Next-generation multiplex surrogate neutralization test

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Abstract

Surveillance of zoonotic viruses in wildlife has revealed that bats harbor multiple zoonotic pathogens that pose a high risk of zoonotic spillover due to their use of well-conserved molecules as receptors. Preexisting human adaptive immunity, such as neutralizing antibodies, plays a crucial role in preventing zoonotic spillovers from becoming pandemics. The devastating impacts of the pandemic are well-evident in the current COVID-19 pandemic, highlighting the inadequacy of existing pandemic preparedness strategies. Therefore, active surveillance of population immunity and antigenic characterization are essential for timely public health interventions. This study aims to establish a high-throughput serological platform for the rapid detection of neutralizing antibodies, facilitating disease risk assessment for multiple viruses with pandemic potential in a single reaction.

Results

SARS-CoV-2 surrogate neutralization test

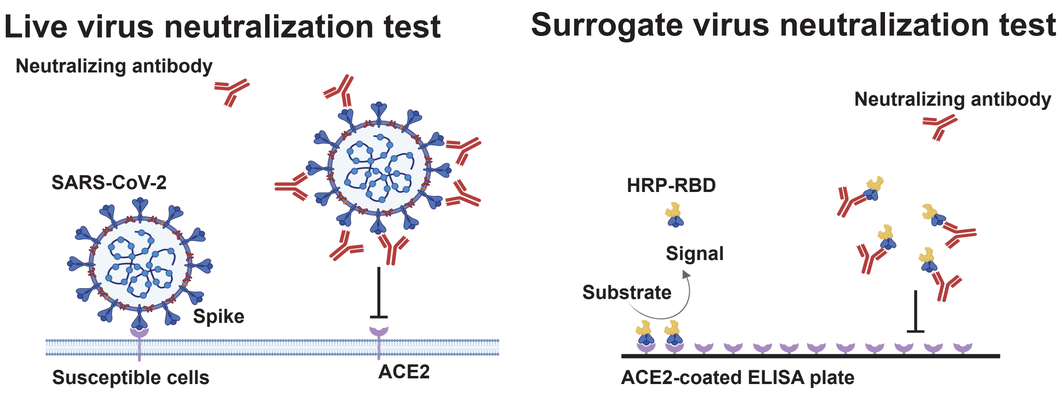

Fig 1. Schematic illustration of SARS-CoV-2 live virus neutralization test. Live virus neutralization test required infectious SARS-CoV-2 and ACE2 overexpression cell line. Surrogate virus neutralization test (sVNT) uses HRP-conjugated receptor-binding domain (RBD) and ACE2-coated ELISA plate to determine neutralizing antibodies that block ACE2-RBD interactions.

Good correlation between sVNT and PRNT

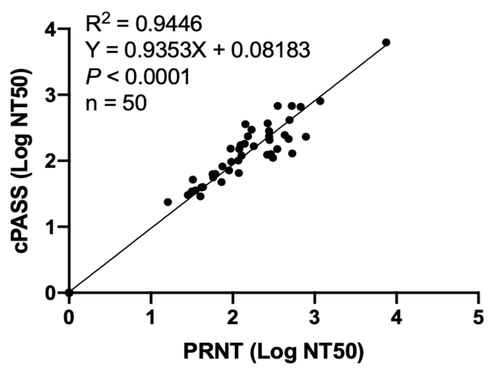

Fig 2. Pearson correlation analysis between sVNT and plaque reduction neutralization test (PRNT). The neutralization titer 50% (NT50) of 50 convalescent sera were determined using ancestral SARS-CoV-2 sVNT and PRNT. Correlation analysis was performed using Graphpad Prism 8.

High-resolution characterization of SARS-CoV-2 neutralizing antibodies

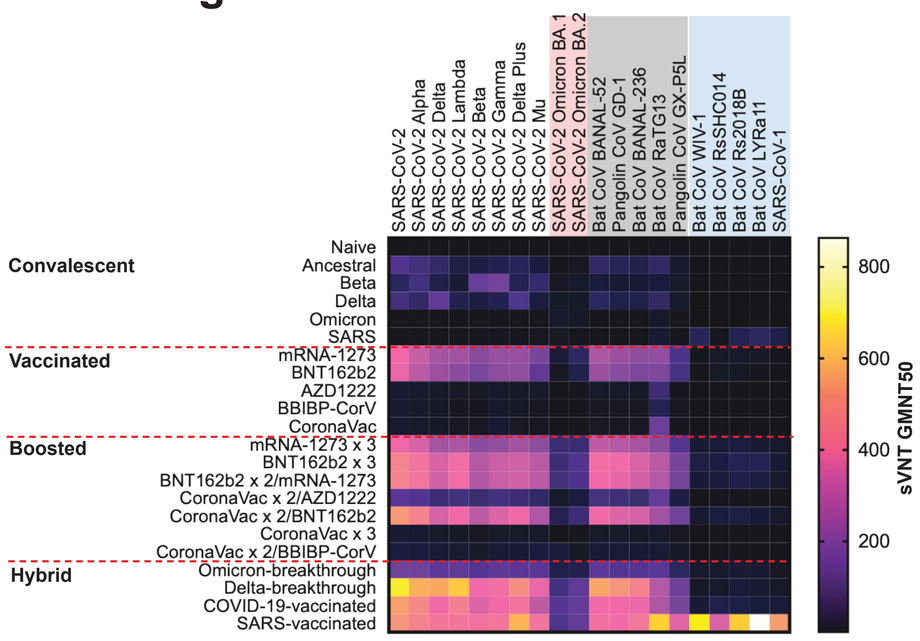

Fig 4. High-resolution characterization of SARS-CoV-2 neutralizing antibodies. A heat map of sVNT GMT50 of 20 serum panels (n = 402) derived from convalescent, vaccinated, boosted and hybrid individuals for immunity against 20 sarbecoviruses using multipex sVNT.

Multiplex SARS-CoV-2 surrogate neutralization test

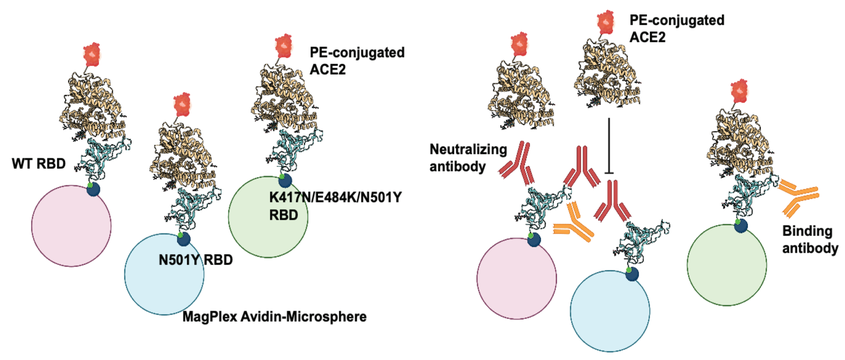

Fig 3. Multiplex sVNT platform. Enzymatic Avi-Tag biotinylated SARS-related CoV RBD were coated onto different bead region of MagPlex Avidin Microsphere. PE-labeled ACE2 were used to detect the RBD. The mean fluorescence intensity was acquired using MAGPIX instrument (Luminex).

Next-generation all-in-one multiplex sVNT

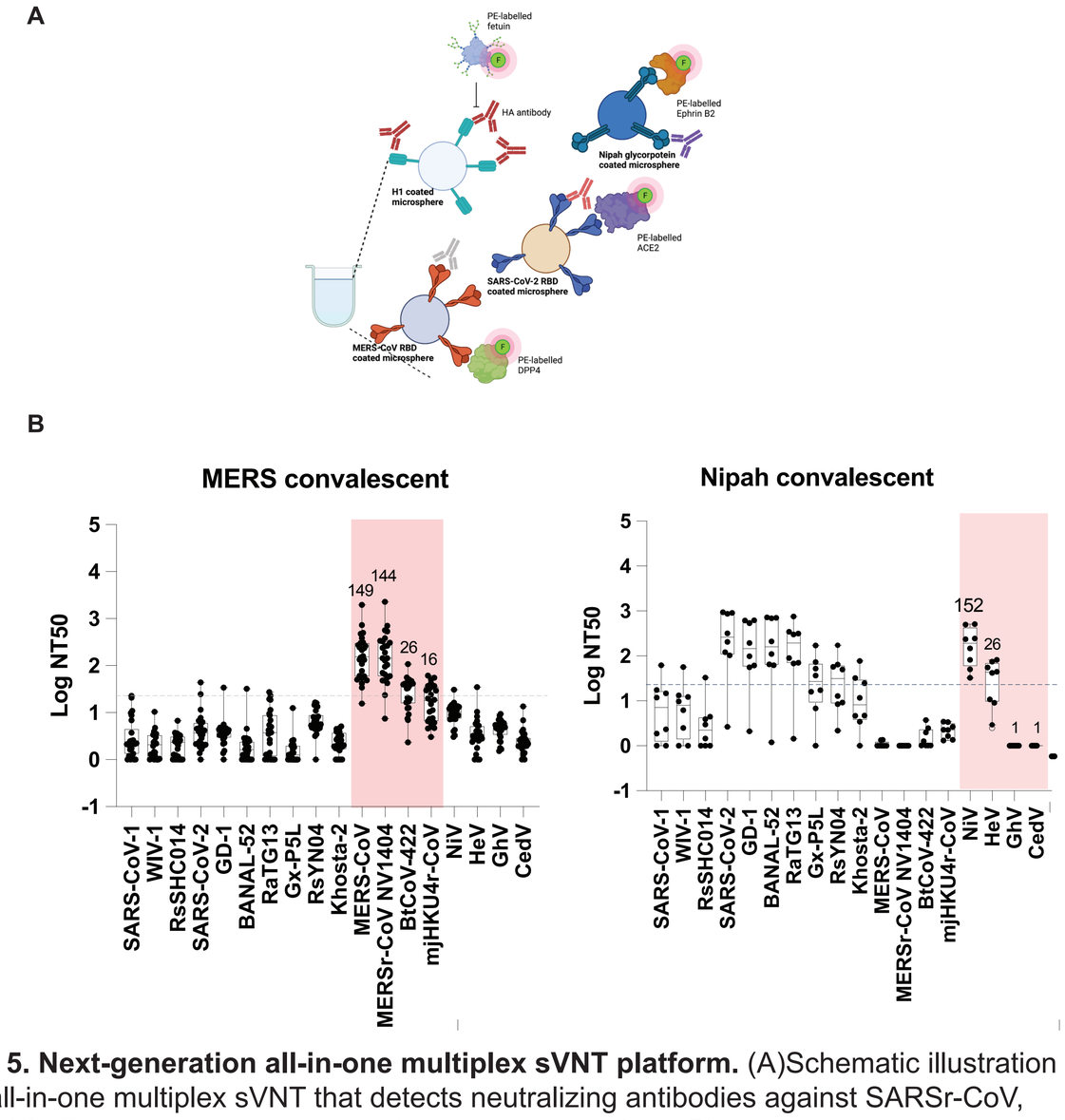

Fig 5. Next-generation all-in-one multiplex sVNT platform. (A)Schematic illustration of all-in-one multiplex sVNT that detects neutralizing antibodies against SARSr-CoV, MERSr-CoV, Henipaviruses and influenza viruses. (B) NT50 generated from multiplex sVNT showing neutralising antibodies of the samples derived from MERS and Nipah convalescent individuals against merbecoviruses and henipaviruses.

Conclusions

We demonstrated that Nipah-specific infection/vaccination induces high-level neutralizing antibodies against homologous strains, with limited to no cross-reactivity neutralizing antibodies against antigenic distinct viruses, thus highlighting the zoonotic potential of these closely related animal viruses.

Acknowledgements

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References

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