgG that cross-recognize key COVID-19 variants of concern (VoC), and if this differs in PLWH compared to individuals without HIV

(3) To compare virus-specific T cell responses generated by COVID-19 vaccines in PLWH and compare results with HIV-negative populations

METHODS AND ANALYSIS

Study design

This study is a multi-centre prospective observational cohort study. Approximately four hundred (400) PLWH aged ≥16 years will be recruited from 4 sites in 3 Canadian provinces including 1) McGill University Health Centre/Jewish General Hospital (Montreal), 2) Ottawa Hospital Research Institute, 3) The University Health Network (Toronto), and 4) St. Paul's Hospital (Vancouver). These sites were selected since they are 4 of the largest HIV clinics in Canada and have established research infrastructures to support the recruitment, enrolment and follow-up of a high volume of diverse study participants. These sites also have strong track records for rapid enrollment of participants in CTN studies. Many sites provide HIV care for many clients who are visible minorities and multiple morbidities. This will enable our sites to recruit a study population representative of PLWH most likely to be impacted by detrimental COVID-19-related outcomes.[19]

Data for HIV-negative individuals will be obtained from the Stop the Spread Ottawa (SSO) cohort. Since we will not perform additional analyses on the samples of the SSO cohort then we can include all the participants in the SSO study and matching of HIV-negative and positive participants will not be required.

Determination of which vaccine is administered at any point in time and to which individuals is dictated by Canada's provincial governments, with input from the National Advisory Committee on Immunization (NACI), and is not influenced by study investigators or staff. We will include participants irrespective of the specific type of COVID-19 vaccine. Furthermore, the duration of the interval between 1st and 2nd doses time from when the vaccine was administered will not influence eligibility, since Canada has decided to extend dose intervals for all 2 dose vaccines to 4 months. However, the duration of interval between vaccine doses will be included as an outcome variable.

Methods: Participants, intervention and comparator and outcomes

Inclusion Criteria*:* 1) Age ≥16 years; 2) HIV-positive for HIV group, immunocompetent and generally in good health for the HIV-negative group; and 3) Receiving ≥1 dose of COVID-19 vaccine. Persons are still eligible to participate if they have already received one or two vaccine doses.

Exclusion Criteria: 1) Receipt of any blood product or immunoglobulin preparation within_1 month of vaccine administration and until study completion; 2) signs/symptoms of active COVID-19 at the time of enrollment; 3) for the HIV-negative group: immunocompromised state or on

immunosuppressant medications. Prior receipt of other vaccines ≤12 months or past COVID-19 infection are not exclusion criteria but will be recorded. Detectable HIV viral load on ART is not exclusionary.

The following groups of PLWH will be prioritized for study enrolment:

1. Older age (55 years and above). Older age is associated with immunosenescence and results in lower vaccine efficacy.[35-37] We have selected 55 as the specific age cut-off since PLWH tend to develop comorbidities at an earlier age.
2. Immune non-responders (CD4 T cell count <350 cells/mm^3, CD4/CD8 <0.75 with undetectable viral load for 1+ year). Immune non-responders may be at risk of more adverse COVID-19 related outcomes than HIV immune responders[38,39,40,41].
3. Multi-morbidity (defined as having ≥2 comorbidities). Comorbidities may include cardiovascular disease (CVD), co-infection, hypertension, dyslipidemia, diabetes, chronic obstructive pulmonary disease and obesity among PLWH[2,42-45] and factors that contribute to worse outcomes with COVID-19.[46]
4. HIV-positive "stable" or "reference" group. These persons will have undetectable HIV viral load for >6 months, CD4 T cell counts >350 cells/mm^3, and a maximum of 1 comorbidity. To capture the full spectrum of individuals in the HIV-negative group, we will include this HIV-positive "stable" group so that we can determine whether there are particular characteristics within PLWH which impact on immune response. In comparing the stable and unstable groups, we will be able to determine whether participants with low CD4 counts (<350cells/mm^3) differ in their response to vaccine from those with normal CD4 T cell counts while controlling for other characteristics. We aim to enroll HIV-

negative and HIV-positive "stable" individuals with overlapping characteristics (i.e., some should have multiple comorbidities) so that the groups are comparable.

General Methodology and participant timeline

Participants will attend 5 visits over 12 months: pre-vaccination; 1 month following first vaccine dose; and at 3, 6 and 12 months following the second vaccine dose **(Table 1)**. Each visit will last between 20-60 minutes.

Primary Endpoint: Percentage of PLWH with COVID-19-specific antibodies at 6 months following 2nd vaccine dose.

Secondary Endpoints: Percentage of individuals with 1) COVID-19 neutralization capacity at 6 months following 2nd vaccine dose and 2) COVID-19-specific antibodies at 12 months following 2nd vaccine dose. 3) Proportion and activation status of CD4 T cells, CD8 T cells, B cells, natural killer cells and monocytes, pre-vaccination and 6 months post 2nd vaccine dose.

Exploratory Endpoints: 1) COVID-19-specific antibodies at 6 months following 2nd vaccine dose, stratified by subpopulations. Critically, we will also assess the ability of vaccine-elicited antibodies to cross-recognize SARS-CoV-2 S-protein variants, including N501Y and/or E484K, using in-house assays.

Sample size

Our primary outcome is the proportion of individuals in each group who mount a satisfactory immune response, although the best marker of what constitutes a satisfactory immune response is unclear at the moment and the science is rapidly evolving. We initially defined a successful

immune response as a 4-fold relative rise in IgG production at 6 months.[47] We anticipated that 90% of HIV-negative individuals would mount an adequate IgG response at this timepoint[27,34], versus 70% of PLWH.[12,48] If 20% of the sample were to have the characteristic of interest or predictor variable (e.g., 20% with multimorbidities), enrolling 200 PLWH and 50 HIV-negative participants would provide sufficient statistical power (>80%) to detect a 20% difference in outcomes between groups whatever the exact proportions. This sample size was determined using the UCSF sample size calculator (https://data.ucsf.edu/research/sample-size) which uses the typical normal distribution assumption with the continuity correction as an approximation to the binomial distribution. In addition, we calculated that we would have >80% power to detect a 20% difference in outcome between those with suppressed CD4 count or unsuppressed viral load and the HIV- group. Previous studies of temporal differences in humoral and cellular responses to COVID-19 have shown differences between individuals when sample sizes included 100 participants or fewer.[49-53] We would also have >80% power to detect a 20% difference in outcome between the higher risk PLWH (ie, with CD4 counts <200 cells/mm^3 and/or unsuppressed HIV viral load) and the HIV-negative group. Such recruitment targets would also also provide a sufficient buffer to account for potential drop-outs of 5-10%. However, to increase our ability to detect differences in our primary outcome between the four sub-populations of interest (individuals of older age; immune non-responders; persons with multimorbidity; and an HIV-positive "stable" or "reference" group), we plan to recruit 400 PLWH and use data from the *entire* cohort of HIV-negative individuals in the SSO study (approximately 1000 individuals) to increase power. Inevitably, the higher risk groups will be overlapping, so we will recruit a minimum of 20% of the 400 PLWH per category. By using the entire cohort of HIV-negative individuals in the SSO study, we avoid the need to match PLWH and HIV-negative participants.

Recruitment

Participants will be informed about the study through a recruitment flyer during routine physician visits to their HIV clinic and via established recruitment strategies through our community partners and the CIHR Canadian HIV Trials Network (CTN) via webpages, email, and social media platforms. Individuals followed for routine HIV care at clinics other than the 4 enrolment sites are eligible to participate if they can come to the enrolment site for study visits. Participants will be compensated $40 per study visit to help offset the time commitment and parking fees. We will make a concerted effort, through the use of recruitment quotas, to ensure the HIV-negative and HIV-positive "stable" groups have overlapping characteristics (eg, age >55 years, CD4 count <350 cells/mm^3, comorbidities) so that the groups will be comparable.

Data Collection

Medical history and HIV history will be gathered from both patient interviews and clinic chart reviews following written informed consent. Information extracted will include comorbidities, year of HIV diagnosis, CD4 T cell nadir (if known) in addition to tobacco smoking and cannabis use history. Medications will be recorded in addition to the ART regimen. History of COVID-19 infection and date of confirmatory test will be recorded.

Sample Collection: At each visit, blood will be collected to isolate serum, plasma and peripheral blood mononuclear cells (PBMCs).

Humoral Immunity (SARS-CoV-2 Binding Antibodies): We will evaluate levels of immunoglobulins M and A (IgM, IgA) and IgG targeting the SARS-CoV-2 Spike (S) protein

receptor-binding domain (RBD) and nucleocapsid protein using a high-throughput automated ELISA co-developed and validated by Dr Marc-Andre Langlois,[54] thereby distinguishing vaccine-induced (S only) from infection-induced (S and nucleocapsid (N)) responses. We will also evaluate samples for IgM and IgG antibody cross-recognition of RBD derived from VoCs, including those harbouring *N501Y* and/or *E484K* (e.g., Alpha and Beta strains, respectively) using a commercial multiplex ELISA (V-Plex, Meso Scale Discovery). This assay is updated regularly by the manufacturer to accommodate emerging spike variants. We will test plasma samples for their capacity to block viral entry using a well-established neutralization assay based on retroviruses pseudotyped with the SARS-CoV-2 S protein.[49,51]

Cellular Immunity*:* Flow cytometry will be performed to enumerate CD4 T cells (including helper and regulatory subsets), CD8 T cells and other T cell subsets (including naïve, central memory, transitional memory, effector memory and terminally differentiated cells), B cells (including naïve, memory and antibody-secreting B cells), Natural Killer cells and monocytes (classical, inflammatory and non-classical). We will also evaluate markers of cellular immune activation, senescence and exhaustion. Following high-resolution Human Leukocyte Antigen class I/II typing, we will examine COVID-19-specific T cell responses using an activation-induced marker (AIM) assay. Briefly, PBMCs will be stimulated overnight with pools of SARS-CoV-2 S peptides. Activated CD4 and CD8 T cells will be quantified by flow cytometry-based expression of CD137, OX40 and/or CD69. Gene expression will be assessed by single-cell RNA sequencing of PBMC as described previously.[55] T and B cell epitope specificity will be confirmed using virus-derived antigens (peptide/HLA or RBD dextramers, respectively). Plasma levels of inflammatory markers including interferon (IFN)-y, interleukin (IL)-1β, IL-2, IL-4 IL-6, IL-8, IL-10, IL-13, IL-17, transforming growth factor (TGF)-β, IFN-y-induced protein-10 (IP-10), IL-12p70 and tumour

necrosis factor (TNF)-α will be measured using multiplex Luminex assays, D-dimer, C-reactive protein, and markers of microbial translocation lipopolysaccharide (LPS), beta-d-glucan (ßdG) and soluble CD14 will be evaluated by ELISA.[56]

Exploratory Safety and Tolerability of COVID-19 Vaccines in PLWH

In the prospective cohort, we will explore vaccine safety and efficacy to inform subsequent studies. Reactogenicity: ***Symptoms Diary***: Participants will be asked to document specific local and systemic reactions in a diary for 1 week and 1 month following each injection, as was done in Pfizer-BioNTech Phase 3 studies.[47] We will report the proportion of participants developing local (redness, pain or swelling at the injection site) or systemic effects (fatigue, headache, muscle pain, fever, joint pain, diarrhea) within 7-30 days following each vaccine dose with 95% confidence intervals (Supplementary information).

COVID-19 Questionnaires: We will administer the COVID-19 System Questionnaire to participants who develop a flu-like illness to confirm the illness, along with PCR-based tests for COVID-19. Participants will complete the COVID-19 Immunity Task Force (CITF) Standardized Core Survey Data Elements questionnaire prior to vaccination and a modified CITF questionnaire (minus the demographic information) at follow-up visits (Supplementary information).

If participants develop COVID-19 symptoms 14 days+ after vaccination, we will also collect CITF system questionnaire (length of illness and symptomatology, which vaccine was administered, number of vaccine doses received) and saliva specimens to enable study of COVID-19 variants of concern. (https://www.covid19immunitytaskforce.ca/covid-19-immunity-task-force-releases-standardized-core-survey-data-elements/)

Data management

The study sponsor, the CTN, will be responsible for national project management, database development, data management and data analysis. To facilitate data sharing, we will: 1) use standard encodings for CITF-defined Core Data Elements; 1) request immunogenicity study participants consent to data sharing as guided by the CITF, including collecting survey elements and saliva 14+ days after vaccination on symptomatic participants to determine whether the infecting strain is a COVID-19 VoC and 3) rapidly share interim data and all requested study metadata for cataloguing.

Confidentiality

All participant-related information, including Case Report Forms, laboratory specimens, evaluation forms and reports, will be kept strictly confidential. All records will be held in a secure, locked location and only accessible to research staff. Participants will be identified using a coded number specific to each participant. All computerized databases will identify participants by numeric codes only and will be password protected. Upon request, and in the presence of the investigator or his/her representative, participant data will be made available to the study sponsor, monitoring groups representative of the study sponsor, representatives of funding groups, and applicable regulatory agencies to verify clinical study procedures and/or data, as permissible by local regulations.

Statistical Analyses

We will use regression techniques to compare COVID-19-specific immune responses applying data transformation where necessary to conform with distributional assumptions in order to determine whether there are differences between the "unstable" (CD4<350) PLWH group, the stable PLWH cohort and the HIV- controls, taking into account factors that are believed to be associated with immune response. We will also perform unadjusted analyses to determine whether there are differences which may or not be driven by factors associated with group membership. We will report data from exploratory analyses with descriptive statistics and data for vaccines from different manufacturers separately and combined. We will stratify results by the number of doses received and the time interval between the two doses in case these factors drive response and differ between groups. We will stratify immunogenicity data by sex as females and males have differences in both vaccine-elicited immune responses[57] and adverse effects from vaccines.[58] Furthermore, we will stratify analyses by individuals who are naive to COVID-19 versus those with pre-existing antibodies as a result of prior COVID-19 infection. This will be important since antibody responses (particularly after the 1st dose) will be much higher in convalescent individuals,[59,60] so it is not appropriate to include them with individuals who do not have pre-existing antibodies to COVID-19.

ETHICS AND DISSEMINATION

Ethics approval and consent to participate

Written informed consent will be obtained from all study participants. The study will be conducted in accordance with the Declaration of Helsinki. At the time of initial manuscript submission (June 2021), a very closely related protocol had been approved by the University of British

Columbia/Providence Health Care Research Institute and Simon Fraser University Research Ethics Boards. The present protocol was later approved by the Research Institute of the McGill University Health Centre (2nd review), the Ottawa Hospital Research Ethics Board, the University of Toronto Research Ethics Board. Patient enrollment for this trial began June 2021. Both the protocol and informed forms were reviewed and approved by the CTN Community Advisory Committee.

Availability of data and materials

De-identified participant data will be stored on a secure password-protected RedCap database. Access to the database will be controlled by the CTN. Access to the final study database will be provided upon written reasonable request to the corresponding author/principal investigator following publication and CTN and CITF approval.

We will standardize reagents and analysis strategies, where possible, working with the Immune Sciences Network and Testing Working Party recommendations to enable data sharing and avoid duplication in consultation with other CIHR and CITF-funded vaccine surveillance projects. We will also contribute results to SeroTracker, a knowledge hub that tracks and synthesizes findings from SARS- CoV-2 serosurveillance efforts worldwide (https://www.covid19immunitytaskforce.ca/serotracker/). To inform COVID-19 immunization guidelines and future interventions for PLWH in Canada and internationally, we are committed to sharing results with all stakeholders and will adhere to Wellcome's *Sharing research data and*

findings relevant to the novel coronavirus (COVID-19) outbreak statement.[61] Data sharing agreements will be obtained between CTN sites to use data not sent to the CITF.

Knowledge Translation (KT) & Dissemination Plan

Serology results will be provided to individual study participants at the end of the study, along with a summary of study results and their implication in lay language. We prioritize meaningful community engagement and our Community Advisory Committee includes PLWH and representation from the Canadian Aboriginal AIDS Network. The Community Advisory Committee reviews protocols and informed consent forms and advises on community priorities. Our well-established relationship with CATIE, Canada's source for HIV information, will enhance KT. We will leverage our KT staff to share lay language updates via press releases, media coverage, website, e-newsletters and social media and undertake targeted KT activities to mobilize knowledge across our network and report results to senior policymakers via research summaries and policy briefs, community groups and participants via factsheets. Our team will publish manuscripts, contribute to guidelines, and present to stakeholders. A first manuscript outlining the results of the primary objective will be submitted for publication within 6 months of participants

completing the 6-month post 2nd vaccine dose study visit. A second manuscript outlining the durability results will be submitted for publication within 6 months of participants completing the 12-month post 2nd dose study visit. Data will also be shared with CTN members at the semiannual meetings and through conference abstracts.

Patient and Public Involvement

The CTN Community Advisory Committee (CAC) was involved in the peer review process of this study proposal and deemed that the research questions addressed were of very high priority to PLWH. The CAC's critiques of the initial proposal were taken into account in the revised proposal. Two members of the CAC (SM and EM) were involved in finalizing the study design, inclusion/exclusion criteria, outcome measures and monitoring plans and are formal study investigators and co-authors. Community consultants will receive financial compensation to recognize their time commitment and expertise.

DISCUSSION

Herein, we present the protocol for an observational cohort study to evaluate COVID-19 vaccine-elicited immunogenicity in PLWH, with a priority of determining immunogenicity in PLWH who are of older age, immune non-responders and those with multimorbidity. These 3 groups were

selected since they represent subpopulations most likely to experience poor outcomes following COVID-19 infection and have a weaker immune response to vaccination.

PLWH immune non-responders are at risk of more adverse COVID-19 related outcomes than HIV immune responders. In the study performed by Braunstein *et al*. (2021), PLWH with COVID-19 had a higher proportion of hospital admissions, intensive care unit (ICU) admission and death. Those who experienced these COVID-19-related outcomes had CD4 T cell counts below 500 cells/mm.[3,38] Similarly, the study by Dandachi *et al*. (2021) found that having CD4 T cell counts below 200 cells/mm^3 was associated with severe outcomes such as ICU admission, intubation and death.[39] Furthermore, a multicentre cohort study by Hoffman *et al.* showed that CD4 T cell counts <350 cells/mm^3 were associated with severe COVID-19 (adjusted OR 2.85, 95% CI 1.26-6.44).[40] However, in a group of patients with inborn errors of immunity, Kinoshita *et al.*(2021) they demonstrated robust T cell activity and humoral immunity against COVID-19 structural proteins in some patients with antibody deficiency,[62] underscoring the heterogeneity and complexity of immune response.

A major challenge with planning this study is the unprecedented, rapidly-changing nature of the COVID-19 pandemic and evolving scientific information. As a result, data on the optimal time points to assess immune responses post COVID-19 vaccination administration are rapidly changing, resulting in multiple adjustments in our protocol plans. Within Canada, the vaccination schedule is determined by provincial vaccination programs based on review of evidence, population risk factors and local infection rates, along with input from NACI. However, differences exist between provinces regarding to vaccine supply, eligibility criteria, type of vaccine

administered and time period between vaccine doses. We are mitigating these challenges by holding monthly meetings with teams to discuss these issues over the previous month, troubleshoot and adjust recruitment priorities accordingly for the upcoming months. Ideally, and under non-pandemic circumstances, we would establish, a priori, methods for analyzing data from single vs. two-dose vaccines. However, in the current context with uncertainty of vaccine supply and distribution, such detailed plans are impractical and will depend on the methods of vaccination used in the participants enrolling in this study. This statement holds true for other variables we will likely encounter, such as different dosing intervals amongst persons receiving two-dose vaccines.

The importance of advanced age in impaired vaccine-induced immunogenicity is well-documented. Due to a combination of disrupted posttranscriptional regulation, T cell receptor signalling, and metabolic function, older individuals demonstrate reduced quantity and functionality of T-cells[63,64]. When this balance is disrupted, T-cells exhibit shorter-lived effector phenotypes rather than memory or follicular helper T cells and vaccine-induced antibodies are less protective than in younger persons[63,64]. As the elderly are considered a priority vaccination group, many of them were eligible for vaccination in early 2021 in Canada, before this current study began, meaning that baseline plasma, serum and PBMCs will not be available for participants in this subpopulation of interest. Another drawback with starting this study in May 2021 is that we may miss other important groups of PLWH, including Indigenous persons, who were prioritized

as high-risk populations for immunization and were eligible to receive COVID-19 vaccines at a younger age than the general population.

Another major challenge with the planning of this study was the need to ensure an adequate sample size to meet the primary objective in the group as a whole, but also in important subpopulations of PLWH. Enrollment of such a large number of individuals, with follow-up until 12 months following the second vaccine dose, is very resource-intensive and requires dedicated study participants. We will provide individualized antibody results to participants at study completion to increase study engagement and prevent drop-outs. Furthermore, due to the need to rapidly enroll participants to match the pace of the COVID-19 vaccine rollout, we decided to use CTN study sites with large clinic volumes and proven capacity to recruit. However, these clinics are based in urban centres and, therefore, may follow individuals who differ systematically from PLWH who live in rural areas. PLWH who live in cities may be of higher socioeconomic status and consist of more men who have sex with men (MSM). Therefore, each site will need to make a concerted effort to recruit sufficient participants with other profiles. For this reason, we will employ a flexible recruitment strategy, whereby sites that can recruit the required participants with characteristics of interest more easily than other sites can help to make up for lower recruitment flexibility at other sites. As with many studies, recruitment of women living with HIV may be challenging. Due to our connection with other studies within the CTN and the help of our community advisory board, we are encouraging clinics with predominantly female clients to ensure they inform their female patients living with HIV about this study.

Currently, the immune correlate of protection against COVID-19 is undefined. T cell and B cell responses are usually used as surrogates of protection.[65] We decided to use a 4-fold rise from baseline in IgG production following second vaccine dose as the criteria for a successful vaccine response, as was indicated in the Pfizer study[47]. Data from the Pfizer study submitted to the Food and Drug Administration and published by Walsh *et al.,* report geometric mean titers that were compared to those from a human SARS-CoV-2 convalescent serum panel as a benchmark. Increased titers were expressed in logarithmic fold increases.[66] There are currently no national standards for presenting the serology data. Some groups prefer to report the raw signal values, whereas others normalize data as fold increases. Since individual baseline values will not be available for participants who have already received their first vaccine dose, one option is to use the cohort baseline data derived from non-vaccinated participants. Alternatively, we may opt to examine relative titers, or fold-changes, and therefore baseline data will not actually be required. Since science is continuously evolving, we will use the definition of a successful vaccine response which is most widely accepted at the time of publication.

The best methodology to match PLWH with the HIV-negative group remains unclear, and there is no optimal approach to match participants to controls. As discussed by Wong *et al*. (2014), the ideal comparison group would be individuals who are identical to HIV-negative adults in all aspects except HIV status[67]. As PLWH have distinct characteristics, traditional risk factors, lifestyle factors and socioeconomic factors compared to the general population, the general population may not be the ideal comparison group[67]. Differences include increased tobacco smoking[68], substance use[69] and comorbidities[70] amongst PLWH compared to the general population. However, PLWH also undergo more screening for age-related comorbidities due to

frequent contact with health care providers[67]. Since matching is challenging, we will avoid the need to match individuals entirely, by using a large existing dataset of over 1000 individuals without HIV infection. As previously mentioned, as long as we ensure there are 20% of individuals with the characteristics of interest in the HIV-negative and HIV-positive "stable" cohorts, we can compare groups while adjusting for these characteristics via regression.

The findings from this study will provide valuable insight into COVID-19-vaccine-induced immunogenicity in important sub-populations of PLWH. HIV-positive persons of older age, with CD4 counts <350 cells/mm^3 and multimorbidity were not included in the early clinical trials, yet are most likely to suffer from poor outcomes if infected with COVID-19. These findings will inform clinical guidelines and recommendations for PLWH and, in turn, reduce COVID-19-induced morbidity and mortality.

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Authors' contributions

Co-principal investigators of the study are CTC, CLC and AHA. CTC and CLC conceived the study, led the proposal and protocol development. CTC wrote the first draft of the manuscript. JS is the biostatistician who provided methodological expertise and performed sample size

calculations. All other authors, including ANB, JN, IK, SM, EM, MAO, CMK, DHST, SLW, MH, MWH and JBA contributed to protocol development, study design and development of the proposal. CTC, MAL, MAJ, MAO, MAB, and ZLB designed the laboratory evaluations. MAL will be responsible for studies on humoral immunity. MAJ will be responsible for flow cytometric studies to define proportions of immune cells and their subsets, while CTC will be responsible for cytokine assessment. MAB and ZLB will be responsible for RNA profiling. MAL and MAB will perform the analysis of antibodies to COVID-19 variants. MAO and MAB will perform analyses of T cell responses to vaccine immunogens. Markers of gut barrier damage, microbial translocation and CMV IgG titers will be performed by JPR. HLA typing will be performed as necessary for participants evaluated for T cell responses by MAO, ZLB and MAB. JS will oversee data analysis between groups and subgroup analyses. All authors critically reviewed and approved the final manuscript.

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in viral pathogenesis and immunity. ZLB holds a Scholar Award from the Michael Smith Foundation for Health Research. JPR holds a Lowenstein Chair in Hematology.

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Competing interests

The authors declare that they have no competing interests

Table 1: Visits and Procedure Schedule

Visit number	**1**[1] **(Screen)**	**Vaccine**	**2**	**Vaccine**	**3**[6]	**4**[6]	**5**[6]
Week Number	-12 to 0 weeeks	0	4 weeks		3 mo after dose 2	6 mo after dose 2	12 mo after dose 2
Window	-3 mo						
Inclusion/Exclusion	X		X				
Informed Consent	X						
Medical History	X						
Blood: Immunology[2]	X		X		X	X	X
Blood Draw: CD4/Viral Load[3]	X		X		X	X	X
Vaccination[4]		X		X			
Participant Diary[5]		X	X	X	X		
CITF [7] Questionnaire	X		X		X	X	X
Adverse Events[8]			X		X		
Concomitant Meds	X		X		X	X	X

[1]Screening assessment may be performed same day as vaccination but will be completed prior to vaccination.

[2]Immunoglobulin levels flow cytometry and cytokine secretion (immunogenicity measures); Will be collected at each visit. For participants who have already received a vaccine dose prior to study enrolment, "baseline" immunoglobulin levels (i.e., pre-vaccination) may not be available.

[3]Blood work such as CD4 and Viral load can be collected as part of standard of care

[4]Participants will receive the COVID-19 vaccine outside of the study per standard of care as part of their provincial immunization program.

[5]Participants will be given a printed diary after vaccination during which they will record their vaccine reactions, oral temperature and any febrile respiratory tract symptoms as well as general changes to health and medications. The diary will be evaluated up to 30 days following each injection.

[6]Visits 3, 4, 5 will be conducted at 3, 6, 12 months after dose 1 respectively, for COVID-19 vaccines administered as a one-dose schedule.

[7]The full CITF questionnaire will be completed at visit 1 and the modified CITF questionnaire will be completed at subsequent visits.

[8]Adverse events will only be collected at 7 and 30 days post vaccination and for participants who receive a vaccine while currently enrolled in the study (i.e. adverse events will not be collected retrospectively)

For participants who develop COVID19-symptoms 14+ days following vaccination.

Participants will be asked to complete the COVID-19 Symptom Survey (Supplementary materials) and to go for a COVID-19 test at their nearest test centre and notify the study

staff of their test result. If positive for COVID-19, the study staff will mail the participant 6 saliva collection kits by courier in order to collect information on SARS-CoV-2 variants

Participants who have already received 1 vaccine dose: These individuals may be enrolled in the study at any duration of time post first dose as long as the baseline blood drawn is before the second booster. Visits 1 and 2 will be combined.

Participants who have already received 2 vaccine doses: These individuals must be enrolled in the study within 3 months of their 2nd dose. Visits 1 and 3 will be combined and Visit 2 will not be required. Participants will follow-up at visits 4 and 5.

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CTN 328
HIV-COV

DATA COLLECTION WORKSHEET

Participant ID | | | | | | |

☐ **Vaccine 1**

☐ **Vaccine 2**

COVID-19 SYMPTOMS QUESTIONNAIRE

Have you experienced any of the following COVID-19 signs or symptoms?

Sign	Yes	No	If yes, provide date, time
Fever, chills			
Cough			
Shortness of breath			
Acute loss of smell or taste			
Fatigue			
Headache			
Muscle aches			
Nausea/Vomiting/Diarrhea			
General weakness			
Nasal congestion			
Sore throat			

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CTN 328
HIV-COV

DATA COLLECTION WORKSHEET

Participant ID | | | | | | |

☐ **Vaccine 1**

☐ **Vaccine 2**

PARTICIPANT DIARY

Following each injection of COVID-19 vaccine, please indicate whether you experienced any of the following, within 7 days and within 30 days, and indicate the severity. See the Other Signs or Symptoms worksheet for a guide to the severity levels.

	Date:	**Date:**	**Date:**	**Date:**	**Date:**	**Date:**	**Date:**	**Date:**	
Sign or Symptom	Day 0*	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Within 30 days
Pain at injection site									
Redness									
Swelling									
Lymphadenopathy/Axillary swelling and tenderness									
Fatigue									
Headache									
Muscle Pain									
Chills									
Joint pain									
Fever									
Diarrhea									
Nausea and/or Vomiting									

* Day 0 refers to day vaccine received, Day 1 is th**e *following day,*** and so forth

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CTN 328
HIV-COV

DATA COLLECTION WORKSHEET

Participant ID | | | | | | |

☐ **Vaccine 1**

☐ **Vaccine 2**

PARTICIPANT DIARY – Other Signs or Symptoms

Please use this form to record the intensity of signs or symptoms experienced on Days 8 -30, and any other signs or symptoms that are not listed on the chart.

For any sign or symptom, please record the intensity based on the following:

- **Grade 1 = Mild** = does not interfere with participant's usual daily function
- **Grade 2 = Moderate** = interferes to some extent with participant's usual function
- **Grade 3 = Severe** = interferes significantly with participant's usual function
- **Grade 4 = Life-threatening** = life-threatening consequences; urgent intervention indicated

Sign or Symptom	Date	Intensity Grade (1-4)	**Additional notes**-please describe as much as possible (i.e., duration of time the sign or symptom lasted, if you took any medications for it etc.)

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DATA COLLECTION WORKSHEET

Participant ID | | | | | | |

☐ **Vaccine 1**

☐ **Vaccine 2**

PARTICIPANT DIARY – Other Signs or Symptoms (continued)

Sign or Symptom	**Date**	**Intensity Grade (1-4)**	**Additional notes**-please describe as much as possible (i.e., duration of time the sign or symptom lasted, if you took any medications for it etc.)

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CTN 328: HIV-COV

CITF CDE BASELINE QUESTIONNAIRE

This version is only administered at Screening

Please answer all questions unless otherwise indicated

Participant ID: ___ ___ ___ ___ ___ ___

Section 1: Demographics

1. Date (DD-MMM-YYYY):

___ ___ / ___ ___ / ___ ___ ___ ___

2. What is your age?

___ ___ ___ YRS ___ ___ MO OR ☐ Prefer not to answer

3. What was your assigned sex at birth?

- ○ Male
- ○ Female
- ○ Prefer to self-describe (specify) _____
- ○ Prefer not to answer

4. What is your sex now?

- ○ Male
- ○ Female
- ○ Prefer to self-describe (specify) _____
- ○ Prefer not to answer

5. What is your gender (how do you currently self-identify)?

- ○ Male
- ○ Female
- ○ Non-binary, genderqueer, agender or a similar identity
- ○ Two-spirit
- ○ Prefer to self-describe (specify) _____
- ○ Prefer not to answer

6. Are you an Indigenous person originating from North America?

If NO or Prefer not to answer, please proceed to Q9

- ○ No
- ○ Yes
- ○ Prefer not to answer

7. Which of the following groups do you belong to? Please select all that apply.

Only answer if Q6 = YES

- ☐ First Nations
- ☐ Inuit
- ☐ Metis
- ☐ Non-status First Nations
- ☐ Other Indigenous (specify) _____
- ☐ Prefer not to answer

8. Do you live on reserve?

Only answer if Q7 = First Nations

- ○ Yes
- ○ No
- ○ Prefer not to answer

9. How would you describe your ethnicity or race? Please select all that apply.

If you are an Indigenous person and answered YES to Q6, select any other that apply.

- ☐ White
- ☐ South Asian
- ☐ Chinese
- ☐ Black
- ☐ Filipino
- ☐ Latin American
- ☐ Arab
- ☐ Southeast Asian
- ☐ West Asian
- ☐ Korean
- ☐ Japanese
- ☐ Prefer to self-describe (specify)

_____

- ☐ Prefer not to answer

10. What are the first three digits of your postal code?

___ ___ ___ OR ☐ Prefer not to answer

11. What is the highest level of education you have completed?

- ○ Less than high school graduation
- ○ High school graduation
- ○ Trade certificate, vocational school, or apprenticeship training
- ○ Non-university certificate or diploma from a community college, CEGEP
- ○ University Bachelor's degree
- ○ University graduate degree (Master's, Doctorate, etc.)
- ○ Prefer not to answer

12. How many people live in your household, including yourself?

_____ OR ☐ Prefer not to answer

13. How many bedrooms are in your household?

_____ OR ☐ Prefer not to answer

14. How many bathrooms are in your household?

_____ OR ☐ Prefer not to answer

Section 2: COVID-19

15. Do you think you have had COVID-19?

If NO or Prefer not to answer, please proceed to Q18

- ○ No
- ○ Yes
- ○ Prefer not to answer

16. Why do you think you have had COVID-19? Please select all that apply.

Only answer if Q15 = YES

- ☐ Symptom review online
- ☐ Symptom profile
- ☐ Nasal/throat test result
- ☐ Health care provider
- ☐ Contact with case
- ☐ Other (specify) _____
- ☐ Prefer not to answer

17. Were you hospitalized due to COVID-19?

Only answer if Q15 = YES

- ○ No
- ○ Yes
- ○ Prefer not to answer

18. Have you ever been tested for an active COVID-19 infection (using nasopharyngeal/throat swab, saliva, or gargle test)?

If NO or Prefer not to answer, please proceed to Q21

- ○ No
- ○ Yes
- ○ Prefer not to answer

19. If yes, how may times have you been tested?

Only answer if Q18 = YES

_____ OR ☐ Prefer not to answer

20.1 Answer the following questions about the **first COVID-19 test**, if applicable.

20.1.a What was the date of the **first** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

20.1.b What was the result of the **first** test?

- ○ Negative
- ○ Positive
- ○ Don't know

20.1.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

20.1.d If yes, what symptoms did you have?

Only answer if Q20.1.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

20.2 Answer the following questions about the **second COVID-19 test**, if applicable.

20.2.a What was the date of the **second** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

20.2.b What was the result of the **second** test?

- ○ Negative
- ○ Positive
- ○ Don't know

20.2.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

20.2.d If yes, what symptoms did you have?

Only answer if Q20.2.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

20.3 Answer the following questions about the **third COVID-19 test**, if applicable.

20.3.a What was the date of the **third** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

20.3.b What was the result of the **third** test?

- ○ Negative
- ○ Positive
- ○ Don't know

20.3.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

20.3.d If yes, what symptoms did you have?

Only answer if Q20.3.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

20.4.a Have you **tested positive** for COVID-19 (using nasopharyngeal, throat swab, saliva or gargle test) on a test that wasn't included the questions above (that is, on the **4th or later test**)?

If NO, please proceed to Q21

- ○ No
- ○ Yes

20.4.b If yes, what was the date the first time you tested positive?

Only answer if Q20.4.a = YES

___ ___ DD / ___ ___ MO ___ ___ ___ ___ YR

Section 3: Exposure

21.a Have you traveled outside of your home province since **January 2020**?

If NO, please proceed to Q23

- ○ No
- ○ Yes
- ○ Prefer not to answer

21.b If you think you had COVID, did you travel in the 6 months before your symptoms began?

Only answer if Q15 = YES

- ○ No
- ○ Yes
- ○ Prefer not to answer

22. What province(s)/territory(ies) or country(ies) did you travel to? Select all that apply.

Only answer if Q21.a or Q21.b = YES

- ☐ Alberta
- ☐ British Columbia
- ☐ Manitoba
- ☐ New Brunswick
- ☐ Newfoundland and Labrador
- ☐ Northwest Territories
- ☐ Nova Scotia
- ☐ Nunavut
- ☐ Ontario
- ☐ Prince Edward Island
- ☐ Quebec
- ☐ Saskatchewan
- ☐ Yukon

OR ☐ Prefer not to answer

List countries you travelled to (separated by a comma):

_____

_____

_____

_____

_____

23.a Do you do either paid or unpaid work in an environment where you work in close proximity to other people?

If NO or Prefer not to answer, please proceed to Q24

- ○ No
- ○ Yes
- ○ Prefer not to answer

23.b If yes, have you been working in any of the following occupations or worksites in the past year? Please select all that apply.

Only answer if Q23.a = YES

- ☐ Hospital or health care facility
- ☐ First responder (paramedic/firefighter/police officer)
- ☐ Childcare worker
- ☐ Correctional officer
- ☐ Teacher or other school staff
- ☐ Transit driver
- ☐ Food service industry
- ☐ Grocery store
- ☐ Pharmacy
- ☐ Hairdresser or barber
- ☐ Aesthetician
- ☐ Flight attendant
- ☐ Factor worker
- ☐ Other (specify)

_____

- ☐ Prefer not to answer

24.a How many times have you been in a gathering of 10 or more since **March 2020**?

_____ OR ☐ Prefer not to answer

24.b If you think you have had COVID, how many times were you in gatherings of more than 10 people in the 6 months before your symptoms began?

Only answer if Q15 = YES

_____ OR ☐ Prefer not to answer

Section 4: Health and Health Behaviours

25. Do you currently smoke tobacco?

- ○ No
- ○ Yes
- ○ Prefer not to answer

26. If yes, how often do you smoke tobacco?

Only answer if Q25 = YES

- ○ Less than daily
- ○ Daily

27. Do you currently use e-cigarettes (vape)?

- ○ No
- ○ Yes
- ○ Prefer not to answer

28. If yes, how often do you use e-cigarettes (vape)?

Only answer if Q27 = YES

- ○ Less than daily
- ○ Daily

29. Have you been diagnosed by a physician with any of the following chronic medical conditions? Please provide an answer for each condition.

		Yes	No	Don't Know	Prefer not to answer
a.	Hypertension				
b.	Diabetes				
c.	Asthma				
d.	Chronic Lung Disease				
e.	Chronic Heart Disease				
f.	Chronic Kidney Disease				
g.	Liver Disease				
h.	Cancer				
i.	Chronic Blood Disorder				
j.	Immune Suppressed				
k.	Chronic Neurological Disorder				

30. What is your current weight (circle units)?

_____ kg / lbs OR ☐ Prefer not to answer

31. What is your current height?

____ . ____ ____ m OR ____ ft ____ ____ in OR ☐ Prefer not to answer

32. Do you have a family physician/primary care provider?

- ○ No
- ○ Yes
- ○ Don't know
- ○ Prefer not to answer

33. Do you usually get a flu shot?

- ○ No
- ○ Yes
- ○ Prefer not to answer

34. Indicate if, or how often you have done the following since **March 2020**?

		Never	Rarely	Occasionally	Often	Always	Prefer not to answer
a.	Worn a mask in public places						
b.	Practiced physical distancing in public places						
c.	Avoided crowded places/gatherings						
d.	Avoided common greetings (such as a handshake or hug)						

		Never	Rarely	Occasionally	Often	Always	N/A	Prefer not to answer
e.	Limited contact with people at higher risk (e.g., an elderly relative)							

		No	Yes	N/A	Prefer not to answer
f.	Self-isolated because you thought you were infected with COVID-19				
g.	Self-quarantined because you may have been exposed to COVID-19, but did not show symptoms				

35. **If you think you have had COVID**, have you done the following in the **6 months before your symptoms began**? (indicate how often).

Only answer if Q15 = YES

		Never	Rarely	Occasionally	Often	Always	N/A	Prefer not to answer
a.	Worn a mask in public places							
b.	Practiced physical distancing in public places							
c.	Avoided crowded places/gatherings							
d.	Avoided common greetings (such as handshake or hug)							
e	Limited contact with people at higher risk (e.g., an elderly relative)							

		No	Yes	N/A	Prefer not to answer
f.	Self-isolated because you thought you were infected with COVID-19				
g.	Self-quarantined because you may have been exposed to COVID-19, but did not show symptoms				

Section 5: Vaccine

36. Have you been vaccinated against COVID-19? Answer YES if you have received at least one dose of the COVID-19 vaccine.

If NO or Prefer not to answer, proceed to Q43

- ○ No
- ○ Yes
- ○ Prefer not to answer

37. How many doses of the COVID-19 vaccine have you received so far?

Only answer if Q36 = YES

- ○ One
- ○ Two
- ○ More than two

38. When did you receive the **first dose** of the COVID-19 vaccine?

Only answer if Q36 = YES

___ ___ DD / ___ ___ MM / ___ ___ ___ ___ YR

39. When did you receive the **second dose** of the COVID-19 vaccine?

Only answer if Q37 = TWO or MORE THAN TWO

___ ___ DD / ___ ___ MM / ___ ___ ___ ___ YR

40. Which vaccine did you receive?

Only answer if Q36 = YES

- ○ Pfizer and BioNTech mRNA vaccine
- ○ Moderna mRNA vaccine
- ○ AstraZeneca Oxford vaccine
- ○ Other (specify) _____
- ○ Janssen (Johnson & Johnson) vaccine
- ○ Don't know
- ○ Prefer not to answer

41. Were you pregnant when you received the vaccine?

If NO or N/A, proceed to Q43

- ○ No
- ○ Yes
- ○ N/A

42. If yes, what trimester were you in when you received the vaccine?

Only answer if Q41 =YES

- ○ First
- ○ Second
- ○ Third

Section 6: Cannabis Use

43. Have you used cannabis (even once) in the past 12 months?

If NO, proceed to the end of this questionnaire

- ○ No
- ○ Yes

43.a If yes, how do you use cannabis? Please check all that apply.

Only answer if Q43 = YES

	Yes	No
Smoked dried plant		
Vaporized		
Oil		
Pills		
Added to baked good or other foods		
Other (specify)		

43.b If you smoke cannabis, please specify how you smoked/took cannabis:

☐ I do not smoke cannabis (proceed to Q44)

	Yes	No
Smoked as joint		
Smoked as joint mixed with tobacco		
Smoked as pipe		
Smoked as water pipe (bong)		
Inhaled using a vaporizer		
Eaten (e.g. as brownies, cake, cookies, etc.)		
Other (specify)		

44. Which response **best** describes how often you **currently** use cannabis?

Only answer if Q43 = YES

- ○ Rarely (2-3 times a year)
- ○ Monthly
- ○ Weekly
- ○ Daily
- ○ More than once a day

45. How many grams per week do you consume?

Only answer if Q43 = YES

- ○ Less than 1 gram
- ○ 1-5 grams
- ○ 6-9 grams
- ○ 10 or more grams
- ○ Unknown

46. If you smoke cannabis, on average how many joints/cigarettes do you smoke per day?

Only answer if Q43 = YES

_____ OR ☐ I do not smoke cannabis

END OF QUESTIONNAIRE

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CITF CDE FOLLOW-UP QUESTIONNAIRE

This version is administered at Visits 2, 3, 4, 5, and at unscheduled visits

Please answer all questions unless otherwise indicated

Participant ID: ___ ___ ___ ___ ___ ___

1. Date (DD-MMM-YYYY):

___ ___ / ___ ___ / ___ ___ ___ ___

Section 1: COVID-19

2. Do you think you have had COVID-19 since your last visit?

If NO or Prefer not to answer, please proceed to Q5

- ○ No
- ○ Yes
- ○ Prefer not to answer

3. Why do you think you have had COVID-19 since your last visit? Please select all that apply.

Only answer if Q2 = YES

- ☐ Symptom review online
- ☐ Symptom profile
- ☐ Nasal/throat test result
- ☐ Health care provider
- ☐ Contact with case
- ☐ Other (specify) _____
- ☐ Prefer not to answer

4. Since your last visit, have you been hospitalized due to COVID-19?

Only answer if Q2 = YES

- ○ No
- ○ Yes
- ○ Prefer not to answer

5. Since your last visit, have you been tested for an active COVID-19 infection (using nasopharyngeal/throat swab, saliva, or gargle test)?

If NO or Prefer not to answer, please proceed to Q8

- ○ No
- ○ Yes
- ○ Prefer not to answer

6. If yes, how may times have you been tested since your last visit?

Only answer if Q5 = YES

_____ OR ☐ Prefer not to answer

7.1 Answer the following questions about the **first COVID-19 test since your last visit**, if applicable.

7.1.a What was the date of the **first** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

7.1.b What was the result of the **first** test?

- ○ Negative
- ○ Positive
- ○ Don't know

7.1.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

7.1.d If yes, what symptoms did you have?

Only answer if Q7.1.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

7.2 Answer the following questions about the **second COVID-19 test since your last visit**, if applicable.

7.2.a What was the date of the **second** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

7.2.b What was the result of the **second** test?

- ○ Negative
- ○ Positive
- ○ Don't know

7.2.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

7.2.d If yes, what symptoms did you have?

Only answer if Q7.2.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

7.3 Answer the following questions about the **third COVID-19 test since your last visit**, if applicable.

7.3.a What was the date of the **third** test?

___ ___ DD / ___ ___ MO /___ ___ ___ ___ YR

7.3.b What was the result of the **third** test?

- ○ Negative
- ○ Positive
- ○ Don't know

7.3.c Did you have any symptoms of COVID when you had this test?

- ○ No
- ○ Yes
- ○ Don't know

7.3.d If yes, what symptoms did you have?

Only answer if Q7.3.c = YES

- ☐ Cough
- ☐ Fever
- ☐ Shortness of breath
- ☐ Sore muscles
- ☐ Headache
- ☐ Sore throat
- ☐ Diarrhea
- ☐ Decreased sense of smell or taste
- ☐ Other (specify) _____

7.4.a Have you **tested positive** for COVID-19 since your last visit on a test that wasn't included the questions above (that is, on the **4th or later test**)?

If NO, please proceed to Q8

- ○ No
- ○ Yes

7.4.b If yes, what was the date the first time you tested positive since your last visit?

Only answer if Q7.4.a = YES

___ ___ DD / ___ ___ MO ___ ___ ___ ___ YR

Section 2: Exposure

8. Have you traveled outside of your home province since your last visit?

- ○ No
- ○ Yes
- ○ Prefer not to answer

9.a Since your last visit, have you worked (either paid or unpaid) in an environment where you work in close proximity to other people?

If NO or Prefer not to answer, please proceed to Q10

- ○ No
- ○ Yes
- ○ Prefer not to answer

9.b If yes, have you been working in any of the following occupations or worksites since your last visit? Please select all that apply.

Only answer if Q9.a = YES

- ☐ Hospital or health care facility
- ☐ First responder (paramedic/firefighter/police officer)
- ☐ Childcare worker
- ☐ Correctional officer
- ☐ Teacher or other school staff
- ☐ Transit driver
- ☐ Food service industry
- ☐ Grocery store
- ☐ Pharmacy
- ☐ Hairdresser or barber
- ☐ Aesthetician
- ☐ Flight attendant
- ☐ Factor worker
- ☐ Other (specify) _____
- ☐ Prefer not to answer

10. How many times have you been in a gathering of 10 or more since your last visit?

_____ OR ☐ Prefer not to answer

Section 3: Vaccine

11. Have you been vaccinated against COVID-19 since your last visit? Answer YES if you have **received at least one dose of the COVID-19 vaccine since your last visit**.

If NO or Prefer not to answer, proceed to the end of this questionnaire

- ○ No
- ○ Yes
- ○ Prefer not to answer

12. How many doses of the COVID-19 vaccine have you received since your last visit?

Only answer if Q11 = YES

- ○ One
- ○ Two
- ○ More than two

13. When did you receive the **first dose** of the COVID-19 vaccine since your last visit?

Only answer if Q11= YES

___ ___ DD / ___ ___ MM / ___ ___ ___ ___ YR

14. Which vaccine did you receive for this **first dose** since your last visit?

Only answer if Q11= YES

- ○ Pfizer and BioNTech mRNA vaccine
- ○ Moderna mRNA vaccine
- ○ AstraZeneca Oxford vaccine
- ○ Other (specify) _____
- ○ Janssen (Johnson & Johnson) vaccine
- ○ Don't know
- ○ Prefer not to answer

15. Were you pregnant when you received this **first dose** since your last visit?

If NO or N/A, proceed to Q17

- ○ No
- ○ Yes
- ○ N/A

16. If yes, what trimester were you in when you received this **first dose** since your last visit?

Only answer if Q15 =YES

- ○ First
- ○ Second
- ○ Third

17. When did you receive the **second dose** of the COVID-19 vaccine since your last visit?

Only answer if Q12 = TWO or MORE THAN TWO

___ ___ DD / ___ ___ MM / ___ ___ ___ ___ YR

18. Which vaccine did you receive for this **second dose** since your last visit?

Only answer if Q12 = TWO or MORE THAN TWO

- ○ Pfizer and BioNTech mRNA vaccine
- ○ Moderna mRNA vaccine
- ○ AstraZeneca Oxford vaccine
- ○ Other (specify) _____
- ○ Janssen (Johnson & Johnson) vaccine
- ○ Don't know
- ○ Prefer not to answer

19. Were you pregnant when you received this **second dose** since your last visit?

If NO or N/A, proceed to the end of this questionnaire

- ○ No
- ○ Yes
- ○ N/A

20. If yes, what trimester were you in when you received this **second dose** since your last visit?

Only answer if Q19 =YES

- ○ First
- ○ Second
- ○ Third

END OF QUESTIONNAIRE

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-9
Objectives	3	State specific objectives, including any prespecified hypotheses	9-10
Methods			
Study design	4	Present key elements of study design early in the paper	10-11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10-15, Table 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	11-12
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A (No matching)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	13, 15-17
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11 (SSO cohort-same time points and methods). 12-13, 15-16,
Bias	9	Describe any efforts to address potential sources of bias	14-15, 22-26 (limitations discussed)
Study size	10	Explain how the study size was arrived at	14
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	19
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	14,19
		(*b*) Describe any methods used to examine subgroups and interactions	19

		(*c*) Explain how missing data were addressed	19
		(*d*) If applicable, explain how loss to follow-up was addressed	19
		(*e*) Describe any sensitivity analyses	19
Results			Protocol paper thus results not yet available
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	N/A protocol paper results not yet available
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22-26
Generalisability	21	Discuss the generalisability (external validity) of the study results	22-26
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on	32

		which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-9
Objectives	3	State specific objectives, including any prespecified hypotheses	9-10
Methods			
Study design	4	Present key elements of study design early in the paper	10-11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10-15, Table 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	11-12
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A (No matching)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	13, 15-17
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11 (SSO cohort-same time points and methods). 12-13, 15-16,
Bias	9	Describe any efforts to address potential sources of bias	14-15, 22-26 (limitations discussed)
Study size	10	Explain how the study size was arrived at	14
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