Review of: "Comparison of two T cell assays to evaluate T cell responses to SARS-CoV-2 following vaccination in naïve and convalescent healthcare workers"

Chris Dark, Ruth Brignall[1]

1 Oxford Immunotec

Potential competing interests: The author(s) declared that no potential competing interests exist.

Article review

Review panel: Chris Dark (Oxford Immunotec), Ruth Brignall (Oxford Immunotec)

Article: Comparison of two T cell assays to evaluate T cell responses to SARS-CoV-2 following vaccination in naïve and convalescent healthcare workers

Authors: Eloise Phillips, Sandra Adele, Tom Malone, Alexandra Deeks, Lizzie Stafford, Susan L. Dobson, Ali Amini, Donal Skelly, David Eyre, Katie Jeffery, Christopher P. Conlon, Christina Dold, Ashley Otter, Silvia D'Arcangelo, Lance Turtle, PITCH Consortium, Paul Klenerman, Eleanor Barnes, Susanna J. Dunachie

Review

Overall, the experimental design is good, however we raise four points for consideration.

The authors do not appear to have determined sensitivity with this study and therefore we would suggest the conclusions should be edited. The authors have looked at concordance between the two tests but neither is a reference test and therefore it is not possible to carry out a true sensitivity analysis. Higher spot counts do not therefore translate into higher sensitivity for one assay or another. A possible reason for lower T cell counts with T-SPOT.Discovery SARS-CoV-2 when compared to the PITCH assay is that the T-SPOT test uses peptide pools (S1 and S2) from which homologous antigens have been removedI. The removal of these potentially cross-reactive peptide sequences with high homology to other endemic coronaviruses enhances the specificity of the T-SPOT test. It is not clear whether high homology regions have been removed from the peptide panels used in the PITCH assay. Retaining these regions would lead to higher spot counts which may explain the discrepancy. This is supported by the closer correlation seen between T-SPOT.Discovery SARS-CoV-2 peptide pool 14 (whole spike with no homologous regions removed) and the PITCH assay S1/S2. As a result the PITCH assay may be detecting more non-specific T cell responses. This is further supported by the fact that the authors detect high T cell responses in nucleocapsid and membrane panels which are known to be highly conserved across coronaviruses. The authors do state that a previous study found that the PITCH ELISpot identified no or minimal responses in pre-pandemic samples suggesting that the assay is relatively specific. However, it is clear from Figure 1 that

the PITCH study is detecting a higher level of responses than the T-SPOT test even in the “Naïve cohort” (approximately 2x higher median spot counts), suggesting that the PITCH assay is detecting a higher level of background noise than the T-SPOT test. With these factors in mind, there is not enough evidence to conclude that one assay is more sensitive than the other. Detection of higher responses in the PITCH assay may be due to higher background noise, which may be an indication of a lower signal-to-noise ratio compared to the T-SPOT assay.

Further, the authors have acknowledged that the cohort they are defining as “Naïve” is a healthcare worker cohort and state that some of them may have been infected asymptomatically or with very mild symptoms without ever testing positive. On that basis we suggest it may be more accurate to define this cohort as “no confirmed infection”.

The authors also state that they observed T cell responses in the absence of antibodies in individuals who did not seroconvert. We believe it is possible that these individuals may have seroconverted but since, in some individuals, antibodies may persist transiently, the antibody levels could have declined whilst T cell responses remain robust. The different kinetics of antibodies and T cells in SARS-CoV-2 is now well described in the scientific literature.

The authors use the term ‘matched samples’. It is not clear whether the samples were taken at the same time, frozen for the PITCH assay and used fresh for the OI assay. Use of frozen samples can lead to significant assay variability, and any comparison between fresh and frozen may lead to misleading conclusions. Taking the samples at different times would also add variability. Perhaps a better description of these samples is that they have come from the same donors. Additionally, the comparison of Fresh vs. Frozen cells introduces another source of spot count variability that is outside of the assay design. Consequently, this must reduce the strength of any assertion that spots count differences between the PITCH assay and the T-SPOT Discovery assay are purely due to assay design.