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Letter to the Editor

Characterising differential antibody response is integral to future SARS-CoV-2 serostudies


Dear Editor,

We read with interest data reported in this Journal by Tré-Hardy et al.,¹ where they provide timely insight to challenges associated with implementing serological testing as an adjunct to SARS-CoV-2 surveillance. In areas where reporting of new cases has declined, consideration is being given to relaxation of social-distancing restrictions and considerable attention has been given to the supportive potential of serological testing in delayed identification of cases.

Tré-Hardy et al. demonstrate the urgent need to determine the optimal time from symptom onset for individual serological assays in order to develop suitable guidance on interpretation of results. As part of this process, we highlight further concerns around the potential interpretation of positive and negative results alike. A plethora of tests, designed for use as either point-of-care or laboratory-based assays have rapidly been developed, many having undergone limited validation in cases of severe, hospital infection and information around potentially inferred immunity has been at times vague. Increasing access to serological testing for SARS-CoV-2 has been available not only through private medical practices but also via high street providers, online, and in some cases via postal services. While national guidelines have gone some way to reduce the risk of confused messaging where clinicians are not directly involved in the resulting process, delayed reporting risks misinterpretation that may affect social-distancing behaviours.

The degree of immunity conferred from infection with SARS-CoV-2 remains unclear. Four major viral structural proteins have been identified (spike, envelope, nucleocapsid, and membrane).² Initial B cell response in SARS-CoV-2 infection appears to occur prior to the recovery phase,³ and while antibody responses are thought to arise first to the nucleocapsid (NP) in SARS-CoV-1, it is the spike protein (essential in virus attachment and cell entry) and specifically the receptor-binding-domain (RBD) for which neutralizing antibodies have been identified.⁴ Early work has also shown anti-RBD antibodies have some correlation with neutralization in SARS-CoV-2.⁵ In addition to this humoral response, there is evidence of a multi-faceted reaction in the early phase of SARS-CoV-2 infection involving not only antibody-secreting B cells, but also follicular T helper cells, activated CD4⁺ cells and CD8⁺ T cells prior to the recovery of symptoms.³

In addition to observations by Tré-Hardy et al. we would further state that the antibody target employed is integral to the potential interpretation of results. Currently available serological assays tend to target either non-neutralizing antibodies or rarely the likely neutralizing anti-RBD antibody. Differentiation between test targets by the wider public is an unrealistic expectation. Using an

online repository of available assays,⁶ and cross-referencing against regulatory body and advisory lists, we identified 284 currently available assays (Fig. 1). Having analysed product descriptions and, where available, instruction leaflets and regulatory documentation submitted for approvals we find that the majority (168/284, 59.2%) make only vague reference to SARS-CoV-2 antigens or antibodies and some no reference whatsoever (49/284, 17.3%). Where manufacturers specify antibody targets, this is most commonly to the NP (36/284, 12.7%). 19/284 (6.7%) specify spike protein targets, 12/284 (4.2%) specify combination targets, with only five (1.8%) providing clear descriptions of targeting the (tentatively neutralising) anti-RBD antibody. The secondary structure of the highly conserved NP protein also raises the possibility of identifying cross-reactive results with other known coronaviruses.

Further, indiscriminate screening among low seroprevalence populations has a poor positive predictive value.^{7,8} among health-care workers seroconversion is seen in those with and without symptoms,⁸ with a greater titre response seen in those with symptomatic, and specifically, severe illness. Longitudinal studies are now needed to determine the comparative persistence of neutralizing antibodies in mild and severe illness as well as determine the degree to which any sterilizing effect is provided. A failure to identify seroconversion in a proportion of PCR positive cases should demand further caution in serology interpretation.⁹ A surge in uptake of serological testing among the general public is likely to ensue if neutralizing antibodies are further characterised *in vitro*, yet mapping of long-term *in vivo* immune protection is still required. As the current wave of SARS-CoV-2 declines, opportunity to assess assay performance, and concordance across different targets, is limited. If, for example, the anti-RBD antibodies are confirmed as having neutralizing activity and direct comparison finds complete concordance with anti-nucleocapsid assays then this concern becomes less immediately urgent. If considerable variation between assays exists however, then it is crucial for assay manufacturers to be more explicit in their target design and more specific in advice around test utility. Those assays utilizing non-neutralizing targets could then have limited utility in delayed case identification but should never then confer a sense of immunity. Regardless, it must be remembered that these tests primarily offer a qualitative detection of antibody at a single time point and are unable to infer any sense of memory, or whether the humoral response to the primary infection is sufficient to lead to any level of protection. Even assays that do detect anti-RBD antibodies, presuming they are shown to have some lasting neutralizing effect *in vivo*, could only be considered a suggestion of immunity. Observation of reduced levels of neutralizing antibody in convalescent patients at just 2–3 months precludes antibody detection testing as a form of ‘immunity passport’.¹⁰ Where the rate of acute infection is significantly declining in some areas, longitudinal studies characterizing the immune response in both symptomatic and asymptomatic individuals with

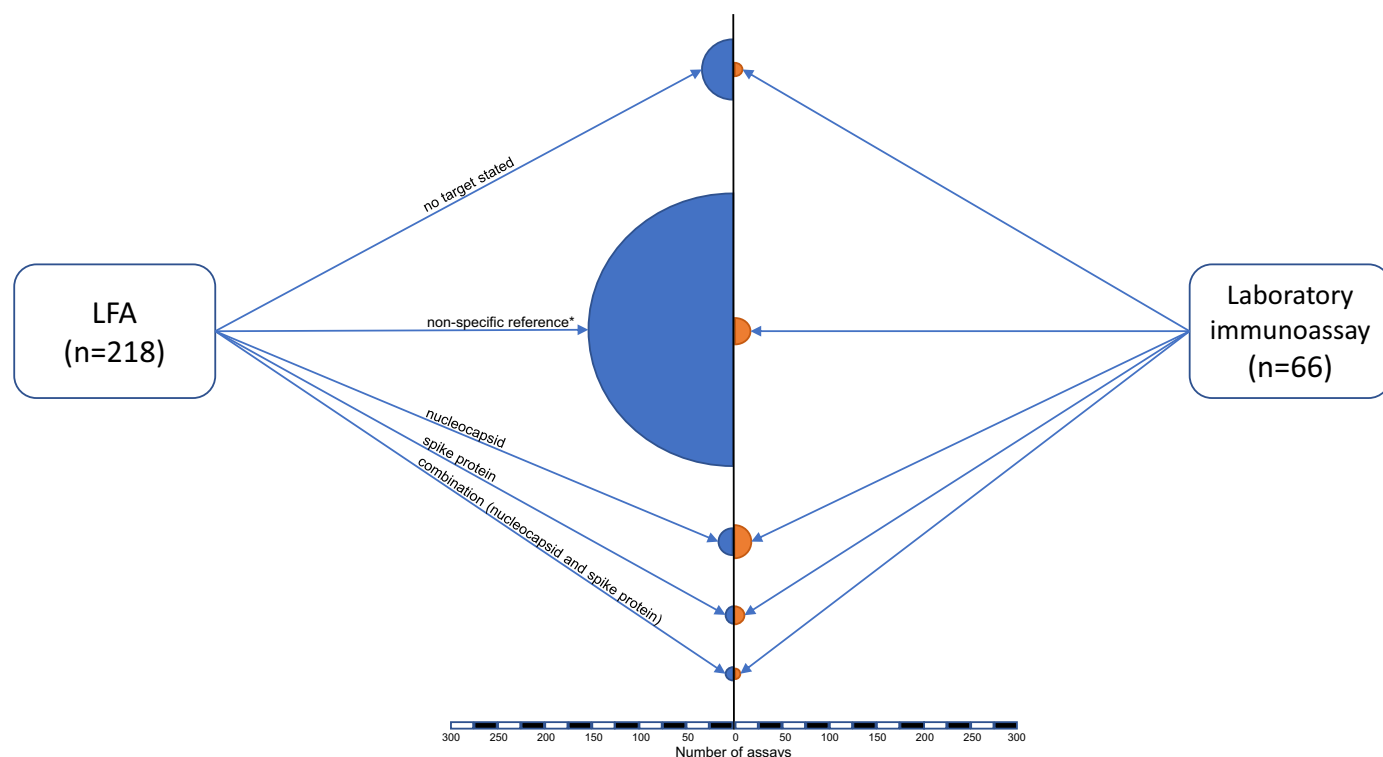


Fig. 1. Information available on serological targets from commercially available lateral flow assays (LFA) and laboratory immunoassays (enzyme-linked immunosorbent assays or chemiluminescence immunoassays) for SARS-CoV-2. Assays identified via an online repository⁶ and cross referenced with regulatory and advisory body online lists for current commercially available serology-based tests for SARS-CoV-2 (US Food and Drugs Administration, Australian Therapeutic Goods Administration, Health Information and Quality Authority, World Health Organisation). Information was taken directly from product listings and inserts on manufacturer and/or distributor sites and cross-referenced against documentation available for product regulatory approval. Circle size is relative to number of assays found within that category and recorded as:

- (i) no identifiable reference to any target found
- (ii) non-specific reference to assay targets including SARS-CoV-2 antigens, recombinant proteins, SARS-CoV-2 peptides, SARS-CoV-2 antibodies
- (iii) nucleocapsid protein
- (iv) spike protein, including specifically the S1 protein, N-terminus and RBD separately specified and
- (v) combination targets including to both nucleocapsid and spike proteins.

confirmed infection is now urgently required. Full benefit of antibody screening programmes will only be realised where clear information is available for researchers around assay targets.

We further calls by Tré-Hardy et al. for the need to establish appropriate timeframes for serological testing in order to minimise misinterpretation but also feel better understanding of the differential antibody response and transparent assay target information is essential to inform this process. While comparative, longitudinal studies between assay targets are urgently required, messaging on social-distancing and the appropriate use of personal protective equipment, regardless of a result, must remain a mainstay. If this is unable to be effectively delivered where kits are being used in the community, then testing for the purposes of delayed case identification alone should be limited to where direct clinical counselling can be undertaken.

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Authors' contributions

SJCP & LSPM designed the methodology. SJCP & MAP collected the data. All authors reviewed the results and data analysis and contributed comments. SJCP drafted the initial manuscript with all authors contributing significantly to revising this for submission. All authors agreed on the final version for submission to the journal.

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Consent

No patient information is involved in this submission.

Declaration of Competing Interest

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Availability of data and materials

The data analysed during the current study and further details on the assays are available from the corresponding author (SJCP; scott.pallett@nhs.net) on reasonable request, as long as this meets local ethical and research governance criteria.

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