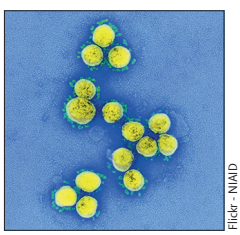
The complexities of SARS-CoV-2 serology



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Diagnosing previous infection with respiratory viruses is challenging. Our understanding of individual and population-level immunity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains incomplete and developing reliable serological assays to detect previous infection has been an intense focus of the global scientific effort. For public health planning we need scalable assays validated against large banks of samples from individuals who had proven seasonal (non-severe acute respiratory syndrome) coronaviruses and those who had well characterised symptomatic and asymptomatic confirmed SARS-CoV-2 infection. False-positive results, due to cross-reactivity with seasonal coronaviruses, are important to avoid, particularly if seropositive-individuals consider themselves immune. In *The Lancet Infectious Diseases,* the National SARS-CoV-2 Serology Assay Evaluation Group[1] provide the first large comparative investigation of the performance of four widely available commercial assays and a single in-house assay.

Antibody responses to SARS-CoV-2 are predominantly directed at the spike glycoprotein, which the virus requires for entry, and the nucleocapsid protein, which binds the viral RNA genome. The SARS-CoV-2 IgG assay (Abbott, Chicago, IL, USA) and Elecsys Anti-SARS-CoV-2 assay (Roche, Basel, Switzerland) assays detect antibody to the nucleoprotein, whereas the LIAISON SARS-CoV-2 S1/S2 IgG assay (DiaSorin, Saluggia, Italy), and SARS-CoV-2 Total assay (Siemens, Munich, Germany) detect antibodies to the spike glycoprotein. The Abbott and Diasorin assays detect IgG only, whereas Roche and Siemens detect total antibody. The diverse approaches taken by the four commercial assays highlight the challenge of choice posed to laboratories: all manufacturers report similarly high sensitivity and specificity.

The authors compared these four assays and a novel 384-well ELISA detecting total IgG to a trimeric spike protein and used all five assays on 976 pre-pandemic samples presumed to be negative, collected between 2014 and 2016, and 536 serum samples from patients with laboratory-confirmed COVID-19 from research studies in Oxford, UK, or plasma donors. The authors report that all assays had a high sensitivity (92·7-99·1%) and specificity (98·7-99·9%). The most sensitive test assessed was the in-house ELISA. The Abbott, Roche, and Siemens assays were the most specific. The benefit of the huge sample bank available to these authors was the clearly documented time since PCR positivity, which allowed them to optimise the manufacturers' cut offs and improve sensitivity. Only three cases did not give rise to any detectable antibody responses in all five of the assays, possibly because of a genuine lack of response in infected individuals, or a false-positive quantitative PCR result.

A limitation of this work is the small number of pauci-symptomatic and asymptomatic cases analysed. Antibody responses in these individuals are likely to be lower, and therefore the sensitivity of all assays might be somewhat less than that reported. Also, data on sex, age, and immunocompromise status were incomplete, meaning that the results could be limited in their application to specific patient groups. This limitation could be especially important in children, who are more likely than adults are to have had a recent infection with a seasonal coronavirus.

The expectation is that the best predictor of antibody-mediated protection will come from neutralisation assays, in which the ability of patient serum to prevent live virus infecting cell cultures is measured. These assays are impractical to deploy at scale. The presence of antibodies against the spike protein of SARS-CoV-2 correlates well with neutralisation.[2,3] The DiaSorin, Siemens, and in-house assays measured these potentially protective antibodies, with the in-house ELISA using trimerised spike protein, which shows a high correlation with neutralisation.[4,5,6] Further work is required to investigate what titre of neutralising antibodies correlates with protection, how long neutralisation activity persists, and which assay best predicts that. Identifying an appropriate assay will be crucial for assessing vaccine responses, and for assessing potential risk of reinfection, which has been shown with seasonal coronaviruses,[7] but not so far for SARS-CoV-2. Consistent with this future possibility, the neutralisation potency of serum declines in the months post infection.[8,9]

As our understanding of immunity and the correlates of protection (both cellular and humoral) increases and the range of immunoassays multiplies, we will probably

use different assays to answer specific questions. For example, most vaccine candidates elicit responses to spike rather than nucleocapsid protein. Measuring antibodies to spike will therefore indicate whether there has been a good response, whereas measuring antibodies to nucleocapsid would help identify whether the individual had nonetheless become infected. Measuring the different antibodies might also have prognostic value; a report showed that a predominant humoral response to nucleoprotein is associated with poor outcome in patients admitted to hospital, compared with that of spike.[10] Further investigation is required and the possibility of a one-size-fits-all immunological assay looks less and less likely.

We declare no competing interests.

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