Assessing methods to adjust for assay differences across COVID-19 serological surveillance studies in Canada

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**Corresponding author:**

**Key words:**

**Running title:**

# Abstract

**Background:** A

**Methods:** A

**Results:** A

**Conclusions:** A

# Introduction

Test of citation: [1]. See [Figure 1](#fig-sample), [Table S 1](#cor-sample), [Table 1](#thm-sample), [Table 2](#thm-data), and [Figure S 1](#lem-sample).

[Be concise in this section]

[The Covid-19 pandemic overview]: Brief, general intro on Covid-19. Serosurveillance programs in Canada, and its importance. Use previous serosurveillence studies as examples.

[Two types of antibodies to be detected by assays]: Anti-spike and anti-nucleocapsid. Why we treated them differently in data analysis, one for quantitative and anothor one for qualitative test results.

* Anti-S (humoral response to infection or vaccination) and anti-N (marker of natural infection)
* Anti-S level elevates after infection and can be boosted by vax, while anti-N level elevates with infection (even drops) but didn’t change so much with vax.
* Anti-S level wanes slower than anti-N level after infection

[Ab level and immunity]: Previous studies have found an association between seropositivity/ab levels and intensity of immunity (humoral + cellular). Declining ab level suggesting waning of immunity against SARS-COV-2 after infection and immunization.

[Our measurement and adjustment]: Seropravalence and rolling incidence, why we need to employ RG equation or Bayesian model to estimated seropravalence, and why we also need probabilistic models to predict past infection using early 2020 and pre-Covid neg ref data.

# Methods

## Dataset

To estimate the SARS-CoV-2 seroprevalence and rolling incidence in Alberta, we used data from the Alberta Outpatient Lab (APL) and Canadian Blood Services (CBS). Alberta Health Services (AHS) tested >160,000 APL blood draws for SARS-CoV-2 anti-N and anti-S antibodies from June 2020 until October 2022. CBS has tested >450,000 blood donations for both antibodies levels since March 2020. The CBS Alberta subset has tested 124,008 samples in the same data collection period as CBS. Besides of assay results, both datasets include demographic variables such as donor’s sex, self-reported race/ethnicity, age, and Forward Sortation Area (FSA) codes. From their FSA codes, neighborhood material/social deprivation and urban/rural indicator have been derived.

Two datasets have different data collection time frames. The CBS dataset has the most extended period, ranging from December 2020 to February 2023. Meanwhile, APL’s data collection spans from June 2020 to October 2022. However, the anti-N assay results are only available starting from December 2021 in the APL data. [Table 2](#thm-data) summarized the differences among two datasets.

[Table 2](#thm-data) CBS and APL

[insert 2](#thm-data)

Regarding the data we used to train the regression models, we identified several studies conducted in the early phases of the Covid-19 pandemic. Blood samples are collected from France, Jamaica, Japan and UK [**add citation**], with known sample positivity at the time of collection (more details are available at the supplemental methods section). The samples in Jamaica, Japan and UK were tested both for Abbott anti-N and Roche anti-N assay, which makes them suitable for transformation model training.

## **Seroprevalence adjusted by RG and Bayesian models**

To evaluate the severity of Covid-19 pandemic overtime in Alberta province, first we calculated the anti-N assay test positivity. Then, to bridge the gap between test positivity and population-level seroprevalence, we employed the adjustment using Rogen-Gladen (RG) equation and Bayesian models. We conducted analysis separately for CBS and APL data then presented the results in figure X ([Figure 2](#fig-seroprevalence)).

Rogan Gladen equation:

Where as “Prevalence” indicate RG adjusted seroprevalence estimates. “Test Positivity” indicates the proportion of test samples with positive results. “Spec” and “Sens” indicate the specificity and sensitivity of the assay, respectively.

Bayesian models adjusting for sensitivity:

[Bayesian models adjusting for waning sensitivity]: Equation (above) and what each factor indicates (What model input and output. What covariates we’ve adjusted for…).

## **Seroprevalence based on raw/transformed data**

Following seroprevalence estimation, we further estimated monthly rolling incidence proportion of SARS-CoV-2 infection. Using matched data from samples tested with both assays early in the pandemic, we developed log-linear regression models to transform CBS Roche qualitative results to Abbott and applied the Abbott cutoff index and made the opposite transformation to convert APL to Roche. The seroprevalence estimates based on raw and transformed data are presented in figure X ([Figure 3](#fig-transformed)).

## **Rolling Incidence**

Next, we developed multinomial logistic regression models using data collected in the early pandemic to estimate the probability a tested sample was tested 0-3, 4-6, 7-9, or >9 months from infection based on the qualitative anti-N assay results. Then we applied this model to the CBS and APL datasets to estimate rolling incidence from 2021 to 2023.

We presented the rolling incidence proportion estimates in a paneled figure ([Figure 4](#fig-rolling-incidence)), displaying regression-based estimation of proportion of population infected in the last ever or in the past 3, 6, or 9 months.

## Attack rates - TBD

The cumulative attack rates is no longer of interest, as the majority of the Canadian population has been infected at least once since the Omicron wave. As the comparison rolling incidence proportion is a measurement with more meaningful public health implications.

## Mixture Models - TBD

In mixture models, we assume the dist of certain variables (in our case it is S/Co of Anti-N/S assay results) has two (or maybe three) distinct (though partially overlapping) distributions. Mixture models were utilized by previous studies to estimate cumulative incidence.

<https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1008728>

<https://www.sciencedirect.com/science/article/pii/S175543652200024X>

## **NT50 and corresponding antibody level:**

In evaluating whether participants possess humoral immunity levels sufficient to shield them from future infections, from either vaccination or infection, we applied a threshold of protection to the quantitative Anti-S assay results. The threshold is cited from the package insert of each assays [Roche and Abbott], indicating sufficient antibody level to neutralize over 50% virus, with certain times of dilution (1:20). We also presented the fluctuation of the proportion sufficiently protected over time in another figure ([Figure 5](#fig-immunity)), and we conducted sensitivity analyses in which we compared alternative threshold levels. The details of the sensitivity analysis is presented in the **Fig**. Y (haven’t scratched this one yet).

# Results

[Describe fig: seroprevalence] A paragraph about test sample positivity, Rogen-Gladen adjusted seroprevalence and Bayesian model adjusted seroprevalence. Describe the trend of changing overtime, difference and consistency among different adjusting methos, regions and datasets. (Cite figures accordingly if necessary.)The datasets had similar seropositivity earlier in the pandemic but diverged after May 2022 due to greater waning sensitivity in the Abbott assay used by APL (Figure X).RG and Bayesian adjustments had minimal impact, increasing sero-prevalence estimates by 2.04% for the CBS Alberta subset and 7.76% for APL (Figure X).

[Describe fig: seroprevalence based on raw/transformed data] Our regression-based approaches increased concordance between estimated seropositivity (Figure X). However, we still observed divergence after May 2022.The gap between CBS and APL seropositivity estimates for October 2022 was 48% using raw seropositivity, 15% after transforming CBS to Abbott, and 14% after transforming APL to Roche.

[Describe fig: rolling inc prop] In the rolling incidence estimates, we obderved more similar estimates of rolling incidence in each panels. The difference on rolling incidence estimates ar October 2022 are X%, X%, X% and X%, for ever infected %, 3-month, 6-month, and 9-month rolling incidence, respectively.

[Describe fig: NT50 and corresponding Ab level] A paragraph about estimated proportion with sufficient humoral immunity to protect them from future possible SARS-CoV-2 infection. This proportion is also adjusted by Rogen-Gladen equation and Bayesian models.

# Discussion

The gap between Rogen-Gladen adjusted sero-prevalence and unadjused sero-positivity: In our case, the sensitivity is where most variance come from. As the proof of excellent assay performance, assay manufacturer published the sensitivity and specificity of their assay. However, their test performance figures derived from hospitalized patients with more severe symptoms, who were samples at the time when sero antibody level reached its highest level. Due to the representativeness issue, it is not recommended only take the test performance figures published by the assays manufacturer into consideration.

Since sens is imperfect (0.85–1.0) and specificity is mostly 100% trustworthy (~1.0), the R-G equation can be simplified as:

According to the equation above, the worse the sensitivity (smaller in value), the larger the gap between the adjusted and unadjusted. We found larger gaps among the Abbott assay results, as the sensitivity we used for Abbott assays are smaller, when compared to sensitivity we used for Roche assays.

[Difference/Consistence among adjusting methods, regions in Canada] Is our finding consistent with previous studies?]

[Strength and Limitation] In our sensitivity analysis, we found the regression models used for Roche–>Abbott transformation is sensitive to the ratio of positive vs negative cases in the training data.

[Public health implications]

1.      Without any adjustment, the public health inference on test positivity is very limited. It only estimates the proportion of test positive results among blood samples collected in the serosurveillance study.

2.      Serosurveillance estimates are sensitive to choice of assay, and the most common adjustment methods (RG) do little to make estimates more concordant, particularly later in the pandemic when differences in waning sensitivity become important.

3.

2.      Adjusting for certain factors will change the public health inference, meanwhile it will also improve its generalizability. When the imperfect nature of the seroassays is taken into account, the adjusted proportion of positive test results (seroprevalence) will be able to represent the target population in our target population, from which our sample has been collected. Upon the seroprevalence, after adjusting for seroreversion using Bayesian models, we get attack rates of SARS-CoV-2, which estimates the proportion of population infected by the virus since the beginning of pandemic.

3.      There’s some differences among all those different quantitative measurements, and also they have different public health inference. Future researchers should be cautious when making references to those different measurements.

Future studies should… When using matched data to train regression models for assay results transformation, negative samples should be down-weighted if the proportion of negative cases is unbalanced high.

# Declarations

**Funding:** A

**Conflicts:** A

**Ethics/Consent:** A

**Data and materials:** A

**Code availability:** A

**Authors’ contributions: A**

# References

1. Langham S, Wright A, Kenworthy J, Grieve R, Dunlop WCN. Cost-effectiveness of take-home naloxone for the prevention of overdose fatalities among heroin users in the United Kingdom. *Value in Health*. 2018;21(4):407-415. doi:[10.1016/j.jval.2017.07.014](https://doi.org/10.1016/j.jval.2017.07.014)

# Tables

**Table 1** This is a sample table

| **col1** | **col2** |
| --- | --- |
| **A** | |
| 1 | Yes |
| 2 | Yes |
| **B** | |
| 1 | No |
| 2 | No |

**Table 2** The table below displays the distribution of age groups and gender across all three datasets. It also details the provinces where the data was collected, along with the corresponding data collection periods.

| **...1** | **CBS** | **CANPATH** | **APL** |
| --- | --- | --- | --- |
| character | character | character | character |
|  | N (%) | N (%) | N (%) |
| **Age Groups** |  |  |  |
| 18-35 | 200 (33.33%) |  |  |
| 36-64 | 201 (33.33%) |  |  |
| 65+ | 202 (33.33%) |  |  |
| **Gender** |  |  |  |
| Male |  |  |  |
| Female |  |  |  |
| **Provinces** |  |  |  |
| Data Collecting Period |  |  |  |
| n: 10 | | | |

# Figures

|  |
| --- |
| **Figure** 1: Figure caption here. |

|  |
| --- |
| **Figure** 2: Figure 1 presents the raw and Rogen Gladen-adjusted seroprevalence estimated from the APL and CBS data with 95% credible intervals. The calculations of seropositivity, seroprevalence are based on the qualitative results of the anti-N assay. |

|  |
| --- |
| **Figure** 3: Figure 2 depicts raw seroprevalence and seroprevalence transformed from Roche to Abbott (APL) and Abbott to Roche (APL) using a log-linear regression model. |

|  |
| --- |
| **Figure** 4: Figure 3 presents regression-based estimate of rolling incidence (proportion of population infected in the last ever or in the past 3, 6, or 9 months). |

|  |
| --- |
| **Figure** 5: The figure panel above depicts the temporal fluctuations in quantitative anti-S assay results, grouped by province and data source. Two cutoff indices (COIs) are utilized for different public health interpretations. The COI of 0.8, recommended by the manufacturer (Roche), is optimal for identifying the presence of antibodies in the blood sample. Meanwhile, a COI of 15 is indicative of a threshold for sufficient humoral immunity against the SARS-CoV-2 virus. We also applied Rogen-Gladen equation and Bayesian models to adjust for test sensitivity. (Just one fig, will add more to build a panel) |

# Supplemental materials

# A. Supplement section

## Supplement Methods

(to-be-deleted: we do not utilize most of the demographic variabes in our analysis) Besides of serology test results and demographic data, CBS has linked the administrative data for the cohort, which provided additional information on their hospitalizations, clinic visits, diagnoses, COVID vaccinations, and long-term disability claims. Similarly, AHS and CANPATH have completed linkages and included additional vaccination history.

A short paragraph about weighting: Our analysis is based on individual level. As we are calculating measurements at a monthly scale, samples sampled at different months were unweighted as they contributed independently to each month’s point prevalence estimates. While in all of those three dataset, it’s possible to have one individual being sampled multiple times within the same month, in this case we applied weights to each individual according to the number of samples they have in the same month. In regression model training, individuals who provided multiple samples were also down-weighted according to the number of samples they provided.

A paragraph about the training data: Cite the table summarizing the features of training data (Table S1). Clarify which data has matched assay results.

# Supplemental tables

Add a table here summarizing the sample size, data source of the training data we used.

**Table S 1** Table caption here.

| **col1** | **col2** |
| --- | --- |
| **A** | |
| 1 | Yes |
| 2 | Yes |
| **B** | |
| 1 | No |
| 2 | No |

# Supplemental figures

**Figure S 1** Figure caption here.

