The Authors' Reply

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In reply to Samuel A. Concerns about "Evidence of early circulation of SARS-CoV-2 in France: findings from the population-based "CONSTANCES" cohort". *Eur J Epidemiol.* 2022

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We thank Dr Samuel for the comments (1), which gives us the opportunity to clarify.

Dr Samuel first mentions the lack of consideration in our work of the uncertainty about the specificity of the serum neutralization (SN) test. He estimated a lower bound of the specificity of the SN test, and used it to infer that our SN positive findings were likely false positive.
The SN test has indeed been validated on 2387 blood donor samples (between 2015 and 2018, none tested positive, and these data have not been fully published) but it is true that the cited reference (2) reports validation on 464 samples from 2017 and 2018.
However, Dr Samuel's conclusion that our findings could easily be explained by a lack of specificity of the neutralization test is not correct. First, there is no valid statistical argument for choosing the lower bound of the 95% confidence interval as the reference value for estimating the probability of false positive results in our sample. Indeed, a value as low as (or lower than) the lower bound had an a priori probability of 2.5%. Second, in our study, 6,020 samples were collected between November 2019 and January 2020, 176 had a positive Elisa-S of which 13 were also positive for SN. Assuming that all 176 Elisa-S samples were false positive (i.e. none of the 176 subjects were "truly infected"), and taking the lower bound of the 95% confidence interval for SN specificity estimated from the 464 published samples (lower bound Specificity= 99.2%, 95% Clopper-Pearson exact Confidence Interval), the probability of observing 5 or more false positive SN tests out of 176 samples was 1.4%, and 13 or more false positive tests out of 176 was $2.6\ 10^{-9}$. Using the lower bound of specificity estimated from the 2387 samples (99.85%), the probability of 5 or 13 false positive would dramatically decrease. Third, it can be recalled that SN is frequently used as a gold standard to identify false positive serological tests in many diseases (dengue fever, Japanese encephalitis etc...), and in our study, SN tests were replicated 6 times to limit the risk of misclassification by measurement error.

In a second part, Dr Samuel raises an interesting point that cross-reactive immunity with seasonal human coronaviruses (HCoV) might have led to a selection bias when restricting our SN tests to sample with Elisa-S ≥ 0.7. In other words, all Elisa-S ≥0.7 that were also SN positives were false positive by cross-reaction, and none of the Elisa-S <0.7 remaining samples would have been SN positive, if they had been tested. This assumption seems hardly realistic. In reference with our response to the first comment, all 2387 samples that were tested negative by SN were not selected according to a prior history of HCoV infection or given their Elisa-S test value - therefore, our initial estimate of 100% SN specificity applied to people with or without prior history of HCoV infection and irrespective of their unknown Elisa-S value.
Second, we performed additional analyses on 4704 samples collected in September and October 2019 (the biological collections in CONSTANCES started in 2018 and additional analyses are underway). Preliminary findings suggest positivity to Elisa-S and other SARS-CoV-2 serological tests (IgG against RBD or Spike evaluated with high-throughput multiplex technology on a Luminex® platform) in some participants, while only 1 participant with other positive tests had SN antibodies at 40 - this participant was sampled on Oct 10, 2019. We also found some evidence of cross-reactivity between seasonal HCoV and SARS-CoV-2 with higher levels of IgG anti-RBD against HCoV (229E, KHU1, NL63) in anti-SARS-COV-2 Elisa-S positive

samples than in negative ones, but we did not identify association between serological tests results of HCoV and anti-SARS-CoV-2 SN.
Although, as already discussed in our paper, we cannot totally exclude that part of our SN positive were false positive results, we do not believe that all our SN positive samples identified between November 2019 to January 2020 were false positive. Importantly, detailed questioning of the exposure circumstances of some participants shows the occurrence of suggestive symptoms in the days preceding the date of sampling or possible exposure in China.

In another comment, Dr Samuel points out the increase in Elisa-S positivity from early January while SN positivity appeared quite stable before and after January 2020. We have no clear explanation for this result. Remind that the increase of antibody titers occurred within 2 weeks after infection, the increase of Elisa-S positivity in early January is unlikely explained by a wide spreading of SARS-CoV-2 from mid-December 2019. Rather, it could correspond to cross reaction with HCoV as we indicated above (HCoV peaks are observed between December and March in Europe (3)) while SN positivity among Elisa-S positive sample could correspond to real SARS-CoV-2 infection.

In a last comment, it is unclear how Dr Samuel came to a "prevalence" of 5%, while we clearly never assumed that all Elisa-S positive samples were true infected persons. If we restricted our selection to the 13 participants with both SN and Elisa-S positivity, the cumulative incidence of SARS-CoV-2 in end-January 2020 would be 0.3%. In addition, if we assumed that 5 to 6 participants among these 13 patients were indeed true positive, it means that fifty thousand people would have been infected with SARS-CoV-2 between November 2019 and January 2020 in France, which based on estimated infection hospitalization or death ratios would have converted into one thousand hospitalizations and approximately two hundred and fifty deaths (4). It is however possible that such events if they were caused by SARS-CoV-2 infection, may have been missed as the average yearly number of hospitalization or deaths for influenza is >10 times larger and there was a concurrent influenza epidemic at this period in France (5).

Finally, although a recent report clearly shows that the SARS-CoV-2 outbreak was originated from the Huanan market (6), our findings do not contradict with this hypothesis. It is indeed possible that a progenitor of the virus has been circulating worldwide giving low-level infections and not inducing severe respiratory disease to the same extent as the Wuhan virus. A recent study reports early identification of SARS-CoV-2 RNA in a patient with measles-like syndrome in September 2019 in Italy (7), and indicated other SARS-CoV-2 RNA sequences obtained from samples collected in Brazil in November 2019. They concluded that a potential progenitor of the B.1 strain may have circulated worldwide since June-July 2019, before the Wuhan outbreak. We agree with these conclusions.

For all these reasons, our conclusion remains unchanged and suggests an early circulation of SARS-CoV-2 in France.

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