WP N°3 Deliverable N°3.2,

WANING ANTIBODIES IN GENERAL POPULATION, TESTED POSITIVE FOR SARS-COV2 SEROLOGY

INSERM

WP3_ D3.2

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History of Changes

Version	Date	Created/Modified by
0.1	29/05/2022	Jérome Le Chenadec, Carmelite Manto, Sophie Novelli, Anne Pastorello, Alexandra Rouquette, Laurence Meyer, Josiane Warszawski

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0.2	07/06/2022	Evelina Tacconelli (UNIVR)
1.0	29/07/2022	For **EpiCov**: Josiane Warszawski, Laurence Meyer, Carmelite Manto For **CON-VINCE**: Rejko Krueger, Marc O'Sullivan, Joelle Fritz, Michel Vaillant, Dmitry Bulaev, Alexey Kolodkin For **Koco-19**: Noemi Castelletti, Christina Reinkemeyer

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Rationale for modifying D3.2

The ORCHESTRA project proposal (including D3.2) was written at the beginning of the pandemic in March 2020 and kicked off in December 2020. At the time the scientific collaboration effectively started, a brief review of new scientific evidence showed that seroprevalence in European population based-cohorts had already been extensively described and compared. Some evidence of that below:

- *Grant R, Dub T, Andrianou X, Nohynek H, Wilder-Smith A, Pezzotti P, Fontanet A. SARS-CoV-2 population-based seroprevalence studies in Europe: a scoping review. BMJ Open. 2021 Apr 1;11(4):e045425.*
- *Rostami A, Sepidarkish M, Leeflang MMG, Riahi SM, Nourollahpour Shiadeh M, Esfandyari S, Mokdad AH, Hotez PJ, Gasser RB. SARS-CoV-2 seroprevalence worldwide: a systematic review and meta-analysis. Clin Microbiol Infect. 2021 Mar;27(3):331-340.*
- *Lai CC, Wang JH, Hsueh PR. Population-based seroprevalence surveys of anti-SARS-CoV-2 antibody: An up-to-date review. Int J Infect Dis. 2020 Dec;101:314-322.*
- *Vaselli NM, Hungerford D, Shenton B, Khashkhusha A, Cunliffe NA, French N. The seroprevalence of SARS-CoV-2 during the first wave in Europe 2020: A systematic review. PLoS One. 2021 Nov 2;16(11):e0250541.*

In the meantime, the question regarding the antibodies decline was raised with the following hypothesis: Levels of Sars-CoV-2 specific IgG antibodies decline over time; decline would depend on factors such as age, symptomatic forms of Covid-19. To add a real value with our data from different population-based cohorts in Europe (and not only from cohorts of ill or hospitalized people, in whom the decline could be different) and address new research questions, we proposed to measure the speed of decay of anti-Sars-CoV-2 IgG antibodies and evaluate factors associated with a rapid or slow decay of antibody levels. According to this, the new title suggested for this Deliverable is: "**Waning antibodies in general population, tested positive for sars-cov2 serology** ».

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Executive summary

Background: Here, we aimed to study the waning in anti-SARS-Cov2 ELISA-S IgG antibodies, using EuroImmun test, among people infected during the first wave of the Covid-epidemic, that is before July 2020, in adult general population, among people who were tested at least twice in the Epicov cohort (France), the Koco19 cohort (Munich, Germany), the CON-VINCE cohort (Luxembourg).

Methods: Home self-sampling on dried blood was proposed to a random subsample in May and November 2020 in EpiCov. Blood sampling was collected at 6 visits after initial test between April 2020 and May 2021 in Con-Vince. Blood sampling was collected at several visits after initial positive PCR in Koco-19. For the three cohorts, we selected here all adult individuals aged 18-79, having a positive ELISA-S result, defined by an Euro-immun IgG optical density ratio ≥ 1.1, between February and July 31th 2020, with at least one subsequent IgG measurement during the following year. The main studied outcomes were: 1) the median IgG at initial and subsequent time point measurement, 2) the relative proportion of individual changes in IgG at each point; 3) the proportion of people who became Elisa-S negative, defined as optical density ratio <0.7. Risk factors of waning antibodies at 6 months could be studied in the EpiCov cohort.

Results: The number of participants included here were: 599 in EpiCov, 16 in Con-Vince and 65 in Koco-19. The overall median initial IgG level in 18-79 years old was 2.6 (1.6-4.5) in EpiCov, 3.6 (1.6-5.4) in CON-VINCE, and 3.7 (2.1-5.7) in Koco-19. Overall, the relative proportion of individual changes in IgG level was +12% (IQR: -8;35) at 1 month, +14% (IQR: -11;48) at 2 months and -1% (IQR: -10;10) at 4 months in Koco-19, -55% at 6 months (IQR: -70;-30) in EpiCov, and -63% (IQR: -78;-33) in non-vaccinated in CON-VINCE at 1 year. Among the people positive (IgG ratio >1.1) in May-July 2020, the proportion who became negative (<0.7) was 0 % within the 4 subsequent months in Koco-19 (with respective upper CI bounds: 8.8%, 13.2%, 19.5%), 31.3% at 6 months (95%CI: 24.4-39.1) in EpiCov, and 31.3% (95%CI: 11.0-58.7) at 1 year in non-vaccinated in CON-VINCE.

Initial level of IgG antibodies and seroneutralization positivity were the only independent factors of seroreversion. Age, a history of covid-like symptoms at first round, especially if onset was recent, and subsequent persistence of long covid symptoms were also associated with a lower risk of seroreversion in univariate analysis, though the association did not remain significant after adjustment for initial level of IgG serology.

Conclusion: The level of IgG increased at 1 month after the initial positive PCR, to remain quite stable during the first 60-120 days as seen in Koco-19. Most waning may occur during the first six months after the initial IgG peak, with near one-third people who became negative, similar at 6 months (EpiCov) and 12 months (Con-Vince). in EpiCov, a lower initial anti-SARS-CoV-2 ELISA IgG level was a strong predictor of waning antibodies, which may be explained at least partially by a less recent infection, as suggested by Koco-19 results.

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Context and objective

European countries have been severely affected by COVID-19.

In France, Germany and Luxembourg, cohorts of general population using Euro-immun serological tests and longitudinal follow-up, were set up early after the first peak of the epidemy.

The EpiCov cohort is a large French national random population-based public health study, set up in May 2020 during the first national lockdown to study the diffusion of Covid-19 virus in the population and to study relations between the epidemic, health and living conditions, with a second round including serological test in November 2020 among participants of the first round in May.

The CON-VINCE cohort is a statistically representative sample of a Luxembourg-based adult population and began in April 2020. The project aimed to evaluate the dynamics of the spread of COVID-19, with a specific interest in the asymptomatic and oligosymptomatic individuals, within the Luxembourgish population by testing a representative panel of over 1,800 people for the presence of the SARS-CoV-2 virus. The CON-VINCE participants were followed up four times every 2 weeks after the initial visit and then again twice more around 12 months after the initial visit.

The KoCo19 cohort is a large community-based prospective cohort study with a representative sample based on 5,000 participants randomly chosen within 2,994 households within the Municipality of Munich. The aim of the KoCo19 study and substudies was to monitor the true number of infected individuals, to measure and detect changes in immune status of antibody titer and to understand the serological short-, medium- and longterm immune response in unvaccinated, vaccinated, infected and uninfected individuals. Study participants were enrolled from April 2020 on and followed up on a regular basis.

Here, we aimed to study the waning in anti-SARS-Cov2 ELISA-S IgG antibodies, using EuroImmun test, among people infected during the first wave of the Covid-Epidemic, that is before July 2020, in adult general population aged, among people who were tested at least twice in the Epicov cohort (France), the Koco19 cohort (Munich, Germany), the CON-VINCE cohort (Luxembourg).

Study design of Epicov, Koco-19, CON-VINCE

The EpiCov cohort

EpiCov is a population-based cohort, based on a probability sample, which combines a questionnaire survey with Covid-19 serological tests performed on home blood self-samples of respondents.

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Individuals aged 15 years or older living in France were randomly selected from the national FIDELI administrative sampling frame. FIDELI is the national database on housing and individuals issued from tax files, containing demographic information on people and household structure and income, and additional contextual data about the living place of people. Differential sampling was used to ensure oversampling of the less densely populated "*départements"* (i.e. French administrative districts), and lower-income categories. Residents in nursing homes for elderly persons were not included.

The two first rounds were conducted in 2020. The first round (May 2nd to June 1rst) included home capillary blood self-sampling for serological testing proposed during the web/telephone questionnaire to a national random subsample. The second round (October 26th to December 14th) was proposed to all respondents, all being eligible for home self-sampling. The third round was conducted in summer 2021 (June 24th to August 9th), with no self-sample.

Home capillary blood self-sampling for serological testing

Dried-blood spots were collected on 903Whatman paper (DBS) kits. The test is considered as positive (ELISA-S+) with an optical density ratio ≥1.1, according to the threshold specified by the manufacturer. All samples with an ELISA-S test optical density ratio ≥ 0.7 were also tested with an in-house microneutralization assay to detect neutralizing anti-SARS-CoV-2 antibodies. The seroneutralization was considered as positive (SN+) with titer ≥ 40.

The Koco-19 cohort

The KoCo19 cohort is a population-based cohort, based on 5,000 participants amongst 2,994 households within the Municipality of Munich, and includes several substudies which aim to understand the serological short-, medium- and longterm immune response in unvaccinated and vaccinated individuals. Participants were enrolled from April 2020 on and followed up over 114 days after natural infection, proven by a positive RT-PCR. EDTA-plasma was collected as soon as possible after the first positive RT-PCR and at multiple occasions thereafter to describe time to seroconversion, peak value and longevity of the serological markers investigated.

Euroimmun Anti-SARS-CoV-2-ELISA anti-S1 IgA/IgG (hereafter called EI-S1-IgG, EI-S1-IgA; Euroimmun, Lübeck, Germany) test kits were used according to the manufacturer's instructions. Measurement raw values were obtained using the quotient of the optical density measurement provided by the manufacturer's software. The positivity threshold is set to 1.1 by the manufacturer.

Quantitative Euroimmun Anti-SARS-CoV-2-QuantiVac ELISA (IgG) (hereafter called EI-S1-IgG-quant; Euroimmun, Lübeck, Germany) test kits were used according to the manufacturer's instructions. Measurement values were obtained using the manufacturer's software taking into

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account the correction formula for BAU provided. The positivity threshold is set to 1.1 by the manufacturer.

The CON-VINCE cohort

Blood, nasopharyngeal swab, and stool sampling was completed at 6 of the 7 visits, figure 1.

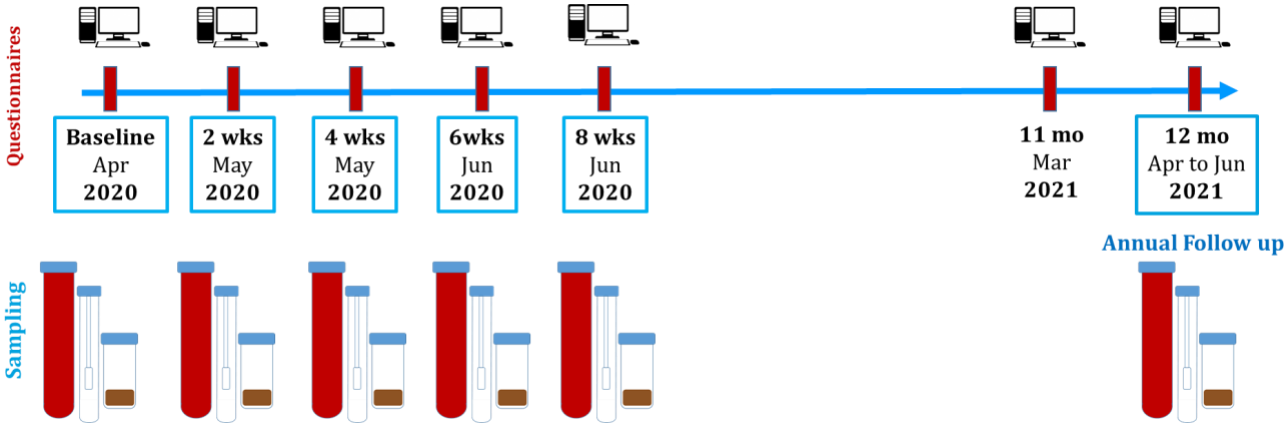

Figure 1. CON-VINCE timeline.

Anti-SARS-CoV-2 IgG were determined by CE-labelled enzyme-linked immunosorbent assay (ELISA) kits (most recent versions of Euroimmun Anti-SARS-CoV-2 ELISA IgG) according to the manufacturer's instructions and as described by others (Streeck et al., 2020). The optical density (OD) was measured at 450 nm from which background OD measured at 650 nm was deducted. OD ratios were calculated by dividing the resulting OD by the OD of the calibrator, which is included in the kit. In house quality controls, prepared to give an expected OD ratio of approximately three times the threshold for positivity, were included in all assays. Samples with OD ratios <0.7 were considered negative, OD ratios ≥1.1 were considered positive and samples with intermediate OD ratios (>0.7, <1.1) were judged borderline positive.

Study population, criteria and statistical analysis for the current analysis

Study population

For this analysis, we selected all 18-79 years-old adult individuals having a positive ELISA-S results, defined by an Euro-immun IgG optical density ratio ≥ 1.1, before July 31th 2020, and who had at least one subsequent IgG measurement during the following year.

Main outcome criteria

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1°) Median IgG with interquartile range (IQR) at initial point and at each subsequent time point studied in the three cohorts

2°) Relative proportion of individual changes in IgG at each time points studied in the three cohorts

3°) The proportion of people who became ELISA-S negative **defined as optical density ratio <0.7 at each timepoint studied in the three cohorts**

Exposure: the analysis was performed overall, and according to gender and age.

Initial time point serological measurement

The initial time at measurement was the first time with Elisa-S ≥ 1.1, from February 2020 to 31 July 2020.

Subsequent time point serological measurement

They differed for each cohort.

- For EpiCov, there was only one repeated measurement, all being performed in the second round in November 2020, that is at 6 months from the first round
- For Koco-19, it was repeated several times, and we defined three periods: 1st month (< 30 days), 2 months (30 - < 60 days), 4 months (60- <120 days)
- For CON-VINCE, it was performed in May-June 2021, that is 1 year after first measurement. For this point, we considered only those who had not received Covid vaccination.

Statistical analysis

Analyses were performed separately for each cohort:

- Overall, median with interquartile of IgG optical density ratio were estimated at initial measurement ≥ 1.1 and each subsequent time measurement.
- For each participant, the relative % change in IgG level was estimated at each subsequent point measurement as the difference in IgG density ratio between this point and the time point, divided by IgG ratio at initial time point, multiplied by 100. The median of relative % change with IQR was then estimated in each cohort at each time point measurement.
- The prevalence, with 95% confidence intervals, of people which became negative (ratio <0.7) was estimated, using exact binomial calculation if necessary

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***In the EpiCov cohort*,** the unequal probabilities sampling design were considered, and final calibrated weights were calculated to correct for non-reponse, as detailed elsewhere (Warszawski et al., 2021), with the specific design-based “proc survey” procedures of SAS and “svy” procedures of STATA. Proportions with confidence interval and median with IQR were estimated, using weighted percentages, and logit transformed confidence limits were used to remain within the interval [0,1].

Since part of people tested in May 2020 had been infected before the lockdown with decreasing IgG level from that time, a sensitivity analysis was extended to people tested non-negative (ELISA-S+ $\geq$ 0.7) in May. This sensitivity analysis was done only in EpiCov for this deliverable.

***In the CON-VINCE Cohort*,** statistical analyses were performed in accordance with the methodology for all cohorts described above, using R (version 4.0.2) and RStudio (version 2022.02.0). No weighting procedures were applied. Medians and IQRs were calculated using the *summary()* function from the “*base*” library. Library “*binom*” (function *binom.confint()* was used to compute the confidence intervals for prevalence summaries, with the choice of exact binomial option (Pearson-Klopper method).

***In the Koco19 cohort*,** statistical analyses were performed in accordance with the methodology for all cohorts described above, using R (version 4.0.2) and RStudio (version 2022.02.0). No weighting procedures were applied. Medians and IQRs were calculated using the *summary()* function from the “*base*” library.

Complementary analysis from the EpiCov cohort

We studied risk factors of waning at 6 months, defined as becoming ELISA-S negative at the second round (November 2020), according to the main outcome criteria.

The factors studied here were age (categorized into 5 classes), gender, initial level of IgG antibodies and seroneutralization (positive versus negative) at first round, initial covid-like symptoms reported at first round, and whether having developed or not a subsequent long-covid.

The level of IgG antibodies among people with ELISA-S+ tests in May 2020 was categorized according to the quartile of positive results, the lower bounds of each categories being respectively :0.7, 1.7, 2.9, 4.7.

Covid-like symptoms definition at first round was adapted from the ECDC definition (ECDC 2020) comprising anosmia or dysgeusia, and/or fever with at least cough, dyspnoea or thoracic pain; there were categorized among three groups: no, covid-like symptoms started in 2020 before the first lockdown confinement (March 17th 2020), covid-like symptoms started during or after first lockdown before sampling.

Long-covid symptoms among people who reported Covid-like symptoms was defined as persistence in November 2020 of symptoms (anosmia or dysgeusia, fever, cough, dyspnoea, headache, breathing

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difficulties, fatigue, muscular pain) which had occurred less than 3 months after the first initial covid-like symptoms, adapted from the OMS definition (Soriano JB 2021).

For complementary analysis, we performed two multivariate logistic regressions, with negative ELISA-S test in November as the dependent variable. Both included age, gender, initial level of IgG and seroneutralization positivity. The first model included also the time at first initial covid-like symptoms and the second one the development of long covid symptoms. Each of these variables had no initial covid-like symptoms as commune category.

The design-based Pearson chi-squared test statistic developed by Rao was used for multiway contingency tables (Rao JN, 1984). Crude and adjusted odds ratios were estimated with logistic regression models based on design-based methods (Skinnet CJ 1989). The significance threshold was 0.05.

Findings

Participants

EpiCov

We selected for this study the 599 individuals with positive ELISA-S optical density ratio ($\geq$ 1.1) in May 2020 at first round (May 2020) and had a second measurement at second round in November 2020.

CON-VINCE

Similar to Epicov, we selected the 16 participants who with positive ELISA-S optical density ratio ($\geq$ 1.1) in April-June 2020 and had a second measurement in May-June 2021 without being vaccinated.

Koco-19

We selected 65 unvaccinated individuals who were PCR-tested positive for COVID-19 from February 2020 on, were tested positive (IgG $\geq$ 1.1) before 31.07.2020 and had at least one second measurement.

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Initial level of IgG May-July 2020 at first measurement >1.1 (Table 1)

The overall median initial IgG level in 18-69 years old was 2.6 (1.6-4.5) in EpiCov, 3.6 (1.6-5.4) in CON-VINCE, and 3.7 (2.1-5.7) in Koco-19.

Changes in IgG level over time (Table 2)

Overall, the relative proportion of individual changes in IgG level was +12% (IQR: -8;35) at 1 month, +14% (IQR: -11;48) at 2 months and -1% (IQR: -10;10) at 4 months in Koco-19, -55% at 6 months (IQR: -70;-30) in EpiCov, and -63% (IQR: -78;-33) in non-vaccinated in CON-VINCE at 1 year.

Proportion of subsequent Covid seronegativity (Table 3)

Among the people positive (IgG ratio >1.1) in May-July 2020, the proportion who became negative was 0 % within the 4 subsequent months in Koco-19 (with respective upper CI bounds: 8.8%, 13.2%, 19.5%), 31.3% at 6 months (95%CI: 24.4-39.1) in EpiCov, and 31.3% (95%CI: 11.0-58.7) in non-vaccinated in CON-VINCE at 1 year.

Complementary analysis in EpiCov: Factors associated with the probability to become seronegative in November 2020 when seropositive in May 2020

In univariate analysis (Table 4), the probability to become seronegative in November was significantly associated with serological status, reporting symptoms at first round, and age. It was not associated with gender.

The probability to become seronegative in November was the highest (61.0%) in people with lowest quartile of IgG ratio in May and the lowest (0.1%) with highest quartile ($p<0.001$). It was also higher when the seroneutralisation status in May was negative compared with positive (54.7% versus 20.1%; $p<0.001$).

Overall, 36.9% of seropositive people in May first round who did not report a history of covid-like symptoms became seronegative in November. The risk tended to be lower in people who reported previous covid-like symptoms, especially if they had occurred more recently (12.0% if occurred after midmarch versus 24.7% before) or if they were followed by developing subsequent long-covid (17.0% versus 36.9%).

Becoming seronegative in November was associated with age ($p<0.001$), with the lower risk in younger people aged 15-29 years (19.1%) compared with 30-39 (54.7%), and 40-49 years (32.1%). The risk was quite similar in youngest and oldest people (14% in 50-59 years, and 22.0% in 60 years or more), In multivariate logistic regression, only initial lower level of IgG and seroneutralization negativity remained independently associated with a higher risk of becoming seronegative in November, when positive in May, after adjustment for age, gender and covid-symptoms.

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Adding people with initially indeterminate IgG, the median level of IgG decreased from 1.32 (IQR: 0.85-3.11) in May to 0.75 (0.46-1.69). The pattern for age differed among non-negative people (IgG $\geq$0.7) in May, as the risk to become seronegative was much twice higher in the oldest than in the youngest category (53.2% in people aged 60 years or old versus 28.6% in aged 15-29 years) (Table 4). Similar results in multivariate analysis are observed when studying risk of becoming seronegative in November when non-negative people (IgG $\geq$0.7) in May (data not shown).

Discussion and conclusion

In the Koco-19 cohort, initial Euro-immun Elisa-S IgG test was repeated in participants since the first positive PCR. The level of IgG tended to increase at 1 month after the initial positive test, to remain quite stable during the first 60-120 days. However, the relative proportion of individual changes in IgG level stopped to increase at 4 months, which may indicate waning start. None of them became negative during the first 4 months.

In the EpiCov and Con-Vince cohorts, initial Euro-immun Elisa-S IgG test was performed independently of history of symptoms or PCR tests. This initial test had most likely been performed after reaching the peak of IgG in infected participants. In both cohorts, the level of IgG strongly declined on the second sample after the first positive test, with a quite similar individual relative change: in median, -55% at 6 months in EpiCov and -63% at 1 year in Con-Vince, and one third became respectively seronegative in both cohorts. This may indicate that most waning occur several months after the peak.

As observed in the EpiCov cohort, initial level of IgG antibodies and seroneutralization positivity were the only independent factors of seroreversion. Age, a history of covid-like symptoms at first round, especially if more recent, and subsequent persistence of long covid symptoms were also associated with a lower risk of seroreversion in univariate analysis, though the association did not remain significant after adjustment for initial level of IgG serology.

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List of tables and figures will be updated afterwards

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Table 1– Median IgG from first positive serology to subsequent time measurements, among adults having positive serology before August 2020 (Elisa-S Ig ≥ 1.1).

	EPICOV (France) Elisa-S Ig ratio level IgG > 1.1 before May 2020					CON-VINCE (Luxembourg) Elisa-S Ig ratio level IgG > 1.1 before Aug 2020 **(non-vaccinated)**				
	T0 (May 2020)			6 months (Nov 2020)		T0 (Apr June 2020)			12 months (May - June 2021)	
	N	Median	IQR	Median	IQR	N	Median	IQR	Median	IQR
ALL	579	2.6	[1.6; 4.5]	1.1	[0.6; 2.6]	16	3.6	[1.6; 5.4]	1.4	[0.7; 2.1]
Gender										
Men	217	2.6	[1.5; 5.0]	1.0	[0.6; 3.0]	8	3.0	[1.5; 5.7]	0.8	[0.5; 1.6]
Women	362	2.5	[1.6; 4.2]	1.1	[0.6; 2.4]	8	3.7	[2.4; 4.6]	1.5	[1.3; 2.1]
Age (years)										
18-29	72	3.1	[1.7; 5.0]	1.3	[0.7; 2.5]	3	2.8	[2.1; 3.2]	1.4	[0.9; 1.9]
30-39	122	1.9	[1.5; 2.7]	0.6	[0.4; 1.2]	7	3.4	[1.4; 4.1]	1.4	[0.9; 1.8]
40-49	158	2.4	[1.4; 3.4]	0.8	[0.6; 1.3]	4	3.7	[2.1; 6.7]	0.7	[0.4; 1.4]
50-59	133	4.0	[2.0; 7.5]	2.6	[0.9; 5.0]	2	6.7	[6.4; 6.9]	2.9	[2.1; 3.6]
60-79	94	3.9	[1.8; 6.8]	2.0	[0.9; 3.0]	0				

	Koco-19 Elisa-S Ig ratio level IgG > 1.1 before Aug 2020											
	T0			< 30 days			[30-60[days			[60-120] days		
	N	Median	IQR	N	Median	IQR	N	Median	IQR	N	Median	IQR
All	65	3.7	[2.1; 5.7]	40	4.2	[2.8; 5.8]	26	3.4	[2.8; 4.8]	17	4.9	[3.3; 5.6]
Gender												
Men	32	3.1	[2.1; 5.9]	17	3.9	[2.6; 6.2]	14	3.5	[2.9; 4.8]	10	5.1	[3.5; 5.6]
Women	33	3.8	[2.4; 5.5]	23	4.2	[3.0; 5.6]	12	3.4	[2.7; 4.5]	7	4.3	[3.2; 5.3]
Age (years)												
18-29	29	3.8	[1.7; 4.4]	17	3.2	[2.6; 5.1]	12	3.3	[2.4; 4.6]	5	5.2	[4.2; 5.7]
30-79	36	3.7	[2.2; 6.3]	23	5.5	[3.8; 6.7]	14	3.6	[3.1; 4.8]	12	4.6	[3.1; 4.8]

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Table 2– Relative individual changes % of IgG from first positive serology to subsequent time measurements, among adults having positive serology before August 2020 (Elisa-S Ig ≥ 1.1).

	EPICOV (France) IgG > 1.1 before May 2020			**CON-VINCE (Luxembourg)** IgG > 1.1 before Aug 2020		
	Individual %relative IgG change at 6 months			Individual %relative IgG change at 1 year **(non-vaccinated)**		
	N	Median %	IQR	N	Median %	IQR
ALL	579	-55	[-70; -30]	16	-63	[-78; -33]
Gender						
Men	217	-49	[-65; -37]	8	-78	[-81; -41]
Women	362	-59	[-71; - 26]	8	-56	[-69; -11]
Age (years)						
18-29	72	-52	[-63; -23]	3	-12	[-50; -6]
30-39	122	-66	[-76; - 49]	7	-53	[-63; -26]
40-49	158	-58	[- 72; - 44]	4	-80	[-81; -78]
50_59	133	-40	[-61; -21]	2	-58	[-68; -49]
60-79	94	-49	[- 73; - 21]	0		

	Koco-19 Elisa-S Ig ratio level IgG > 1.1 before Aug 2020								
	Individual %relative IgG change at < 30 days			Individual %relative IgG change at [30-60[days			Individual %relative IgG change at [60 -120 [days		
	N	Median %	IQR	N	Median %	IQR	N	Median %	IQR
ALL	40	12	[-8; 35]	26	14	[-11; 48]	17	-1	[-10; 10]
Gender									
Men	17	9	[-8; 35]	14	18	[-1; 56]	10	-5	[-19; 6]
Women	23	15	[1; 31]	12	-6	[-15; 45]	7	0	[-2; 27]
Age (years)									
18-29	17	11	[-8; 24]	12	7	[-13; 32]	5	-3	[-8; -1]
30-79	23	12	[-6; 43]	14	18	[-10; 56]	12	1	[-13; 13]

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Table 3 – % of negative IgG serology (<0.7) at subsequent time measurements, among adults having positive serology before August 2020 (Elisa-S Ig ≥ 1.1).

	EPICOV (France) IgG > 1.1 before May 2020				**CON-VINCE (Luxembourg)** IgG > 1.1 before Aug 2020			
	% IgG negative <0.7 at 6 months (November 2020)				% IgG negative <0.7 at 12 months (May June 2021) **(non-vaccinated)**			
	N	%	(n)	95%CI	N	%	(n)	95%CI
ALL	579	31.3	(141)	[24.4 – 39.1]	16	31.3	(5)	[11.0 – 58.7]
Gender								
Men	217	29.1	(43)	[19.1 – 41.7]	8	50.0	(4)	[15.7 – 84.3]
Women	362	32.4	(97)	[23.7 – 42.4]	8	12.5	(1)	[0.3 – 52.7]
Age (years)								
18-29	72	25.1	(11)	[8.6 – 54.4]	3	33.3	(1)	[0.8 – 90.6]
30-39	122	54.7	(40)	[39.9 – 68.8]	7	28.6	(2)	[3.7 – 71.0]
40-49	158	32.1	(47)	[21.9 – 44.5]	4	50.0	(2)	[6.8 – 93.2]
50-59	133	14.0	(25)	[7.3 – 25.4]	2	0.0	(0)	[0.0 – 84.2]
60-79	94	23.1	(17)	[11.4 – 41.3]	0			

	Koco-19 Elisa-S Ig ratio level IgG > 1.1 before Aug 2020								
	% IgG negative <0.7 at < 30 days			% IgG negative <0.7 at [30-60[days			% IgG negative <0.7 at [60 -120 [days		
	N	%	Exact 95%CI	N	%	Exact Upper bound 95%CI	N	%	Exact Upper bound 95%CI
All	40	0.0	[0.- 8.8]	26	0.0	[0-13.2]	17	0.0	[0-19.5]
Gender									
Men	17	0.0	[0-19.5]	14	0.0	[0-23.2]	10	0.0	[0-30.8]
Women	23	0.0	[0-14.8]	12	0.0	[0-26.5]	7	0.0	[0-41.0]
Age (years)									
18-29	17	0.0	[0-19.5]	12	0.0	[0-26.5]	5	0.0	[0-52.2]
30-79	23	0.0	[0-14.8]	14	0.0	[0-23.2]	12	0.0	[0-26.5]

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Table 4 – Complementary analysis - Percentages of people with negative serology in November 2020 (Elisa-S Ig <0.7), among people having positive serology in May 2020 [1] (Elisa-S Ig ≥ 1.1), according to gender, initial level of IgG, seroneutralisation status and symptoms *living in mainland France [2] (the national EpiCov cohort, rounds 1 & 2).*

	% of negative IgG (ratio < 0.7) in Nov 2020												
				UNIVARIATE ANALYSIS [3]				MULTIVARIATE ANALYSIS (1) [3]			MULTIVARIATE ANALYSIS (2) [3]		
	N	%	(n)	95% CI	OR_{crude}	95% CI	p	OR_{adj}	95% CI[3]	p	OR_{adj}	95% CI	p
All with IgG > 1.1 in May 2020	599	29.5	(141)	[22.8 - 37.2]									
Initial level of IgG													
1st quartile [0.700-1.700]	149	61.0	(94)	[47.9-72.6]	ref		<0.001	ref		<0.001	ref		<0.001
2nd quartile]1.700-2.918]	150	34.4	(39)	[21.2-50.5]	0.3	[0.1-0.7]		0.2	[0.1-0.6]		0.3	[0.1-0.6]	
3rd quartile]2.918-4.690]	150	15.1	(7)	[4.1-42.3]	0.1	[0.02-0.5]		0.1	[0-0.6]		0.1	[0-0.6]	
4th quartile > 4.690	150	0.1	(1)	[0.0-0.7]	<0.001	[<-0.005]		<0.001	<0.001-0.001]		<0.001	<0.001-0.001]	
Seroneutralisation status													
Positive (SN >= 40)	427	20.1	(66)	[25.2-39.8]	ref		<0.001	ref		0.040	ref		0.03
Negative (SN <40)	146	54.7	(67)	[14.3 - 27.6]	4.8	[2.3-9.9]		2.7	[1-7]		2.8	[1.1-7.2]	
Gender													
Men	230	25.7	(44)	[16.2-38.1]	ref		0.36	ref		0.53	ref		0.42
Women	369	31.5	(97)	[23.0-41.4]	1.4	[0.7-2.9]		1.3	[0.6-3]		1.4	[0.6-3.2]	
Age (years)													
15-29	86	19.1	(11)	[6.1-46.2]	ref		0.001	ref		0.076	ref		0.075
30-39	122	54.7	(40)	[39.9-68.8]	5.3	[1.2-22.8]		4.8	[0.9-24.6]		4.7	[0.9-24]	
40-49	158	32.1	(47)	[21.9-44.5]	2.1	[0.5-8.7]		1.3	[0.3-6.9]		1.3	[0.2-6.9]	
50_59	133	14.0	(25)	[7.3-25.4]	0.7	[0.2-3.3]		1.5	[0.3-8]		1.4	[0.3-7.9]	
≥ 60	100	22.0	(18)	[11.0-39.2]	1.3	[0.3-6.5]		2.5	[0.4-16.2]		2.4	[0.4-15.7]	
Time at first covid-like symtoms													
During or after first lockdown	191	12.0	(17)	[4.3 - 29.1]	ref		0.034				ref		0.22
Before first lockdown	97	24.7	(15)	[10.8 - 46.9]	2.4	[0.5-10.8]					2.2	[0.5-10.3]	
Did not have covid-like symptoms	311	36.9	(112)	[27.7-47.1]	4.6	[1.4-15.3]					2.9	[0.9-9.9]	
Developed long-covid after covid-like symptoms													
Yes	54	8.1	(5)	[2.2 - 25.6]	ref		0.0044	ref		0.13			
No	234	17.0	(49)	[8.4 - 31.3]	3.1	[0.5-19]		2.4	[0.4-15.5]				
No covid-like symptoms	311	36.9	(278)	[27.7 - 47.1]	9.3	[1.7-50]		4.7	[0.8-26.4]				

1. Home sampling by finger prick/Euroimmun ELISA-S test

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2. People aged 15 years or over residing in mainland France, outside nursing homes for elderly and prisons.

3. The sampling design is considered for the estimation of prevalence, confidence intervals (logit transformation) and statistical tests, with the SAS procsurvey procedure. The percentages are weighted by sampling weight (the inverse of inclusion probability), corrected for non-response weights and calibrated on the margin of the census.

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