Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.

eAppendix. Study Design and Blood Sampling and Matching

The *COVID-19 Occupational Risks, Seroprevalence and Immunity among Paramedics in Canada* (CORSIP) study is a prospective observational cohort study which enrolled paramedics in Canada, starting in January of 2021. Enrolled participants completed sociodemographic and health questionnaires including vaccination status and history of nucleic acid amplification test (NAAT)-confirmed COVID-19 infections. Participants were asked to provide a blood sample at the time of enrollment, and 6-months after their first vaccine dose (if applicable). Enrolled participants received their first vaccine doses December 16, 2020 - February 22, 2021. "Documented COVID-19" was based on a positive NAAT or a reactive test on the Elecsys SARS-CoV-2 nucleocapsid test.

For the "Short vs. Medium" investigation we used samples collected from participants at the time of enrolment. To create groups with comparable time periods since the second vaccine, we matched samples based on the second vaccine-to-blood sample collection interval (days). Comparing outcomes based on the second vaccine-to-blood sample collection interval is a common approach[1,2] to account for short-term rises in antibody concentrations after vaccination[3] (however result in different intervals from the first dose to outcome ascertainment). In addition, we matched samples with the following hierarchy: vaccine type, age, sex, and comorbidities (including hypertension, diabetes, asthma, lung disease, heart disease, kidney disease, liver disease, cancer, hematological disease, and neurological disease).

For the "Short vs. Long" investigation, we used samples collected from participants at 6 months (180+/-10 days) post-first SARS-CoV-2 vaccine, allowing for comparison at a standardized time juncture from the start of the vaccine series.[4] As samples were collected at a standard vaccine-to blood sample collection interval, matching was not performed based on collection timing. We matched samples with the following hierarchy: vaccine type, age, sex, and comorbidities.

Samples were considered for matching (without knowledge of outcome results) in the order they were received and chosen to achieve the best matched groups.

Neutralization Antibody Testing Procedures

Live virus neutralizing antibody titers were measured against the Wuhan "wild type" SARS-COV-2/Canada/VIDO-01/2020 strain. This standard virus neutralization assay, as previously described,[5] was performed at the British Columbia Centre for Disease Control. Each serum specimen was heat inactivated at 56 °C for 30 minutes and duplicate serial 2-fold dilutions from 1:8 to 1:4096 were each incubated with 100 TCID50 of SARS-CoV-2 for 2 hours, then added to monolayers of Vero-E6 cells. Monolayers were examined after 72 hours for characteristic cytopathogenic effect (CPE). The inverse of the highest serum dilution to inhibit CPE was deemed the antibody titre.

Meso Scale Discovery Testing Procedures

Anti-S1 spike and Receptor-Binding Domain (RBD) antibody (IgG) concentrations were measured using the V-PLEX COVID-19 Coronavirus Panel 2 IgG assay (Meso Scale Discovery [MSD], Maryland, USA; #K15383U), reported as Arbitrary Units [AU/mL]). Inhibition of human ACE2 receptor binding onto viral RBD was measured for the Wuhan strain, and alpha (B.1.1.7), beta (B.1.351), delta (B.1.617.2), and gamma (P.1) variants, using the V-PLEX SARS-COV2 Panel 11 ACE2 kit (MSD, Maryland, USA; U/mL; #K15455U).

The MSD assay was performed as per the manufacturer's instructions, at the BC Children's Hospital Research Institute (Golding and Lavoie). Samples were tested at a 1:10,000 dilution, with the diluent provided by the manufacturer (Diluent 100).

Roche Testing Procedures

Anti-S1 spike was also measured with the Elecsys Anti-SARS-Cov-2 S assay (Roche, Indiana, USA; validated range of 0.4 - 2500 U/mL).[6] All plasma samples were also tested with the Elecsys Anti-SARS-CoV-2 nucleocapsid (Roche, USA) assay[7], an immunoassay for the in-vitro qualitative detection of nucleocapsid antibodies (including IgG) to SARS-CoV-2. Both tests were performed at the Canadian Blood Services national clinical laboratory.

The Roche assay was performed as per manufacturer's instructions, using the same lot of reagents. Samples with an anti-A1 spike concentration above the measuring range (250 U/ml) were diluted by the Roche analyzer with Diluent Universal at 1:10, as per manufacturer's instructions. After dilution by the analyzer, the software automatically utilized the dilution value when calculating the sample concentration, up to the maximum value of 2500 U/ml. Heat inactivation was not performed. For quality control, PreciControl Anti-SARS-CoV-2 was used. These were run at least once every 24 hours. Cut-offs were determined automatically by the analyzer software based on calibrated master curves. For calibration, the method is standardized against an internal Roche standard for anti-SARS-CoV-2 provided with the assay. A pre-defined master curve is adapted to the analyzer using the kit calibration reagents. Calibration is preformed once per reagent lot or following servicing of the analyzer.

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