Letter to the Editor

Reliability of SARS-CoV-2 serological testing for influencing public health policies: A reappraisal

Dear Editor,

We read with interest the recent article of Alexopoulos et al. [1], and we would like to express our (partially divergent) opinion on the critical matter of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serological testing.

Although it seems at least theoretically agreeable that serological assessment may be useful for defining potential incidence and prevalence of community infections (i.e., "seropositivity"), and thus for driving public health policies, this concept seems no longer so straightforward after the surge of the new Omicron sublineages as it was during the periods of prevalence of the former SARS-CoV-2 variants. This conclusion is based on some important analytical and biological issues that we have summarized in Table 1.

First, the currently available immunoassays have been manufactured using antigen(s) derived from the prototype (ancestral) SARS-CoV-2 strain identified and sequenced in Wuhan, in 2019 [2]. Since then, the viral genome has undergone such a huge number of mutations, that the identity of the epitopes used to construct the immunoassays no longer reflects that of the circulating variants. The serum levels of anti-SARS-CoV-2 antibodies may thus be variably underestimated, up to the point that some anti-SARS-CoV-2 immunoassays are no longer capable to detect antibodies generated against the most recent circulating Omicron sublineages (e.g., BA.4/5, BA.2.75 and BA.2.75.2, BA.4.6, BQ.1 and BQ.1.1, BF.7, XBB.1 and so forth) as recently shown by two independent studies [3,4]. These aspects are also crucial when assessing vaccine response by serology in selected populations, as endorsed by Alexopoulos et al. [1]. In fact, the neutralizing potential versus Omicron sublineages of antibodies developed after monovalent COVID-19 vaccination is no longer adequately reflected by the anti-SARS-CoV-2 serum levels measured by some commercial immunoassays [4]. For this purpose, plaque reduction neutralization tests and/or live virus micro-neutralization assays are obviously preferable [5].

The progressive waning of natural and vaccine-elicited immunity is another important drawback, in that anti-SARS-CoV-2 antibodies, targeting both the spike and nucleocapsid proteins, become no longer detectable in the vast majority of subjects between 12 and 24 months from previous SARS-CoV-2 infection or COVID-19 vaccination [6,7]. Thus, testing negative (i.e., being "seronegative") during SARS-CoV-2 serological surveys does not always mirror the lack of infection or ineffective vaccination, in that anti-SARS-CoV-2 antibodies may have already waned to undetectable levels, whilst cellular immunity will persist for longer and will also more efficiently protect from the risk of developing severe COVID-19 illness [8]. Finally, both harmonization

Table 1

Main limitations of SARS-CoV-2 serological testing using currently available commercial immunoassays.

- Unreliable detection and measurement of anti-SARS-CoV-2 antibodies elicited by highly mutated SARS-CoV-2 lineages (e.g., Omicron)
- Does not satisfactory reflect neutralization of highly mutated SARS-CoV-2 lineages (e.g., Omicron)
- Progressive waning of anti-SARS-CoV-2 antibodies titer over time
- Lack of harmonization and standardization

and standardization of infectious disease serology are largely unmet targets [9], thus precluding the possibility to compare or pool measures of anti-SARS-CoV-2 antibodies obtained in different clinical laboratories, using different methods, irrespective of the availability of new international standards such as the WHO 21/338. To this end, only anti-SARS-CoV-2 immunoassays showing good concordance with neutralization tests performed with live virus should be used for epidemiological surveys and clinical practice [10].

In conclusion, although we would agree that serological testing has many theoretical advantages, the currently available anti-SARS-CoV-2 commercial immunoassays appear mostly outdated and poorly harmonized (standardization remains a chimera), and should hence be considered presently unfit for achieving most of the goals for which they have been originally designed and commercialized.

Declaration of Competing Interest

The authors declare they have no conflict of interest.

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Abbreviations: SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; COVID-19, Coronavirus disease 2019.

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Giuseppe Lippi[a,*], Mario Plebani[b]

[a] *Section of Clinical Biochemistry and School of Medicine, University of Verona, Verona, Italy*

[b] *University of Padova, Padova, Italy*

[*] Corresponding author at: Section of Clinical Biochemistry, University Hospital of Verona, Piazzale LA Scuro, 37134 Verona, Italy.

E-mail address: giuseppe.lippi@univr.it (G. Lippi).