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From days to hours: detecting SARS-CoV-2 neutralizing antibodies

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After SARS-CoV-2 infection, our body produces antibodies that can defend us against the virus upon infection. However, not all antibodies act similarly - some antibodies can stop virus infection, while others cannot. To quickly differentiate these antibodies, we devised a simple test that can easily determine the presence of neutralizing antibodies within hours.

Coronavirus Disease 2019 (COVID-19) requires no introduction. Since late December 2019, SARS-CoV-2, the virus that causes COVID-19, has infected close to 40 million and claimed over a million lives. Like in any other infection, the body mounts an immune response against SARS-CoV-2. One type of immune response comes in the form of antibodies.

Antibodies differ based on their capacity to stop an infection. Neutralizing antibodies prevent the virus from infecting the cell, while non-neutralizing antibodies bind to the virus but do not prevent infection. For example, SARS-CoV-2 infects our cells by binding to ACE2 receptor - a protein found on the surface of cells. The protein in SARS-CoV-2 binding to ACE2 is the spike protein. If you have antibodies that bind to a specific part of the spike protein and block it from interacting with ACE2, you prevent infection – these are the neutralizing antibodies. On the other hand, antibodies that can bind the virus (or the spike) but cannot stop the interaction between our cells and virus are non-neutralizing.

We can detect antibodies using several methods. Conventional assays are routinely available as clinical tests to detect total binding antibodies. However, these assays cannot distinguish neutralizing from non-neutralizing antibodies.

Assays that specifically detect neutralizing antibodies exist as well. These tests, named virus neutralization tests (VNT), use live viruses and cells

to recreate the virus infection process. When neutralizing antibodies are present, cells will not become infected, nor activate their normal response to infection. The absence of these characteristic changes upon virus treatment indicate the presence of neutralizing antibodies. VNTs take a longer time than conventional tests, as the cells and viruses need to grow and replicate. In addition, the involvement of live SARS-CoV-2 means that laboratories must adhere to biosafety level 3, which require trained personnel and extra safety measures,. These safety measures are what you see in movies – people walking around the lab wearing spacesuit-like clothing!

Our group devised a method to shorten the SARS-CoV-2 VNTs from days to hours. This became the surrogate-VNT, vis-à-vis conventional-VNT. We recapitulated the virus neutralization test setup – with two major changes: instead of using the live SARS-CoV-2, we used the ACE2 receptor, and instead of using live cells, we used a plate coated with the human ACE2 receptor protein. The readout from the surrogate-VNT is "percent inhibition" – which refers to the reduction in signal, coming from the binding of spike protein to ACE2 receptor, with the addition of the sample, compared to when no sample was present. A low readout indicates that neutralizing antibodies exist in the sample.

Surrogate-VNT carries numerous advantages over conventional-VNTs. Not only does it detect neutralizing antibodies in patients' blood, it also reduces the turnaround time for testing neutralizing antibodies. Furthermore, as surrogate-VNT does not require live viruses, biosafety level 3 safety conditions are not necessary to perform the assay.

We tested samples from known COVID-19 patient as well as negative samples and found that surrogate-VNT is highly sensitive and specific - meaning that it can clearly distinguish COVID-19 patients from healthy people with very few mistakes. We also compared surrogate-VNT to conventional-VNTs, and found that both tests showed very similar results, meaning that surrogate-VNT can be a reliable substitute for conventional-VNTs.

Moreover, we tested surrogate-VNT in various animals (e.g. mouse, rabbit, and llamas) that were expressing antibodies against SARS-CoV-2 proteins and found a neutralizing response. This shows that surrogate-VNT may be a useful tool in the hunt for the origins of SARS-CoV-2.

The exact role of neutralizing antibodies is not yet fully understood in terms of immunity against COVID-19. However, neutralizing antibodies may be a good surrogate marker for protection from infection (of course, there are exceptions to this rule). This assay could also test if a vaccinated person mounts a neutralizing antibody response after vaccination, which could help for vaccine trials. Although the cumbersome conventional-VNT can determine the presence of neutralizing antibodies after vaccination, surrogate-VNT offers an easier alternative, especially for large scale testing. Moreover, with the emergence of SARS-CoV-2 variants of concern, it is important to note that the surrogate-VNT platform is easily adaptable to SARS-CoV-2 variants specific neutralizing antibody detection assays if needed. The practicality and specificity of surrogate-VNT may also strengthen our hand in our fight against COVID-19 by allowing the detection of neutralizing antibodies from days to hours.

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