Review 2: "Rapid 'mix and read' assay for scalable detection of SARS-CoV-2 antibodies in patient plasma"

Thomas Moran¹, J. Andrew Duty², Thomas Kraus²

¹Icahn School of Medicine at Mount Sinai, Microbiology, USA,
²Icahn School of Medicine at Mount Sinai

Published on: Nov 15, 2020
DOI: 10.1162/2e3983f5.b8ca0b8d
License: Creative Commons Attribution 4.0 International License (CC-BY 4.0)
Review 2: "Rapid ‘mix and read’ assay for scalable detection of SARS-CoV-2 antibodies in patient plasma"

**RR:C19 Evidence Scale** rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:

The manuscript by Yue et al. documents the development of a TR-FRET assay for measuring concentration of spike/RBD binding antibodies in serum samples using an established patient cohort. Appropriately the authors tested the assay for optimal labeling (donor vs receptor labels), functionality with different antibody classes, specificity and background with RBD vs spike usage, the effect of serum on sensitivity and the degree of labeling of the spike protein. The assay performed excellently and comparable to the standard ELISA assay. The limit of detection is in the low ng/ml level though the presence of serum reduces sensitivity to some degree.

The assay is simple, rapid, and sensitive. A great advantage over ELISA is that there is no need for washing. ELISA give a stronger signal at the lowest concentrations, but the TR-FRET has lower background which allows clear discrimination.

TR-FRET seems to have a broader range in that there doesn’t seem to be a ceiling effect as occurs with ELISA at high antibody concentrations. There is really no discussion of range or quantitation as the point of the assay as described is simplicity, specificity, and speed. The one step preparation without a need for washing contributes to the reproducibility and low background of the assay. However, this is also responsible for the strong prozone effect observed as well as the reduction in sensitivity caused by serum. These are minor problems and, overall, the assay works equivalently to the ELISA but is simpler and more rapid.