



## PHASE 4B OF THE STUDY ON THE SEROPREVALENCE OF ANTIBODIES TO SARS-COV-2 IN QUEBEC

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## Introduction

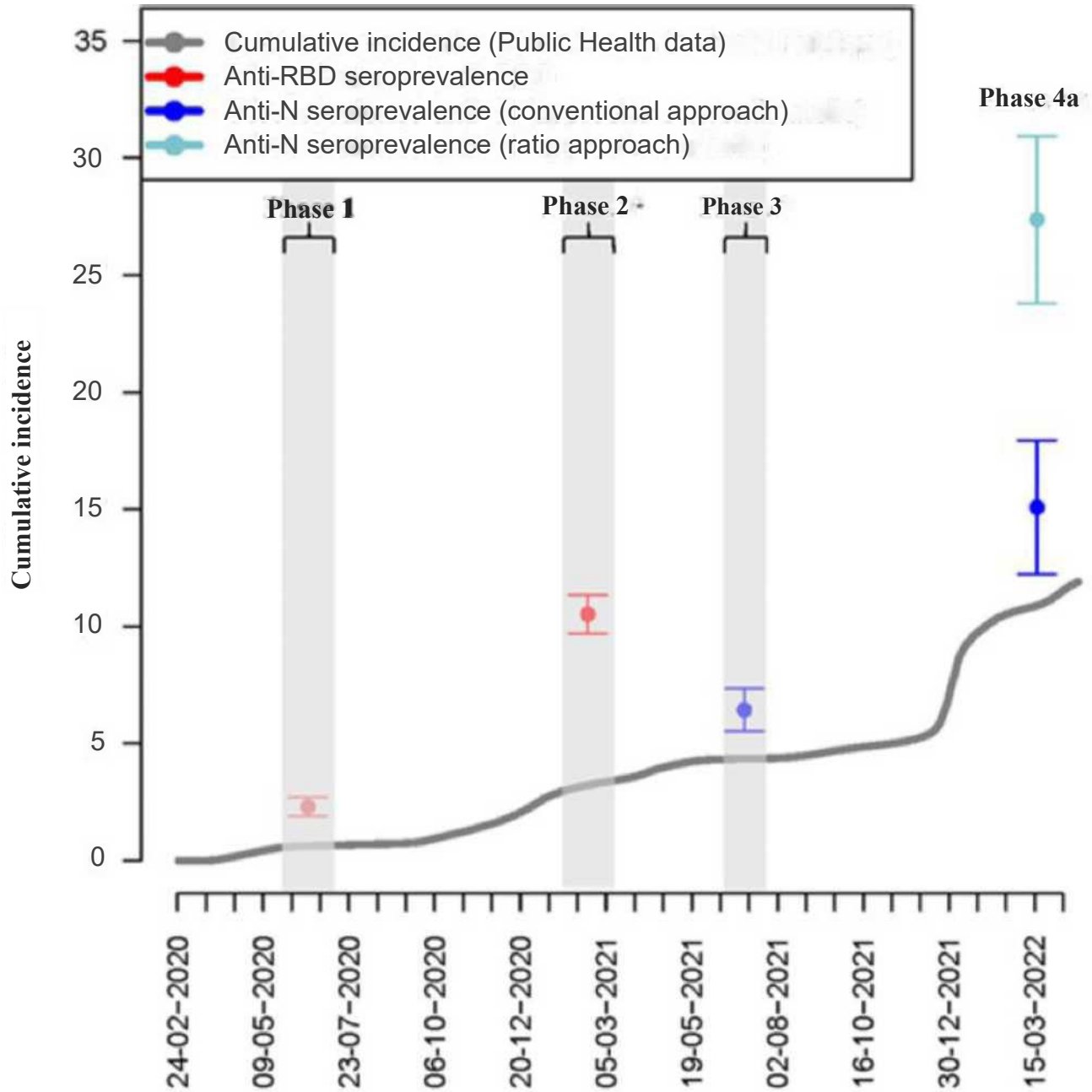
Phase 4b of the study on the seroprevalence of SARS-CoV-2 aims to document the seroprevalence of antibodies to SARS-CoV-2 in Quebec in the last two waves caused by the Omicron variant in 2022, namely the wave between mid-December 2021 and mid-March 2022, dominated by the BA.1 subvariant, and the wave between mid-March and June 2022, dominated by the BA.2 subvariant.<sup>1</sup>

In phase 1 conducted between May and July 2020 (i.e., after the 1<sup>st</sup> wave of the COVID-19 pandemic), the weighted seroprevalence of antibodies targeting the receptor binding domain (RBD) of the spike protein (S) was estimated at 2.23% (confidence interval [CI] 95%=1.90%-2.56%).<sup>2</sup> In phase 2 conducted between January and March 2021 (i.e., after the peak of the 2<sup>nd</sup> wave), the weighted anti-RBD seroprevalence was estimated at 10.52% (CI 95%=9.71%-11.33%; **Figure 1**) in the unvaccinated population and 14.72% (CI 95%=13.81%-15.63%) in the general population studied.<sup>3</sup> In phase 3 conducted between June and July 2021 (i.e., after the 3<sup>rd</sup> wave, when a large part of the adult population had received at least one dose of the vaccine), the weighted anti-RBD seroprevalence was estimated at 89.61% (IC 95%=88.48%-90.75%) and the weighted anti-nucleocapsid (N) seroprevalence at 6.43% (CI 95%=5.52%-7.34%).<sup>4</sup> Anti-nucleocapsid antibodies are only produced by infection with SARS-CoV-2 and not by vaccination and point to a “recent” previous infection.

Phase 4a, conducted at three points during the first wave in 2022, namely mid-January, mid-February and mid-March, showed that a “conventional” approach—where a seropositive threshold distinguishes (at a given time) anti-N seropositive from anti-N seronegative individuals—is not sensitive enough to detect recent infections in vaccinated individuals (i.e., >80% of the Quebec population). A new “ratio” approach which compares anti-N titres in two or more serial samples from one individual was developed during phase 4a of the seroprevalence study and allowed us to identify infection in more than 98% of individuals with recent infections confirmed by PCR using longitudinal samples (i.e., >2 samples/individual). This effective approach was therefore used for the rest of the seroprevalence studies. In phase 4a, the weighted anti-N seroprevalence was estimated at 27.37% (CI 95%=23.80%-30.93%) using the ratio approach (for the period between December 2021 and mid-March 2022) and only at 15.09% (CI 95%=12.23%-17.95%) in mid-March 2022 using the conventional approach (**Figure 1**).

This report presents the preliminary results of phase 4b of the seroprevalence study covering the period studied in phase 4a, namely the BA.1 wave (December 2021-March 2022) and the rise of the BA.2 variant (i.e., March-June 2022). This study was conducted by Héma-Québec with financial support from Quebec’s Ministère de la Santé et des Services sociaux (MSSS) and the Public Health Agency of Canada (through the Vaccine Surveillance Reference Group and the COVID-19 Immunity Task Force) toward collecting the samples used in the study. The opinions expressed in this document do not necessarily reflect those of the MSSS or the Public Health Agency of Canada.

**Figure 1. Weighted seroprevalences observed in phases 1-4a, and cumulative incidence of PCR-confirmed SARS-CoV-2 infections reported by public health<sup>1</sup>**



**Notes:**

1. The error bars represent confidence intervals of 95%; the shaded areas represent the periods during which the samples were collected.
2. Seroprevalence in the unvaccinated population.



## Method

### *Data and donor sources*

Samples were taken from regular plasma donors residing in 12 of the 18 health regions (HR) in Quebec. All the plasma donors whose samples were analyzed for anti-SARS-CoV-2 consented to take part in the study, which was approved by Héma Québec's research and ethics committee. They are all part of the PLASCOV Biobank project.<sup>5</sup> As in phases 1-4a of the study, minors (<18 years) and donors who had COVID-19 symptoms in the 14 days preceding their donation were excluded, as they were not eligible for regular blood donations.

### *Phase 4b study design*

Three samples were collected per participant at the following times (i.e., longitudinal samples): (1) before December 15, 2021, (2) between March 15 and April 1, 2022 and (3) between May 30 and June 5, 2022. Seroprevalences were thus estimated for the periods between December 2021 and March 2022, between March 2022 and June 2022, and between December 2021 and June 2022 (i.e., aggregate of these two periods).

The samples were tested using the anti-N ELISA (also used in phases 3 and 4a of the study) since these antibodies are only present in individuals who were infected with SARS-CoV-2. This test is similar to the anti-RBD ELISA used in phases 1-2 of the study, except that a recombinant form of the N protein (and not the RBD) was adsorbed to the plates. For each donor, the three samples (and their technical replicates) were tested simultaneously on one plate to limit technical variability.

### *Anti-N ELISA— conventional approach*

With the conventional approach, when the test was developed, its seropositive threshold was set to an optical density of 0.350 using logistic regression and a receiver operating characteristic curve. With this threshold, the test has a sensitivity of 98.1% in unvaccinated individuals who previously contracted SARS-CoV-2 (n=52) (i.e., infection confirmed by PCR) and a specificity of 98.5%, as assessed based on pre-pandemic samples (n=66).

### *Anti-N ELISA — ratio approach*

The ratio approach was developed to compensate for the lack of sensitivity of the conventional approach observed in phases 3 and 4a. This method requires at least two longitudinal samples per individual and allows us to assess seropositivity during a given period rather than since the start of the pandemic. Since this phase covers two periods (the BA.1 and BA.2 waves), three samples were collected. A ratio is then calculated between the absorbance after and before the first wave (dominated by BA.1) and between the absorbance after and before the second wave (dominated by BA.2). An aggregate of the two periods (December to June) was also calculated. A ratio is not calculated for a given period if the most recent sample has an absorbance of less than 0.100, since these numbers are considered assay background noise.



We tested this approach on 248 vaccinated frequent plasma donors who contracted SARS-CoV-2 during the Omicron wave (confirmed by PCR according to the provincial register [TSP]). Only 63.3% of these donors were seropositive using the conventional approach. These results show that more than 35% of vaccinated individuals do not have

sufficiently high anti-N levels to be considered seropositive using the conventional approach, even after a recent infection, which shows the major limitations of this approach in analyzing serological anti-N test results and estimating SARS-CoV-2 infection rates. Using the ratio approach, 95.2% of donors were positive using a seropositive threshold of 1.5 for the anti-N ratio. Thus, this new method provides a more accurate estimate of the proportion of individuals with (recent) infections in a given period.

### *Statistical analyses*

Means and standard deviations were reported for continuous variables, and proportions were reported for discrete variables. Anti-N seroprevalence was also weighted based on the age and sex of the Quebec population, and the demographic weight of HRs in Quebec.



## Results

### *Donor characteristics*

A total of 558 regular plasma donors (average age:  $55.87 \pm 14.34$  years) were selected for this phase of the study. The proportion of men (76.0%) was higher than women (24.0%), and donors in the greater Montréal area and its belt were underrepresented (**Table 1**). Nearly all the plasma donors were Caucasian.

**Table 1. Characteristics of the donors used to estimate seroprevalence**

	<b>N=558</b>
Age, average $\pm$ SD	55.87 $\pm$ 14.34
Women, n (%)	134 (24.01)
Montréal and belt, n (%)	74 (13.26)
Caucasian, n (%)	544 (97.49)

*Observed seroprevalences*

With the conventional approach, the age, sex and HR-weighted seroprevalence was 30.74% (CI 95%=26.96%-34.52%) in June 2022 ([Table 2](#)). Note that (unlike with the ratio approach), individuals who are considered seropositive with this approach could have contracted SARS-CoV-2 several months prior.

With the ratio approach, the age, sex and HR-weighted seroprevalence was 45.32% (CI 95%=41.24%-49.40%) between December 2021 and June 2022. Note that the individuals considered seroprevalent with this approach were necessarily infected during this period; this approach is designed to only measure infections during a given period which can span several months, not from the start of the pandemic.

**Table 2. Greater area and approach (conventional vs. ratio)-stratified anti-N seroprevalence**

	June 2022 (conventional approach)		December 2021 - June 2022 (ratio approach)	
	n/N	Seroprevalence (%), <sup>1</sup> (CI 95%)	n/N	Seroprevalence (%), <sup>1</sup> (CI 95%)
<b>Greater area</b>				
Montréal and Montréal’s belt <sup>2</sup>	20/74	28.65 (21.13-36.16)	29/74	41.60 (33.40-49.79)
Other regions	135/484	31.41 (27.04-35.78)	206/484	46.51 (41.82-51.21)
<b>Total</b>	<b>155/558</b>	<b>30.74 (26.96-34.52)</b>	<b>235/558</b>	<b>45.32 (41.24 - 49.40)</b>

**Abbreviation:** CI = confidence interval

**Notes:**

1. Weighted based on health region (HR), and distribution by sex and age in each HR (2011 census).
2. Montréal, Laval and Montréal’s belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes - Mirabel Sud, Rivière-du-Nord - Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie.





### *Health region-stratified seroprevalence*

With the ratio approach, Montréal and its belt had a weighted seroprevalence of 20.14% (CI 95%=13.48%-26.81%) between December 2021 and March 2022, 17.14% (CI 95%=10.87%-23.40%) between March and June 2022 and 41.60% (CI 95%=33.40%-49.79%) between December 2021 and June 2022 ([Table 3](#)). The other regions trend similarly, but with slightly higher rates.



**Table 3. Region-stratified Anti-N seroprevalence (ratio approach)**

	December 2021 to March 2022		March to June 2022		December 2021 to June 2022	
	n/N	Seroprevalence (%), <sup>1,2</sup> (CI 95%)	n/N	Seroprevalence (%), <sup>1,2</sup> (CI 95%)	n/N	Seroprevalence (%), <sup>1,2</sup> (CI 95%)
<b>Health regions</b>						
01-Bas-Saint-Laurent	-	-	-	-	-	-
02-Saguenay-Lac-Saint-Jean	20/92	25.92 (15.21-36.62)	23/92	28.28 (17.28-39.28)	44/92	54.97 (42.82-67.12)
03-Capitale-Nationale	14/74	15.00 (7.78-22.22)	21/74	34.48 (24.86-44.09)	35/74	49.48 (39.37-59.59)
04-Mauricie-et-Centre-du-Québec	27/94	31.03 (19.35-42.71)	14/94	13.86 (5.14-22.59)	41/94	44.89 (32.33-57.45)
05-Estrie	19/83	24.43 (13.90-34.97)	14/83	17.52 (8.20-26.85)	33/83	41.96 (29.86-54.06)
06-Montréal	7/34	20.59 (10.98-30.20)	6/34	17.65 (8.59-26.71)	13/34	38.24 (26.68-49.79)
07-Outaouais	16/92	20.58 (10.97-30.20)	13/92	15.28 (6.72-23.85)	29/92	35.87 (24.46-47.28)
08-Abitibi-Témiscamingue	-	-	-	-	-	-
09-Côte-Nord	1/1	100.0 (15.81-100.0)	0/1	-	1/1	100.0 (15.81-100.0)
10-Nord-du-Québec	-	-	-	-	-	-
11-Gaspésie-Îles-de-la-Madeleine	-	-	-	-	-	-
12-Chaudière-Appalaches	6/20	32.93 (16.96-48.91)	3/20	11.73 (0.79-22.67)	9/20	44.66 (27.76-61.56)
13-Laval	1/14	7.67 (0.00-17.89)	2/14	14.35 (1.07-28.23)	3/14	22.32 (6.34-38.31)
14-Lanaudière	2/5	40.00 (9.64-70.36)	1/5	20.00 (0.00-44.79)	5/5	100.0 (69.15-100.0)
15-Laurentides	1/7	16.00 (0.00-36.32)	3/7	48.00 (20.30-75.70)	4/7	64.00 (37.39-90.61)
16-Montérégie	11/42	27.77 (17.31-38.23)	6/42	15.88 (7.35-24.42)	18/42	46.49 (34.85-58.14)
17-Nunavik	-	-	-	-	-	-
18-Terres-Cries-de-la-Baie-James	-	-	-	-	-	-
<b>Greater area</b>						
Montréal and Belt <sup>3</sup>	14/74	20.14 (13.48-26.81)	12/74	17.14 (10.87-23.40)	29/74	41.60 (33.40-49.79)
Other regions	111/484	24.35 (20.31-28.39)	94/484	22.05 (18.15-25.95)	206/484	46.51 (41.82-51.21)
<b>Total</b>	<b>125/558</b>	<b>23.33 (19.86-26.76)</b>	<b>106/558</b>	<b>20.86 (17.53-24.18)</b>	<b>235/558</b>	<b>45.32 (41.24-49.40)</b>

**Abbreviation:** CI = confidence interval

**Notes:**

1. Weighted based on HR, and distribution by sex and age in each HR (2011 census).
2. An anti-N ratio of >1.5 is considered a recent infection. Samples with anti-N ODS of < 0.10 are directly identified as negative samples.
3. Montréal, Laval and Montréal's belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes - Mirabel Sud, Rivière-du-Nord - Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie.

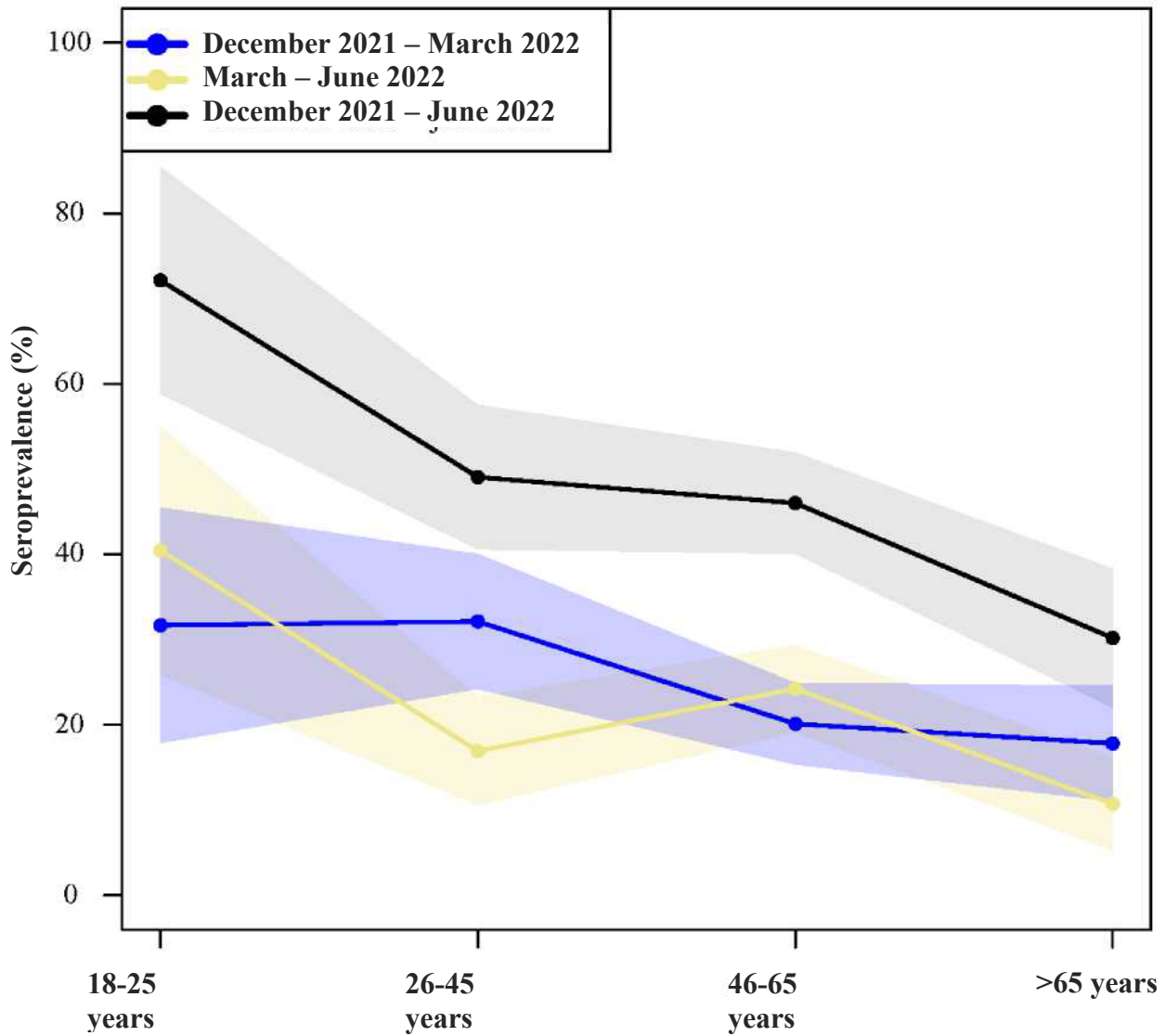


### *Age-stratified seroprevalence*

Using the ratio approach between December 2021 and March 2022, the weighted anti-N seroprevalence was higher among younger donors compared to older donors, namely >30% in individuals aged 18 to 45 and <20% in individuals aged >65 (**Table 4** and **Figure 2**). Between March and June 2022, the weighted anti-N seroprevalence was 40.45% (CI 95%=25.90%-54.99%) in individuals aged 18-25 and 10.73% (CI 95%=5.26%-16.20%) in individuals aged >65. For the entire period between December 2021 and June 2022, the weighted seroprevalence was 72.13% (CI 95%=58.83%-85.42%) in individuals aged 18-25 and 30.18% (CI 95%=22.06%-38.29%) in individuals aged >65.

In the two individual waves, the ratio-weighted seroprevalence was higher in other regions compared to Montréal, Laval and the belt. There is a difference of up to 5 percentage points when comparing regions in the period between December 2021 and June 2022. However, the confidence intervals overlap, which limits the statistical significance.

**Figure 2. Age-stratified anti-N seroprevalence (ratio approach)**



**Note:**

1. The shaded areas represent CIs of 95%.

**Table 4. Region and age-stratified anti-N seroprevalence (ratio approach)**

DEC - MAR	18-25 years		26-45 years		46-65 years		>65 years	
	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)
<b>Greater area</b>								
Montréal-belt <sup>2</sup>	1/4	25.0 (0.00 - 55.01)	3/13	23.08 (6.88 - 39.27)	6/35	17.22 (8.36 - 26.09)	4/22	22.64 (8.84 - 36.44)
Others	8/24	33.18 (17.73 - 48.62)	31/84	34.30 (25.35 - 43.24)	50/239	21.13 (15.50 - 26.76)	22/137	15.88 (8.23 - 23.54)
<b>Total</b>	<b>9/28</b>	<b>31.68 (17.89 - 45.47)</b>	<b>34/97</b>	<b>32.12 (24.23 - 40.02)</b>	<b>56/274</b>	<b>20.12 (15.36 - 24.89)</b>	<b>26/159</b>	<b>17.82 (11.06 - 24.59)</b>

MAR - JUN	18-25 years		26-45 years		46-65 years		>65 years	
	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)
<b>Greater area</b>								
Montréal-belt <sup>2</sup>	2/4	50.00 (15.35 - 84.65)	3/13	23.08 (6.88 - 39.27)	6/35	16.96 (8.15 - 25.78)	1/22	5.66 (0.00 - 13.28)
Others	9/24	38.31 (22.36 - 54.25)	14/84	15.45 (8.65 - 22.26)	52/239	26.74 (20.63 - 32.84)	19/137	21.05 (13.20 - 28.89)
<b>Total</b>	<b>11/28</b>	<b>40.45 (25.90 - 54.99)</b>	<b>17/97</b>	<b>16.93 (10.59 - 23.27)</b>	<b>58/274</b>	<b>24.23 (19.13 - 29.32)</b>	<b>20/159</b>	<b>10.73 (5.26 - 16.20)</b>

DEC - JUN	18-25 years		26-45 years		46-65 years		>65 years	
	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)	(n/N)	Seroprevalence (%), <sup>1</sup> (CI 95%)
<b>Greater area</b>								
Montréal-belt <sup>2</sup>	3/4	75.00 (44.99 - 100.0)	6/13	46.15 (26.99 - 65.32)	14/35	39.93 (28.43 - 51.43)	6/22	33.97 (18.35 - 49.58)
Others	17/24	71.48 (56.67 - 86.29)	45/84	49.75 (40.33 - 59.17)	103/239	48.11 (41.22 - 55.00)	41/137	28.65 (19.18 - 38.12)
<b>Total<sup>3</sup></b>	<b>20/28</b>	<b>72.13 (58.83 - 85.42)</b>	<b>51/97</b>	<b>49.05 (40.60 - 57.51)</b>	<b>117/274</b>	<b>46.01 (40.08 - 51.94)</b>	<b>47/159</b>	<b>30.18 (22.06 - 38.29)</b>

**Abbreviation:** CI = confidence interval

**Notes:**

1. Weighted based on HR, and distribution by sex and age in each HR (2011 census).
2. Montréal, Laval and Montréal's belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes - Mirabel Sud, Rivière-du-Nord - Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie.
3. The total can be higher than the sum of the cases in the periods of December to March and March to June since for some individuals (N=4) the ratio increase (>1.5) could not be observed in the first two periods but was visible for the total period (December to June).



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### Sex-stratified seroprevalence

Using the ratio approach, the weighted anti-N seroprevalence was similar in men and in women. From December 2021 to March 2022, it was estimated at 24.28% (CI 95%=19.99%-28.57%) in men and 21.39% (CI 95%=15.54%-27.24%) in women ([Table 5](#)). From March 2022 to June 2022, the weighted anti-N seroprevalence was 21.63% (CI 95%=17.51%-25.75%) in men and 19.28% (CI 95%=13.65%-24.91%) in women. From December 2021 to June 2022, the weighted anti-N seroprevalence was 46.56% (CI 95%=41.57%-51.55%) in men and 42.79% (CI 95%=35.73%-49.85%) in women.

**Abbreviation:** CI = confidence interval

**Table 5. Sex-stratified anti-N seroprevalence using the ratio approach**

	December 2021 to March 2022				March 2022 to June 2022				December 2021 to June 2022			
	Men		Women		Men		Women		Men		Women	
	n/N	Seroprevalence (%), <sup>1</sup> (CI 95%)	n/N	Seroprevalence (%), <sup>1</sup> (CI 95%)	n/N	Seroprevalence (%), <sup>1</sup> (CI 95%)	n/N	Seroprevalence (%), <sup>1</sup> (CI 95%)	n/N	Seroprevalence (%), <sup>1</sup> (CI 95%)	n/N	Seroprevalence (%), <sup>1</sup> (CI 95%)
<b>Greater area</b>												
Montréal-belt <sup>2</sup>	11/50	24.18 (15.38-32.97)	3/24	12.50 (3.14-21.86)	9/50	19.58 (11.43-27.73)	3/24	12.50 (3.14-21.86)	21/50	45.95 (35.71-56.19)	8/24	33.33 (20.00-46.67)
Others	88/374	24.31 (19.40-29.22)	23/110	24.43 (17.32-31.53)	67/374	22.27 (17.50-27.03)	27/110	21.59 (14.79-28.39)	156/374	46.75 (41.04-52.46)	50/110	46.02 (37.78-54.26)
<b>Total</b>	<b>99/424</b>	<b>24.28</b> <b>(19.99-28.57)</b>	<b>26/134</b>	<b>21.39</b> <b>(15.54-27.24)</b>	<b>76/424</b>	<b>21.63</b> <b>(17.51-25.75)</b>	<b>30/134</b>	<b>19.28</b> <b>(13.65-24.91)</b>	<b>177/424</b>	<b>46.56</b> <b>(41.57-51.55)</b>	<b>58/134</b>	<b>42.79</b> <b>(35.73-49.85)</b>

**Notes:**

1. Weighted based on age in each HR (2011 census).
2. Montréal, Laval and Montréal's belt, which includes the Lanaudière region, the local health and social services networks of Deux-Montagnes - Mirabel Sud, Rivière-du-Nord - Mirabel-Nord and Thérèse-De Blainville in the Laurentides region, and the local health and social services networks of Champlain and Pierre-Boucher in Montérégie.

## **Limitations**

This phase of the study had several limitations. First, due to seroreversion, the anti-N ratio does not remain  $>1.5$  indefinitely in infected individuals. In fact, seroreversion was observed in 12 of the 558 study participants (2.15%). Accordingly, the ratio approach cannot be used over periods of more than six months without a drop in sensitivity. Second, individuals who contracted SARS-CoV-2 several times may not have been able to donate plasma as often as they wished, and these donors may be underrepresented in our sample. Third, the seroprevalences observed in our study more strongly reflect the rate of infections happening at least 1-2 weeks before the most recent samples were collected, since the primary humoral response, which is responsible for anti-N production, is optimal approximately 1-2 weeks after an infection occurs and individuals have to refrain from making a donation for the two ensuing weeks. The seroprevalence observed between December 2021 and June 2022 is thus a stronger reflection of the number of infections that occurred up to mid-May 2022. Fourth, given that the ratio approach only detects recent infections, this method underestimates the proportion of individuals who were infected since the start of the pandemic. One would therefore have to add the seroprevalence (or cumulative incidence of cases reported by Public Health) in late fall 2021 to the number obtained for the period between December 2021 and June 2022 for a more accurate representation of the cumulative incidence of infection since the start of the pandemic. This number would still be an underestimation of the actual total cumulative incidence because of anti-SARS-CoV-2 seroreversion. One must also consider the possibility that some individuals were infected more than once since the start of the pandemic, which could of course skew the incidence estimate. Finally, the number of eligible participants was limited, since each had to have given plasma on three occasions between December 2021 and June 2022. This may have hindered the detection of statistically significant trends in the region and/or age-stratified analyses. Despite this limitation, the ratio approach is far more sensitive than the conventional approach while also being specific, as shown by the analyses described above (see section “Method” subsection “Anti-N ELISA — ratio approach”) and the phase 4a results.

## **Conclusion**

Using the ratio approach, the weighted anti-N seroprevalence was estimated at 45.32% in Quebec between December 2021 and June 2022. This number is far higher than the number obtained using the conventional approach (30.74%), confirming that the latter is not sensitive enough when studying vaccinated individuals. Anti-N seroprevalence was particularly high among younger individuals (e.g., 18-25 years: 72.13%;  $>65$  years: 30.18%), suggesting that this group remains the primary vector of SARS-CoV-2 transmission in Quebec.



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