Rapid Reviews COVID-19

Review 1: "Structural and Functional Comparison of SARS-CoV-2-Spike Receptor Binding Domain Produced in Pichia pastoris and Mammalian Cells"

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RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable, unless the weaknesses are clearly understood and there is other theory and evidence to further support it.

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Review:

Investigators authoring this preprint aim to produce the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, in native form, at low cost and high yield. They expect their RBD to advance vaccinology and serology studies. Here the investigators compare RBD production in yeast and mammalian (293T cell) cultures. RBDs were produced in both systems, with yields from yeast about twice that of mammalian cell culture. The research team reported similar biochemical properties of the yeast and mammalian RBDs, however, the RBDs from yeast are set apart by their higher molecular-weight, high-mannose N-linked glycans. Both the yeast and the mammalian RBDs elicited RBD-specific antibodies in mice.

The findings in this submission come from studies of RBD thermal stability, RBD salt bridges, and RBD aggregation after freezing and thawing. Overall, the results appear reliable and the point to the potential of the yeast cultures for upscaling thermostable, native-folded, monomeric RBD production. However, they might be considered incremental advances, as it is already known that yeast are suitable organisms for robustly producing SARS-CoV RBDs. The findings might also be considered incomplete. The biochemical works are thorough but there are no functional assessments. RBDs were not evaluated for ACE2 binding or for obstruction of SARS-CoV-2 cell entry. Furthermore, antibodies elicited by RBDs were not evaluated for virus neutralizing activities. To elevate the impact of the report, these tests should be done, especially because the high mannose glycans on the yeast-produced RBDs might interfere with ACE2 binding or occlude antibody epitopes. Additional results are necessary to demonstrate that the yeast-produced RBDs have utility in proposed vaccinology and serology studies.

Although many of the results in the preprint are convincing, there are several inaccuracies in the text, and a thorough formal peer review process should be completed. Aligning the text with the stated research goals is also recommended, particularly with respect to the economic objectives. The relative

costs of mammalian and yeast RBD should be stated explicitly, so that the cost savings associated with yeast expression are fully appreciated.