clsa élcv
Canadian Longitudinal Study on Aging
Étude longitudinale canadienne sur le vieillissement

Data Support Document
SARS-CoV-2 Antibodies

1.0 Purpose and Scope

This document provides information on the collection, handling, and processing of samples along with the analytical methods for SARS-CoV-2 antibodies (N and S type).

The samples were collected as part of the CLSA COVID-19 Seroprevalence Study. The purpose of this study was to determine how widespread coronavirus infection is among participants of the CLSA across Canada.

Participants were recruited between November 2020 and July 2021 according to the flowchart described in **Figure 1**.

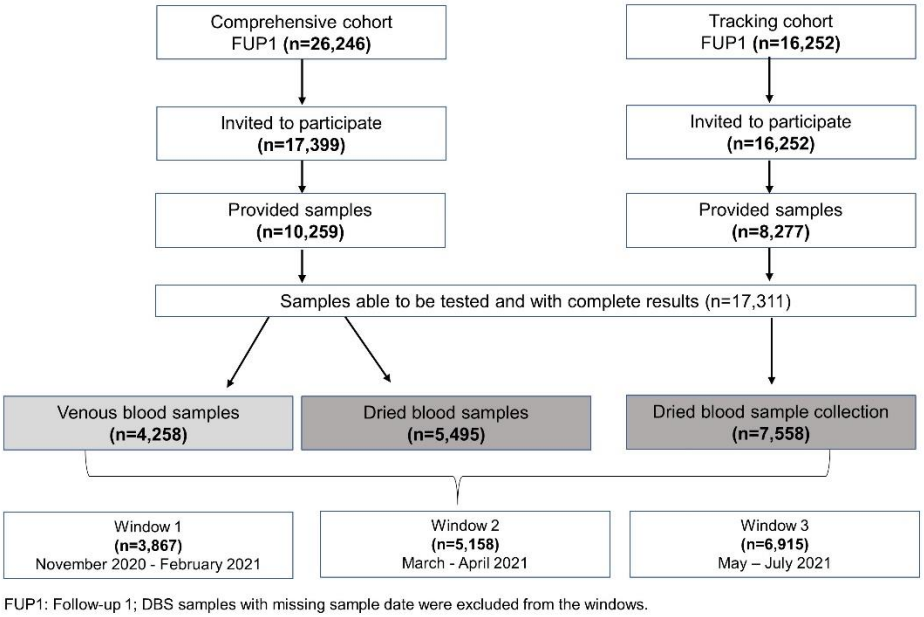

Figure 1: Overview of CLSA comprehensive and tracking participants who participated in the CLSA COVID-19 Seroprevalence Study.

2.0 Instruments and Methodology

2.1 Data Collection

Sample collection

Participants had blood collection by one of two methods, self-collection at home or by venipuncture in a CLSA Data Collection Site (DCS). (**Table 1)**

Table 1: Collection, processing, and storage

	Capillary Blood	Venous Blood
Location	At-home. *Participants were sent a blood collection kit via FedEx or Canada Post*	CLSA DCS.
Collection	One blood collection kit (Velvet™, Weavr Health).	One 10 mL vacutainer containing lithium heparin, 90 USP.
Shipping	Kits were returned by the participant by pre-paid FedEx or Canada Post envelopes to the Biorepository and Bioanalysis Centre (BBC). *Stored at -80˚C freezers until processed (1-198 days).*	N/A
Processing	Plasma was extracted from the plasma portion of the paper substrate. Briefly, the paper was placed into a well of a 12-well plate along with 300 µL of 0.1% BSA and incubated on an orbital shaker set at 350 rpm for one hour at room temperature. The plates were then centrifuged for two minutes at 168 x *g*. The extract, at least 200 µL, was transferred to a false bottom tube.	Samples were processed in the DCS within two hours of collection. Briefly, vacutainers were centrifuged for 10 minutes at 2000 x *g* at room temperature and 500 µL plasma aliquoted into cryovials.
Storage	Extracted material was stored at -80˚C.	Cryovials were stored in the DCS at -80˚C until shipped in cryoshippers to the BBC where they were stored at -196ºC.

Analysis

Sample analysis occurred from May to September 2021. **Table 2** provides assay descriptions.

Table 2: SARS-CoV-2 antibody assays

Assays	**Elecsys Anti-SARS-CoV-2** Qualitative detection of antibodies (including IgG) to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) nucleocapsid (N) antigen (Nucleocapsid Antibody). **Elecsys Anti-SARS-CoV-2 S** Quantitative determination of antibodies to SARS-CoV-2 spike (S) protein-binding domain (RBD) antigen (Spike Antibody)

Instrument	Roche Cobas 8000 series Modular Analyzer (module e 602) *Alberta Precision Laboratories, Calgary AB*
Test principle	Double-Antigen Electrochemiluminescence Immunoassay (ECLIA) Antibody in the sample forms a sandwich complex with two SARS-CoV-2-specific recombinant antigens, one is biotinylated and the other is labelled with a ruthenium complex. The complex binds to streptavidin-coated magnetic microparticles. The sample is transferred to a measuring cell where the magnetic particles bind to the surface of an electrode and unbound substances removed. The chemiluminescent emission is produced after the application of a voltage to the electrode which is measured by a photomultiplier.

Related Documents: *available upon request*

COV_BCP_0001	Collection of Blood by Venipuncture for COVID-19 Seroprevalence Study Participants
BIO - SOP – 3061	Reception and Storage of DBS Kits
BIO - FORM – 2787	Receiving and storing the DBS Kits Quiz
GEN - IFU – 7023	Blood Collection Device – Instructions for Use
LAB - SOP – 3304	Processing of Velvet Blood Collection Device
LAB - FORM – 3290	Processing Velvet Competency Quiz

Cut-off indices

The cut-off indices (COI) for classifying a result as positive or negative for the presence of an antibody was determined for each combination of assay type and sample type. These were calculated using the “cut-off” package in RStudio 4.1.0. The package fits a finite mixture model to bimodal data with Expectation-Maximization algorithm and derives a cut-off value that separates the two peaks for a given type-I error from the fitted mixture model that uses continuous distributions from the exponential family. Values greater than or equal to these derived cut-off values is defined as positive (**Table 3**).

The data collected from the first group of Heparin Plasma participants (n=2,741) was used to calculate COI for Anti-SARS-CoV-2 assay. The same COI value was obtained for the data from the heparin plasma participants whose samples were tested on/after August 9, 2021 (n=879).

Two different COI values were calculated for the dried blood sample (DBS) Anti-SARS-CoV-2 assay: (a) The data collected from the first group of DBS participants (n=2,440) was used to calculate COI for Anti-SARS-CoV-2 results; (b) A reagent lot change was made August 9, 2021 which resulted in a shift of values requiring a second COI value to be calculated for DBS samples tested on/after August 9, 2021 (n=2,305).

For both Heparin Plasma and DBS Anti-SARS-CoV-2 S assays, the same COI (the lowest level of test results) was used.

Table 3: Cut-off indices (COI) for the Elecsys Anti-SARS-CoV assays

	Heparin Plasma COI		DBS COI	
	Anti-SARS-CoV-2	Anti-SARS-CoV-2 S	Anti-SARS-CoV-2	Anti-SARS-CoV-2 S
Roche	≥ 1.0	≥ 0.8	N/A	N/A
Calculated	≥ 0.103	≥ 0.4	≥ 0.155 ≥ 0.353[1]	≥ 0.4

COI: Cut-off index, N/A: not available from the manufacturer.
[1] COI for tests performed post-reagent lot change on August 9, 2021.

2.2 Data Cleaning

Participant selection

At the start of the CLSA COVID-19 Seroprevalence Study, there were 42,498 active CLSA participants (26,246 Comprehensive and 16,252 Tracking) available to recruit. A stratified random sample of 33,651 were selected by age and province for the Tracking cohort, and by age for the Comprehensive cohort (**Figure 2**). Participants were contacted in a predefined random order by e-mail if they had an active e-mail address, or by telephone. If they were interested, they completed a consent questionnaire to assess their safety for venipuncture or self-collection by sampling capillary blood. Venipuncture collection occurred at DCSs if pandemic restrictions allowed. If a DCS site was not able to see participants in-person because of restrictions, they were sent a DBS kit for blood collection.

Test results

A total of 17,610 blood collection kits were sent to 16,707 participants who consented to participate in the study. The discrepancy between the number of sent kits and the number of participants consented occurred as some of the participants received more than one kit for various reasons (e.g., lost kit, insufficient sample). There were 14,776 kits (88.4%) returned by participants to BBC and of these, plasma was extracted from 13,865 (93.8%) with results for both antibodies available for 13,397 samples (96.6%).

There were 4,270 heparin plasma samples collected of which 4,258 (99.7%) had results for both antibodies.

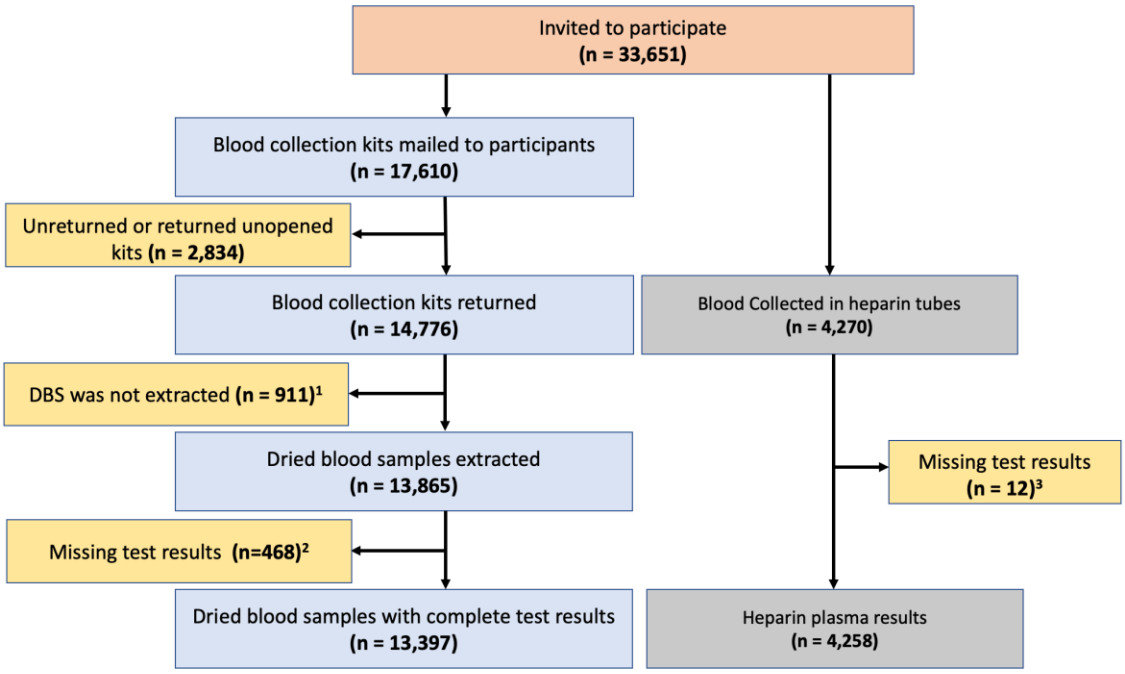

Figure 2: Summary of the number of participants with available COVID-19 antibodies data.

[1] DBS was not extracted from 911 kits due to duplicates (participants received more than one kit).
[2] Missing DBS test results were due to incomplete test results (Nucleocapsid or Spike result only), duplicate extracted samples, results were not imported to LIMS labware, insufficient quantity of the samples, results were not received from the testing laboratory, the participant did not consent for DBS, or results were cancelled due to data entry error.
[3] Missing heparin plasma test results were due to cancelled samples, incomplete test results (Nucleocapsid result only) or results not received from the testing laboratory.

3.0 Quality Controls and Assessment

3.1 Internal Quality Control

Quality control material, non-reactive (negative) and reactive (positive), was run each day for each assay. The instrument calculated COI results were interpreted relative to the manufacturer's COI (**Table 2**) as pass or fail. There were no failures. The signal generated by the instrument is expressed as a relative light unit (RLU). The mean and coefficient of variation (CV) of the RLU values from the quality control material are summarized in **Table 4**.

Table 4: Summary of internal quality control data

Test	Unit	Internal quality control material	Negative		Positive	
			Mean	CV (%)	Mean	CV (%)
Anti-SARS-CoV-2	RLU	Roche PreciControl Anti-SARS-CoV-2	0.107	14	2.8	7.8
Anti-SARS-CoV-2 S	RLU	Roche PreciControl Anti-SARS-CoV-2 S	0.39	38	7.8	2.8

RLU: Relative light unit
CV: Coefficient of variation

3.2 External Quality Assessment

Alberta Precision Laboratories (APL) participated in the new College of American Pathologists (CAP) SARS-CoV-2 serology survey. This survey was created for assays that detect antibodies to nucleocapsid and spike proteins, combined antigens (nucleocapsid and spike), and the receptor binding domain of the spike protein. Each survey includes three serum samples and is conducted twice a year. The Anti-SARS-CoV-2 assay is assessed qualitatively as detected, intermediate/borderline, or not detected. All samples tested by APL agreed with the intended response. The Anti-SARS-CoV-2 S assay is assessed quantitatively by each laboratory relative to the assay group mean and SD. All samples tested by APL were within ± 1.0 SDI.

4.0 Availability of Additional Information

Information was collected relative to each sample to monitor tracking and characterize the environment for quality purposes. These data are available upon request (**Table 5**).

Table 5: Meta-data

Note: Row order in sequence of events.

Type	Category	Description
DBS		
Tracking	Date	Kit sent to participant
Tracking	Date and Time	Blood collected
Tracking	Date	Kit returned to BBC
Environmental	Temperature	Device storage at BBC
Tracking	Date and Time	Storage at BBC
Tracking	Date and Time	Retrieval from storage
Tracking	Date and Time	Extraction
Environmental	Temperature	Storage at BBC
Tracking	Date and Time	Shipped to APL
Environmental	Temperature	Cryoshipper
Tracking	Date and Time	Arrival at APL
Tracking	Date and Time	Analysis
Heparin plasma		
Tracking	Date and Time	Blood collection

Type	Category	Description
Tracking	Date and Time	Blood processing
Environmental	Temperature	Collection and processing
Environmental	Humidity	Collection and processing
Environmental	Temperature	Storage at DCS
Tracking	Date and Time	Shipped to BBC
Environmental	Temperature	Cryoshipper
Tracking	Date and Time	Arrival at BBC
Environmental	Temperature	Storage at BBC
Tracking	Time	Storage at BBC
Tracking	Date and Time	Shipped to APL
Environmental	Temperature	Cryoshipper
Tracking	Date and Time	Arrival at APL
Analysis	Date and Time	Test performed at APL

5.0 Use by Researchers

The conditions of use are described in the CLSA Access Agreement/Data and Biospecimen Access Publication and Promotion Policy.