Development and Accuracy Determination of a Peptide-based Diagnostic Test for SARS-CoV-2 Based on the N-terminal Ectodomain of the Membrane Glycoprotein

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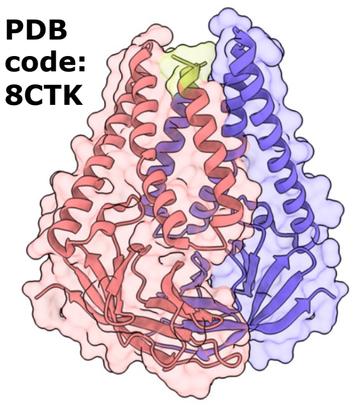
Introduction & Aim



The N-terminal ectodomain (NTE) of the SARS-CoV-2 membrane (M) glycoprotein is a surface-exposed, structurally flexible region with promising potential as a B-cell epitope for peptide-based vaccine development.

Thus, this study aimed to characterize the antibody-binding properties of peptide analogs of SARS-CoV-2 M NTE. Specifically:

- Design synthetic peptide analogs of M NTE;
- Determine binding affinity of cognate antipeptide antibodies for designed synthetic peptide analogs of M NTE; and
- Determine diagnostic accuracy of an assay utilizing peptide analogs on clinical samples.

Methods

Design & synthesis

- On February 26, 2021, **Immune Epitope Database (IEDB)** was queried for linear SARS-CoV-2 epitopes (organism ID: 2697049) that had been experimentally validated as positive in B-cell assays. Retrieved sequences were grouped by cluster analysis to reduce redundancy. Structural and functional interpretation was then performed by mapping the selected epitopes onto available three-dimensional models obtained from the **Protein Data Bank** and **UniProt,** followed by molecular visualization in **PyMOL**.

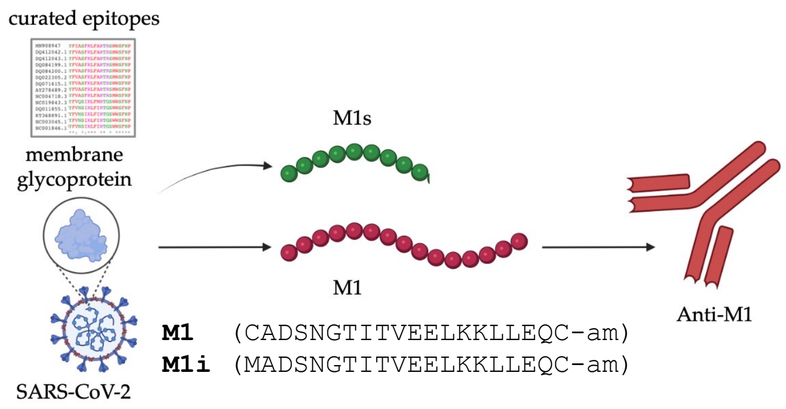
Affinity determination

- An in-house ELISA was performed using the analogs and antipeptide antibodies, affinity-purified from sera of immunized rabbits. Absorbance values were plotted against antibody concentration. Data were subsequently linearized using the method described by Liliom, *et al.* (1991). The **apparent dissociation constant (K_d^{app})** was determined directly from the slope of the resulting linear fit.

Diagnostic accuracy determination

- Eligible participants: adults (≥18 years) admitted to the **Philippine General Hospital**, a 1,335-bed tertiary referral center, during October 2020–February 2021, before SARS-CoV-2 vaccines became available locally. All patients had RT-PCR–confirmed infection from nasopharyngeal swabs. Enrollment followed convenience sampling, with referrals made by the primary physician of each patient. Biobanked plasma controls served as negative control.

Results & Discussion



Graphical abstract. M1 and M1i (SARS-CoV-2 M NTE peptide analogs) were designed from IEDB-curated B-cell epitopes. M1 was prepared in monomeric and polymeric forms. Anti-M1 is an antipeptide antibody affinity-purified from the sera of rabbits immunized with M1i (`-am`: *C*-terminal amidation).

```
 D3G
 D3H
 D3N              Q19E
  |                |
CADSNGTITVEELKKLLEQC (M1)
MADSNGTITVEELKKLLEQC (M1i)
MADSNGTITVEELKKLLEQWNLV... PDB code:
**********----------HHHHHH... 8CTK (A, B)
********--HHHHHHHHHHHHHH... 7VGR (A, B)

D3G:  Omicron BA.1
D3H:  Omicron BA.2.86
D3N:  Omicron BA.5, BQ.1.1
Q19E: Omicron BA.2, BA.2.12.1, BA.2.75,
BA.2.86, BA.4, BA.5
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Figure 1. M1 and M1i represent a largely conserved, antibody-accessible, conformationally disordered region of the SARS-CoV-2 M NTE. X-ray crystallography failed to resolve N-terminal residues of the protein sequence in available PDB structures (*: unresolved; H: α-helix; N: *N*-glycosylation site).

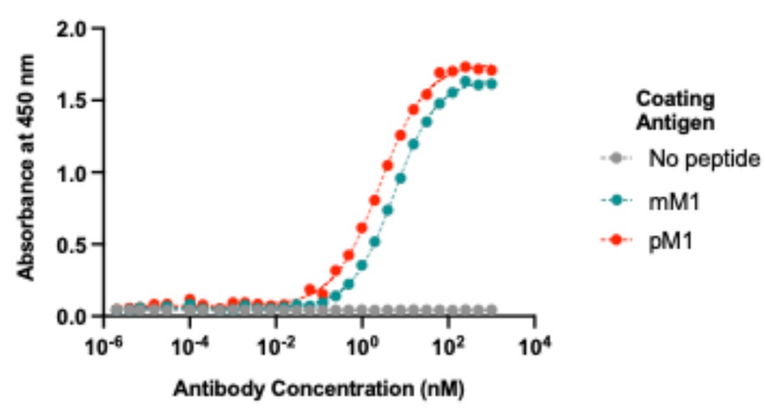

Figure 2. Quantitative analysis of anti-M1 binding to monomeric and polymeric M1s yielded dissociation constants (K_d^{app}) of 8.00 nM and 4.33 nM, representing average affinity and avidity, respectively. This suggests ~2-fold avidity gain over the monomeric form.

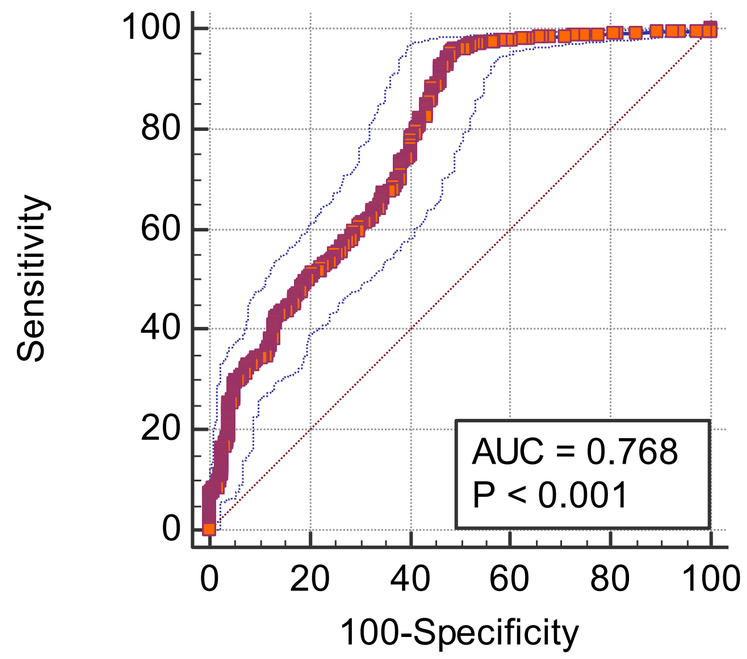

Figure 3. Testing on 1,222 samples and 221 controls revealed a *posthoc* (by Youden J) sensitivity of 95.26% and specificity of 52.27%, positive likelihood ratio of 1.60, negative likelihood ratio of 0.47, corresponding to an accuracy of 88.70%.

Conclusion

The clinical performance exhibited by polymeric M1s, our SARS-CoV-2 M NTE analog suggests its potential usefulness as a highly sensitive immunodiagnostic. Moreover, the strategy of achieving an avidity gain by polymerization may be used to overcome limitations on antibody-binding affinity of peptide analogs of target whole proteins.

References

Liliom, K., Orosz, F., Horváth, L., & Ovádi, J. (1991). Quantitative evaluation of indirect ELISA. Effect of calmodulin antagonists on antibody binding to calmodulin. *Journal of Immunological Methods*, *143*(1), 119–125. https://doi.org/10.1016/0022-1759(91)90280-s

Patent

Philippine patent applications PH 1-2020-050482 (WO 2022/108460), filed 19 November 2020, granted on 11 July 2025; and PH 1-2021-050256 (WO 2022/231442), filed 13 August 2021.

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