dried blood spot vs. venous sample collection on SARS-CoV-2 antibody test results in the CLSA serological study of older Canadians

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# Letter (500 / 500 Words)

Participant-collected dried blood spots (DBS) are a convenient, minimally invasive alternative to phlebotomist-collected venous samples for population serological surveillance of infectious diseases. Several SARS-CoV-2 serosurveillance studies collected DBS samples through the mail, reaching wider geographic areas, relatively healthier population, and minimizing face-to-face contact during the pandemic [1–3]. This sample collection approach avoids the logistic issues associated with specimen handling, a common occurrence in venous samples. Prior studies characterize assay accuracy with DBS as ‘reliable’, based on their high sensitivity and specificity, in paired comparisons with venous samples [3–5]. A recent study comparing SARS-CoV-2 IgG results from paired venous and DBS samples concluded performance was “comparable” [6]. However, another study found markedly lower sensitivity on DBS samples, which could be partially mitigated by using a lower threshold for positivity[7]. Despite low sensitivity observed in some previous studies, it remains valuable for detecting potential emerging outbreaks when seroprevalence exceeds a specific threshold. In a national SARS-CoV-2 serological study of older Canadians conducted by the Canadian Longitudinal Study on Aging [8], participants either mailed a DBS sample or provided a venous sample at a testing center. We analyzed these data to assess for systematic differences in assay findings between DBS and venous

First, we developed a logistic regression propensity model to examine the association of demographics, geography, and health-related factors with the choice to provide a DBS or venous sample. Then, we compared SARS-CoV-2 Anti-N and Anti-S (7,230 assays each) results between participants who provided DBS (n = 3,773) or venous samples (n = 3,450; **Fig. S1**), using inverse probability of treatment weighting to adjust from potential confounding variables (details in **Supplemental Methods**).

Participants who provided in-person venous samples were more likely to be male, white, unvaccinated, and live in an urban area (**Fig. 1**). After balancing for 10 covariates via propensity score weighting, venous samples were 223% more likely to be Anti-N positive (Odds ratio [OR]: 3.33, 95% confidence interval [CI]: 2.78–4.01) and 27% more likely to be Anti-S positive (OR: 1.27, 95% CI: 1.08–1.49). Among Anti-S antibody positive samples, the distribution of quantitative Anti-S levels was systematically lower for DBS samples (p < 0.0001, Kolmogoriv-Smirnov test; **Fig. 2**). Few DBS (0.66%) samples were above the assays upper dynamic range (2500 U/ml), compared to 7.39% of Anti-S positive venous. Province-level sub-analyses yielded consistent findings (**Fig. S3**). Compared to using venous samples, we estimate that DBS for all participants would have underestimated anti-N seropositivity by 69.3% (2.49% positive with DBS vs. 8.12% with venous) and would have underestimated anti-S positivity by 13.7% (43.74% vs. 50.68%).

In contrast to earlier reports [1–3,5], we found substantial differences between SARS-CoV-2 serological findings in DBS and venous samples. One limitation of our study is the use of unmatched samples. Typically, matched sample analysis is preferred, as it allows for better control over variances in other factors. While assay-specific in-house calibration may mitigate these differences [1], more work is needed to understand how use of DBS samples impacts population serosurveillance estimates.

# Declarations

**Funding:** None.

**Conflicts:** No conflicts of interests.

**Ethics/Consent:** REB submitted and approved (REB 23-09-061).

**Data and materials:** Data are all de-identified prior to transfer to our research team by our data-sharing partners (Canadian Blood Services, Alberta Precision Labs, and the Canadian Longitudinal Study on Aging).

**Code availability:** https://github.com/altonrus/dbs-vs-venous-antibodies

**Authors’ contributions:** Finish this part once we got input from all co-authors.

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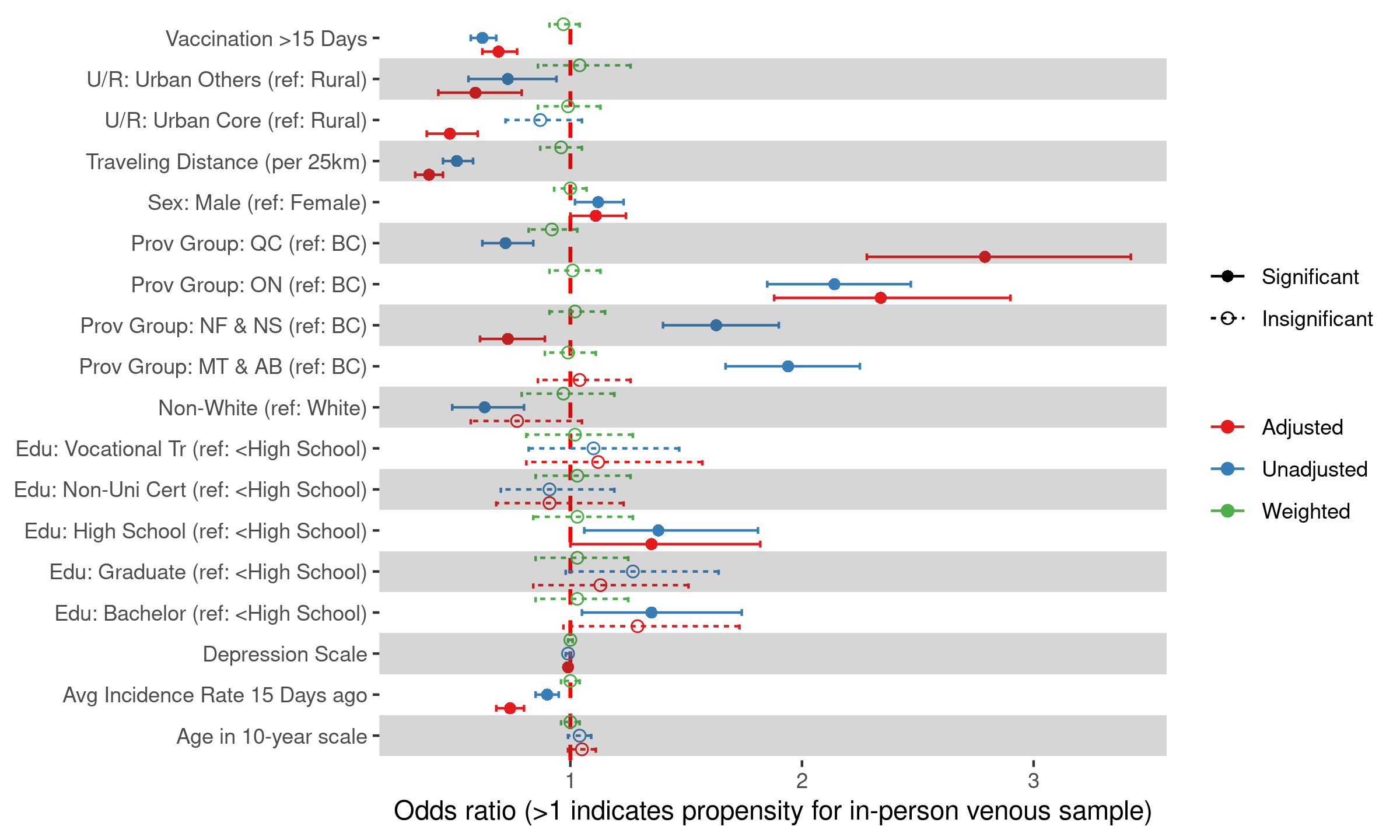
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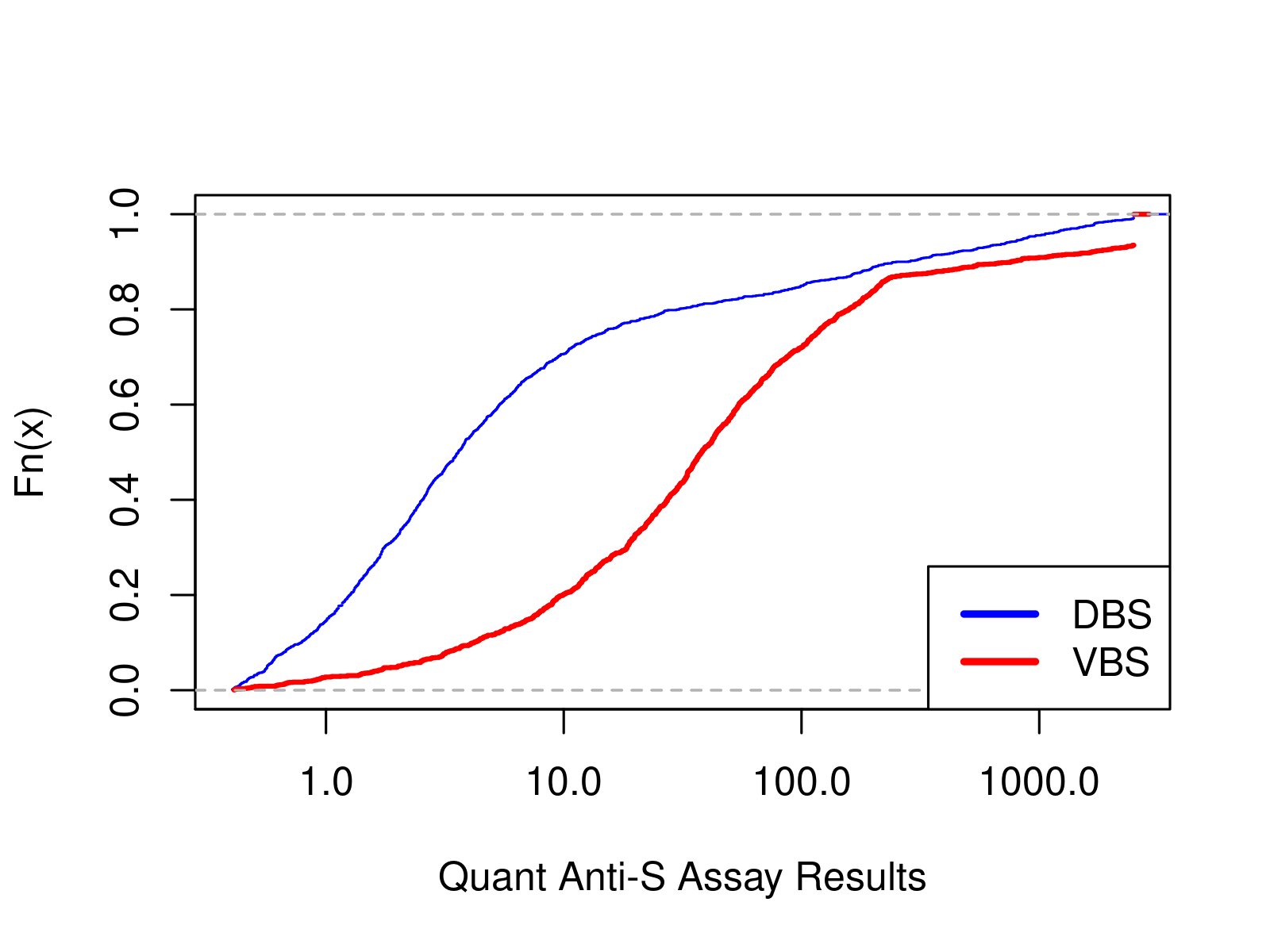
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# Figure



**Figure 1**: Odds ratios for providing an in-person venous sample instead of DBS using a multivariate logistic regression model (‘adjusted’) and univariate model (‘unadjusted’). After applying inverse probability of treatment weighting (‘weighted’), all odds ratios were insignificant, suggesting good balance.



**Figure 2**: Empirical cumulative distribution function for the quantitative Anti-S level among participants who tested positive on the Anti-S assay. Venous samples tended to have higher Anti-S levels (mean: 300.54 U/ml, median: 38.63 U/ml) compared to DBS (mean: 105.75 U/ml, median: 3.43 U/ml). Adjusted Anti-S assay results performed on DBS samples has similar distribution as assay results performed on venous samples. However, this similarity is only observed within the dynamic range of the assay (0.4-2500 U/ml).

# Supplemental materials

# Supplemental methods

## Dataset

The Canadian Longitudinal Study on Aging (CLSA) is a comprehensive national long-term study designed to track approximately 50,000 individuals aged 45 to 85 years at recruitment for a minimum of 20 years [8]. The CLSA COVID-19 Antibody Study has two complementary cohorts. The first one is called “comprehensive cohort”, in which 30,097 participants were randomly selected from locations within 25-50 km of the data collection sites (DCS) and underwent in-person interviews, providing detailed information on demographics, physical and mental health status, as well as vaccination and hospitalization history. The second cohort, known as the “tracking cohort,” was interviewed by telephone. The detailed sampling process of CLSA is presented in the flowchart (**Fig. S1**).

During the COVID-19 pandemic, the CLSA COVID-19 Antibody Study was conducted to evaluate the pandemic’s population-level health impact on older Canadians. The CLSA COVID-19 Antibody Study included over 18,000 CLSA participants across ten provinces from November 2020 to July 2021 [**canadian\_longitudinal\_study\_on\_aging\_covid-19\_2022?**]. Among those participants, 10,259 from the comprehensive cohort provided samples that were suitable for testing, within which N=4,258 contributed venous blood samples, and N=5,495 provided dried blood samples. In addition to demographic and health status variables, the data we acquired included immunoassay tests results.

***Evolution of data collection methods over time*** [In this section we would like to describe how, when and why DBS vs. venous sampling was available to participants at different times of the pandemic in different provinces]

In our analysis, we utilized data exclusively from the comprehensive cohort of the CLSA COVID-19 Antibody Study, as it provides more comprehensive demographic and health condition data. Additionally, we excluded participants residing in regions located more than 50 km from the data collection center, as their extended travel distance is caused by recent relocations subsequent to enrollment in the study. The final sample size included in our analysis was 7,230.

***In-house laboratory procedures for calibrating the assay results***: [In this section we would like to describe the reason why:

1) COIs applied to DBS and venous samples are different;

2) COIs applied are different to the COI published by Roche;

3) COIs changed due to reagent lot change, and how finite mixture models are built to calculate COIs.]

## Statistical analysis

**Analyzing predictors of in-person venous samples vs. DBS:** Before conducting propensity score weighting, we performed an initial unadjusted bivariate analysis to compare the DBS and VBS groups. Additionally, we developed multivariate logistic regression models that regressed VBS/DBS against demographic factors, physical and mental health conditions, travel distance, vaccination status, and time since the last COVID outbreak. The comprehensive results generated by those simple logistic regression models and the full model are displayed in the odds ratio (OR) plot (**Fig. 1**).

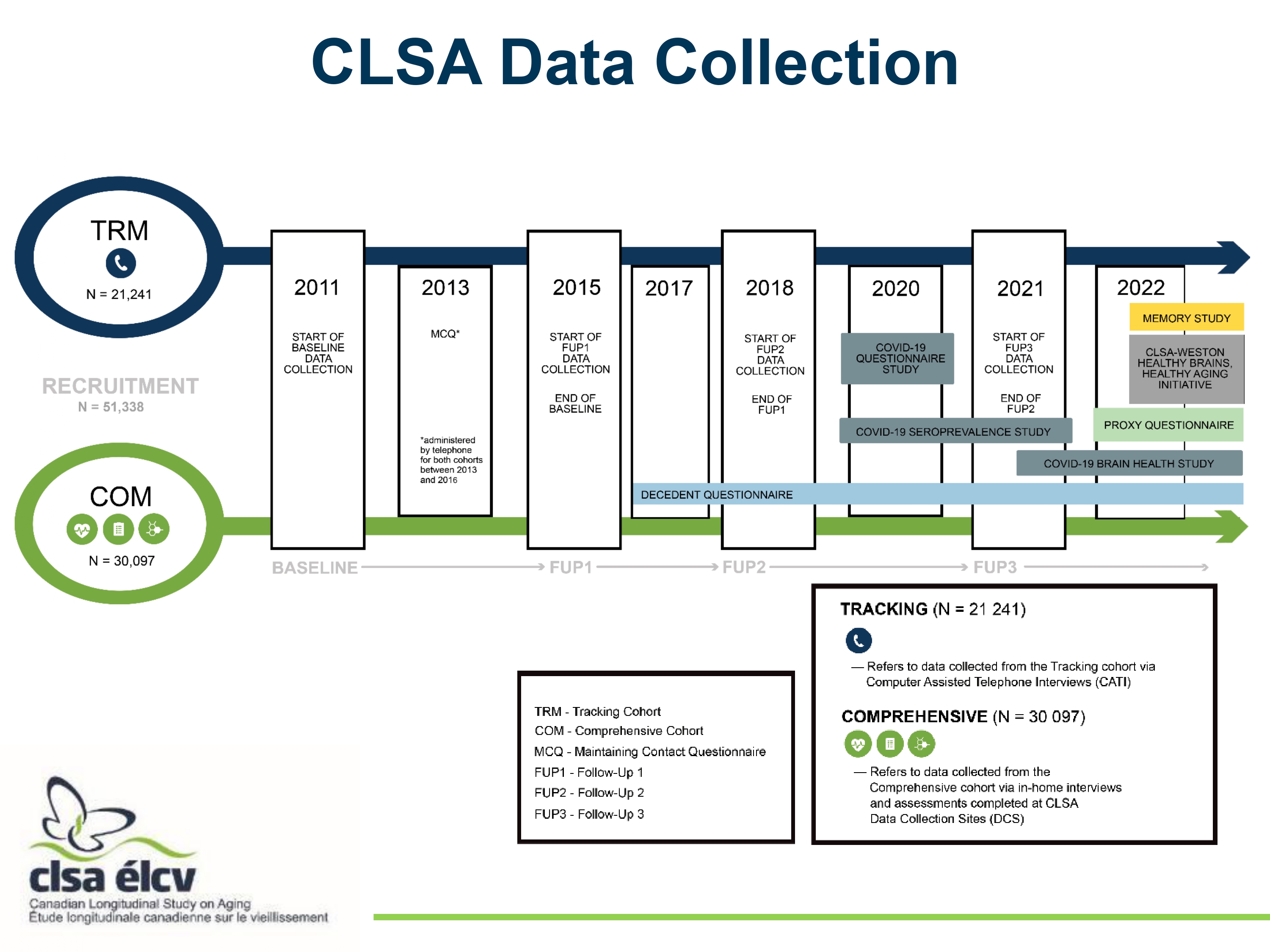
**Propensity score weighting:** Upon completion of the full model, we constructed a best-fit model utilizing a backward stepwise model selection strategy. The model with lowest Akaike Information Criterion (AIC) value was selected as the best-fit model. Subsequently, we applied this best-fit model to calculate propensity scores for the selection of VBS over DBS. Participants from both groups were weighteded based on their propensity scores using inverse probability of treatment weighting (IPTW). IPTW achieved good balance between the DBS and VBS groups, illustrated by the fact that the odds ratios in the model are all not significant after applying the weights.

**Analysis with Weighted Data：** Utilizing weighted data, we further analyzed the differences in assay positivity between the DBS and VBS cohorts. The outcomes of the stratified analysis (**Fig. S3**) aligned with those from the unstratified national dataset. To analyze the variation in quantitative Anti-S immunoassay results, we employed the weighted empirical cumulative distribution function (eCDF) to visualize the distribution in the DBS and VBS cohorts (**Fig. 2**). We used Kolmogorov-Smirnov (KS) Test to assess whether the distributions are statistically-significantly different.

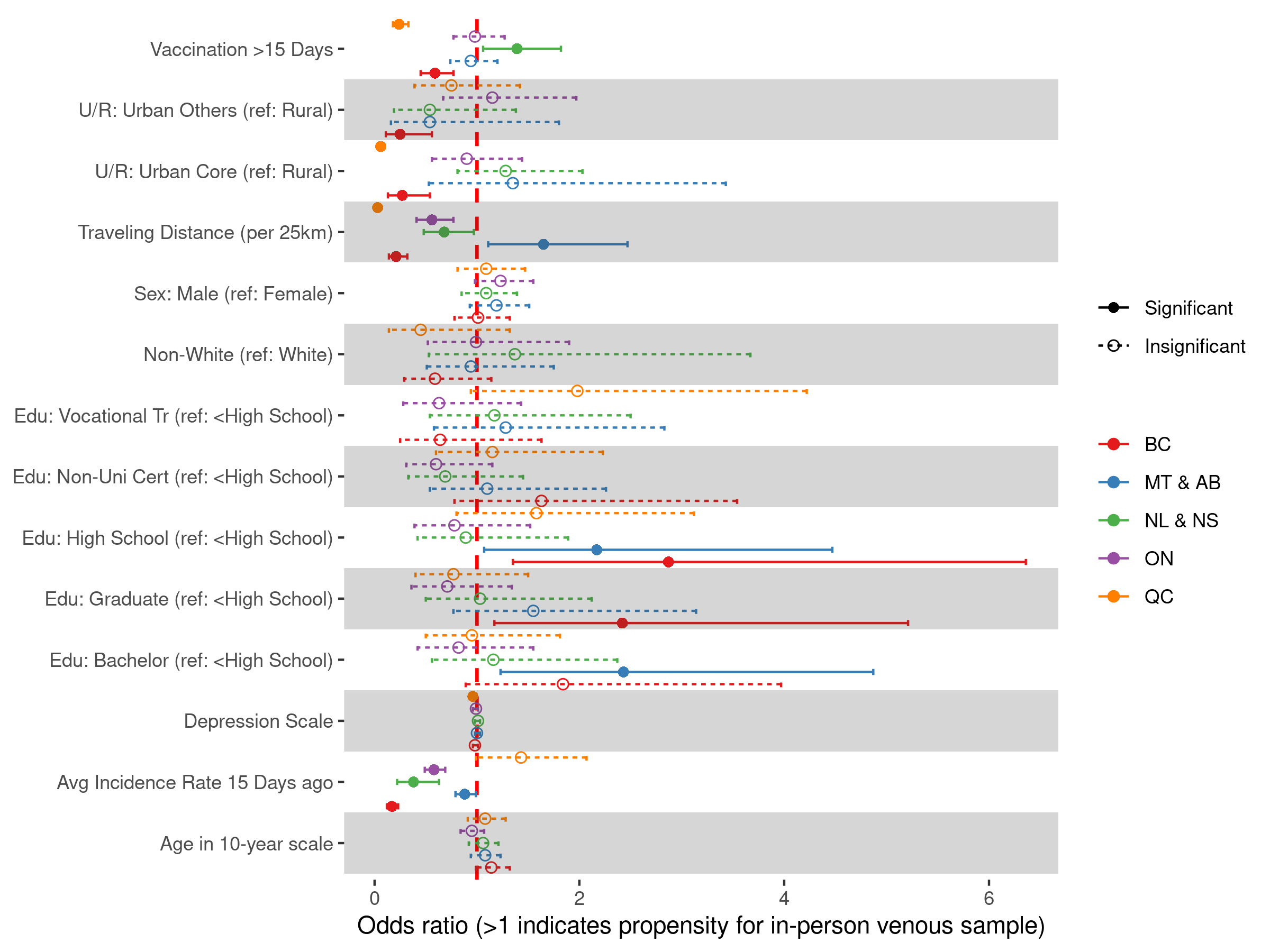
A previous study discovered a linear relationship between the quantitative Anti-S assay results performed on DBS and venous samples [9]. The linear relation is quantified and expressed using the equation above. We applied the same equation to the quantitative Anti-S assay results performed on DBS samples, then presented the distribution of adjusted assay results in the same eCDF figure (**Fig. 2**).

**Sub-analyses:** Based on the national data, we conducted a stratified analysis by province. Our study sample included participants from seven provinces, which were recategorized into five groups according to their locations and geographic proximities: British Columbia (BC), Manitoba (MB) & Alberta (AB), Ontario (ON), Quebec (QC), and Newfoundland (NL) & Nova Scotia (NS). The pandemic’s impact varied across these regions, with residents adapting differently to the diverse policies implemented by their respective health departments [10,11]. **Figure S2** illustrates the variation in odds ratios for the preference of venous blood sampling across provinces.

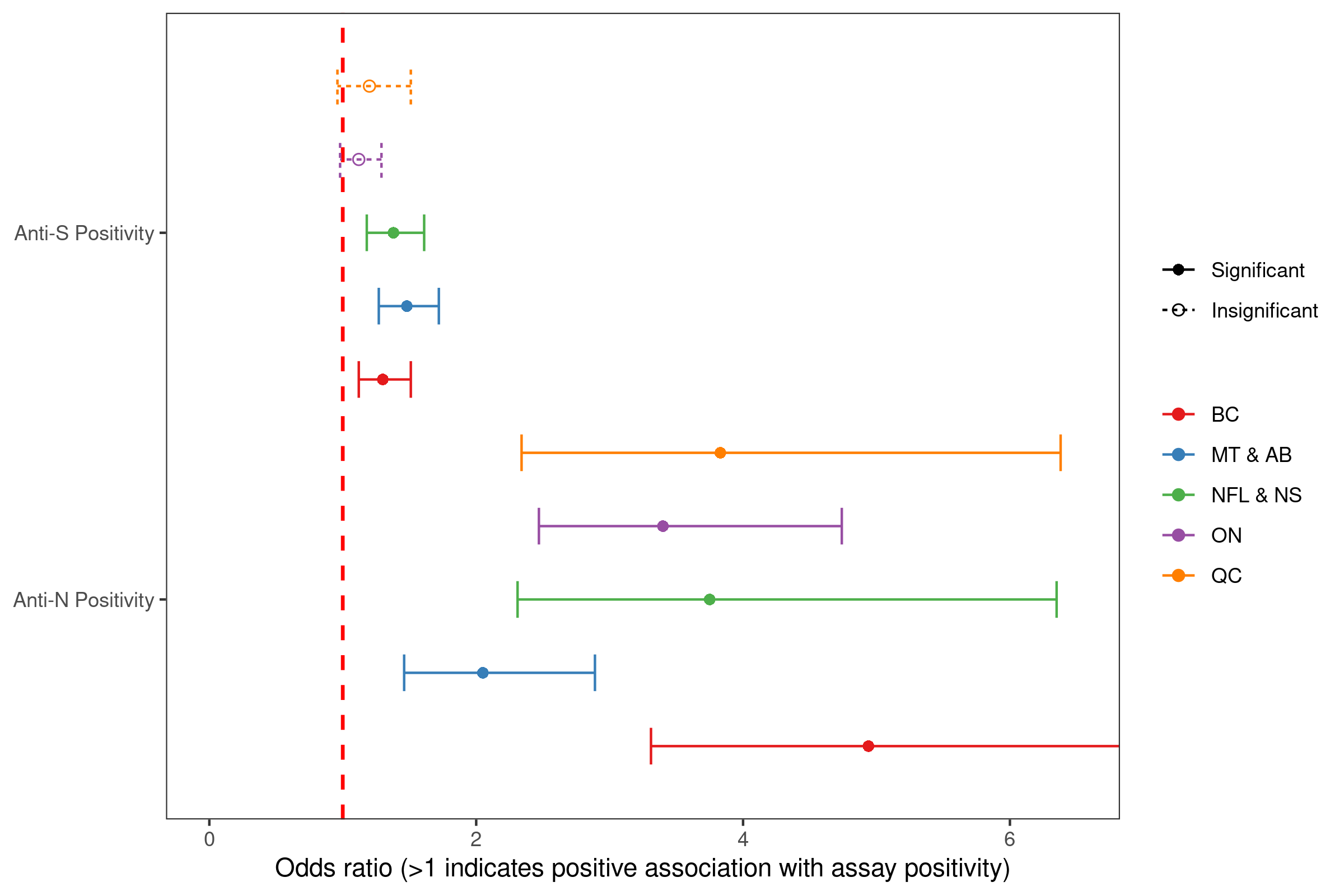
# Supplemental figures



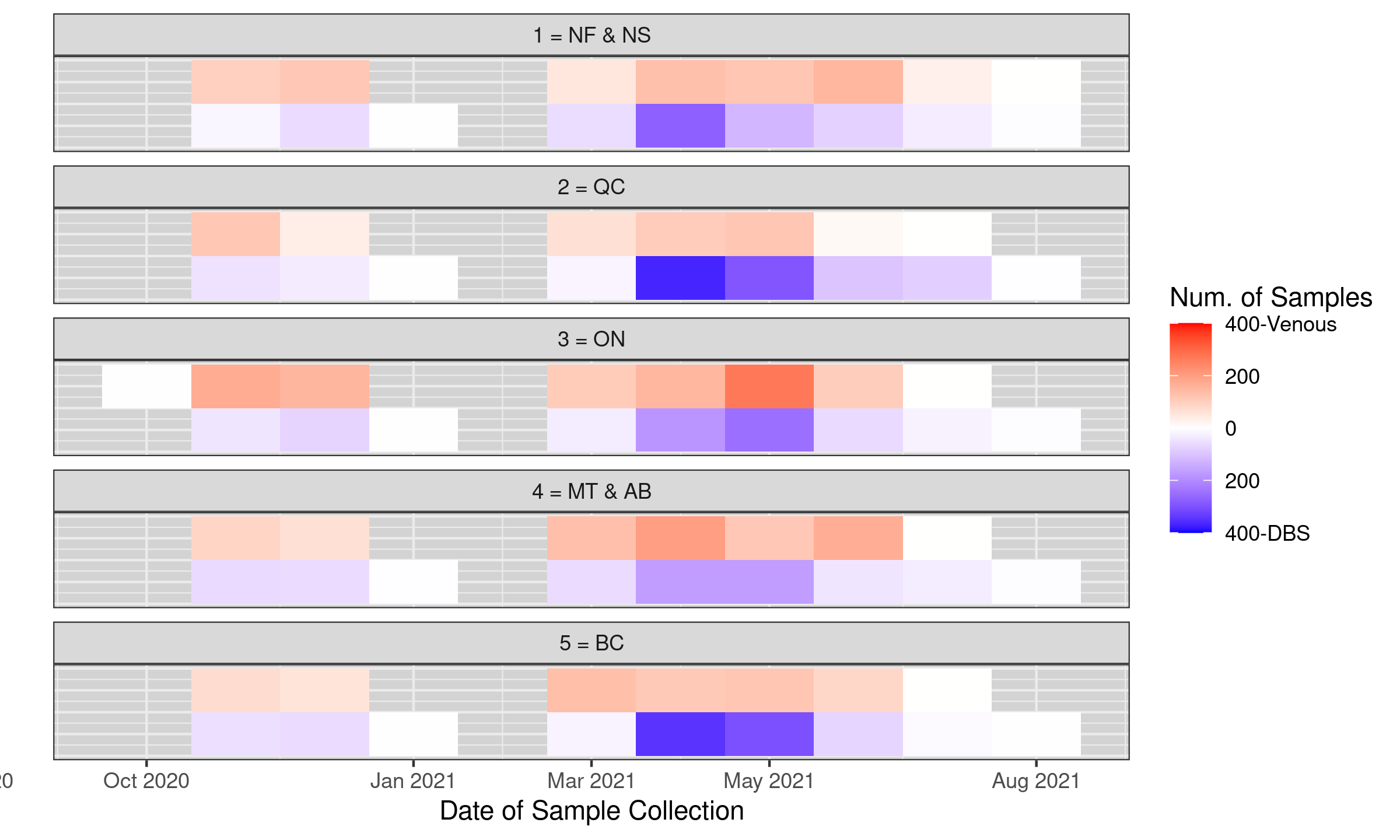
**Figure S1: CLSA COVID-19 Study Sampling Process.** Reproduced from <https://www.clsa-elcv.ca/doc/2429>



**Figure S2. Odds Ratio of Venous Blood Sampling, Stratified by Province or Region.** The odds ratios for venous blood sampling vary among the five provincial groups. We observed significant differences in sampling method preferences across provinces. Compared to British Columbia (BC), participants in Ontario (ON) and Quebec (QC) preferred in-person venous sampling. In the Atlantic provinces, however, there was a preference for at-home DBS sampling over the venous sampling approach.



**Figure S3. Odds Ratio of Assay Positivity, Stratified by Province or Region.** We observed an increased odds of assay positivity in venous blood samples for both anti-N and anti-S assays, which is consistent with the analysis using unstratified national data.



**Figure S4** Add caption here