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A practical approach to SARS-CoV-2 testing in a pre and post-vaccination era

Sean C. Taylor

GENSCRIPT USA INC. 860 Centennial Ave., Piscataway 08854, NJ, United States

A B S T R A C T

As countries globally are in the process of planning, introducing or implementing mass vaccination strategies while continuing to deal with the ongoing SARS-CoV-2 pandemic, an evolution in testing strategies may be required to minimize spread in mixed vaccinated and non-vaccinated populations. This mini-review explores the key public health questions associated with the widely varying efficacy of commercially available vaccines and their persistence of protection in the context of a growing number of variant virus strains. A new strategy for SARS-CoV-2 testing that accommodates the current and evolving pandemic paradigm is proposed.

1. Introduction

Covid-19 has led to the rapid innovation and commercialization of a myriad of tests for early detection of SARS-CoV-2 [1–4]. Unfortunately, there has been little vetting of their efficacy and accuracy with real world samples in rigorous clinical trials due to the urgent population testing requirements to minimize transmission and spread [5–8]. Thus, the government and private clinical diagnostics sectors have been obliged to filter through the flood of available commercial assays and create in house strategies to vet, choose and adopt individual approaches to SARS-CoV-2 population testing without global standards [8].

In the rush to contain the spread of infection through testing, mistakes have been made with the adoption of rapid blood and/or saliva tests with such poor accuracy that they were discarded costing some governments millions of dollars [9–11]. There has also been concern around the cutoff cycle for many qPCR molecular tests that may have resulted in a large number of false positive results upending the lives of those individuals requiring quarantine [12,13].

The mass production and distribution of vaccines from multiple commercial suppliers has raised further questions and concerns pertaining to testing:

(1) Since different commercial vaccines have widely different SARS-CoV-2 strain-dependent efficacies ranging from 10% to 95% [14–16], how is protection assured post-vaccination in the context of circulating wild type and variant strains?
(2) How long does immunity persist after administration of vaccines?
(3) What tests are relevant and provide the most actionable information in populations of mixed vaccinated and non-vaccinated individuals?

The rate of data produced and published pertaining to SARS-CoV-2 has eclipsed most other fields of study since the onset of the pandemic requiring almost daily literature reviews to filter the solid articles that are advancing the field [17–19]. There is now strong evidence correlated from multiple peer-reviewed articles in solid journals to answer the above questions.

2. How is protection assured post-vaccination?

All vaccines are designed to elicit an immune response to either the spike protein of SARS-CoV-2 or the attenuated virus [14,15,20]. The goal being to train the immune system to engage the virus with a robust immune response with front-line, neutralizing antibodies (nAb's) along with T and B cells. Neutralizing antibody (nAb) levels post-vaccination have been shown to be highly predictive of vaccine efficacy and immune protection from symptomatic SARS-CoV-2 infection [21–23]. Furthermore, their early presence within 14 days of infection has been negatively correlated to COVID-19 morbidity [24]. However, two recent studies demonstrated that 30% of elderly people over 80 years of age receiving the Biontech/Pfizer BNT162b2 vaccine and 71.5% of immunocompromised patients receiving the mRNA-1273 (Moderna) vaccine did not elicit a measurable nAb response [25,26]. Also, the level of protection from infection by the native SARS-CoV-2 and emerging variants has been shown to differ greatly between the vaccines [14,15]. Fortunately, there is a growing body of evidence correlating a strong vaccine or infection induced nAb response to immunity from the SARS-CoV-2 wild type and variant forms of the virus [21,27–36]. Taken together, these data warrant the measurement of neutralizing antibodies post-vaccination.

Although a global consensus relating neutralizing antibody titers to vaccine efficacy and protection has not yet been reached, some very recent articles support this premise. Of note, the Gilbert et al. pre-print paper demonstrates that vaccine efficacy drops precipitously below 90% when neutralizing antibody titers are below 100 WHO International Standard (IS) Units/mL [22]. The Feng et al. Nature Medicine article shows that neutralizing antibody titers below about 200 WHO IS Units per mL equates to vaccine efficacy below 80% [23]. Finally, the Khoury et al. Nature Medicine article supports only 50% protection from vac-

E-mail address: sean.taylor@genscript.com

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Table 1
Correlation between Neutralizing Antibody Titer, Vaccine Efficacy and cPass surrogate virus neutralization test (sVNT) (cPass sVNT)% Neutralization.

Titer (IU/ml)	% Efficacy (AstraZeneca) [23]	% Efficacy (Moderna) [22]	cPass sVNT[2]% Neutralization at a 1:20 Sample Dilution Factor
<100	<70	<90	30 to <60
100–300	~70-<90	~90-<93	~60 to ~90
>300	>80	>93	>90

Note: These cut-offs have been interpreted from the above-mentioned articles and have not been Authorized by the US Food and Drug Administration.

cines when neutralizing antibody titers are approximately 54 WHO IS Units per mL. These articles represent some of the first published evidence supporting a neutralizing antibody titer-based dependence on vaccine efficacy and protection summarized in Table 1.

Many commercial manufacturers of serology assays have therefore responded by supplying tests that measure non-functional, binding immunoglobulins (typically IgG's) to spike protein and/or its receptor binding domain (RBD) which are the primary target for nAbs [2,3,37]. However, it has recently been shown that only a fraction of these IgG's are in fact neutralizing [38]. Also, the total IgG immune response to spike protein or RBD from vaccinated individuals does not correlate as close to vaccine efficacy as nAbs [26,39,40] underlining the requirement to specifically measure nAb levels.

The current, gold standard, neutralization antibody tests require live cells and either live SARS-CoV-2 virus or a less dangerous pseudovirus [41–43]. In either case, these tests require days to complete and an elevated biosecurity level laboratory (BSL 3) (when working with live SARS-CoV-2). Therefore, these live virus/cell neutralization tests are expensive, dangerous and low throughput so not amenable to large population testing. A recent high-throughput, ELISA-based test termed cPass surrogate virus neutralization test (sVNT) (cPass sVNT) [2,44] has been designed, commercialized and EUA authorized by FDA to specifically detect nAbs at much lower cost, and with highly correlating data to the live cell/virus assays (Fig. 1) [2,33,37,44–47].

3. The choice between molecular (qPCR) and neutralizing antibody (nAb) tests

Molecular (qPCR) testing has been used exclusively for early detection (within one to two weeks of SARS-CoV-2 infection) where the results represent a snapshot of infection status at the time of testing whereby negatively tested individuals could acquire the virus immediately after their qPCR test [48,49]. In contrast, cPass measures the persistent, functional immune response to infection whereby nAb levels have generally been shown to increase rapidly post-vaccination or infction and then decline over a period of several months [50–55].

The prevailing data from animal models indicates that although vaccinated individuals will elicit nAbs with protection from infection and propagation of SARS-CoV-2, they may still be carriers of the virus that would give a positive result with qPCR [56–60]. These seemingly contradictory data underline the nature of viral infection and immunity whereby that although the virus can enter the nasal passages and lungs (giving a positive PCR test), the trained immune system is effectively blocking it's cellular entry, preventing replication and rendering infection inert. Thus, a strong positive nAb result may be the only requirement to substantiate a robust immune response whereby protection as has been shown in both human and animal studies for SARS-CoV-2 and other viral infections [28,32,56–63]. Hence, in a SARS-CoV-2 post-vaccination era with mixed populations of vaccinated and non-vaccinated individuals, qPCR testing could be reserved for those who have not been vaccinated or infected/recovered (ie: do not have circulating nAbs) to diagnose early infection. Also, for those who exhibit no or low nAb response post SARS-CoV-2 infection or vaccination, qPCR would be an appropriate secondary test to assess early infection.

4. Persistence of protection from vaccination

A major question associated with the mass vaccination efforts globally is the duration of protection [32,55,64]. Longitudinal human stud-

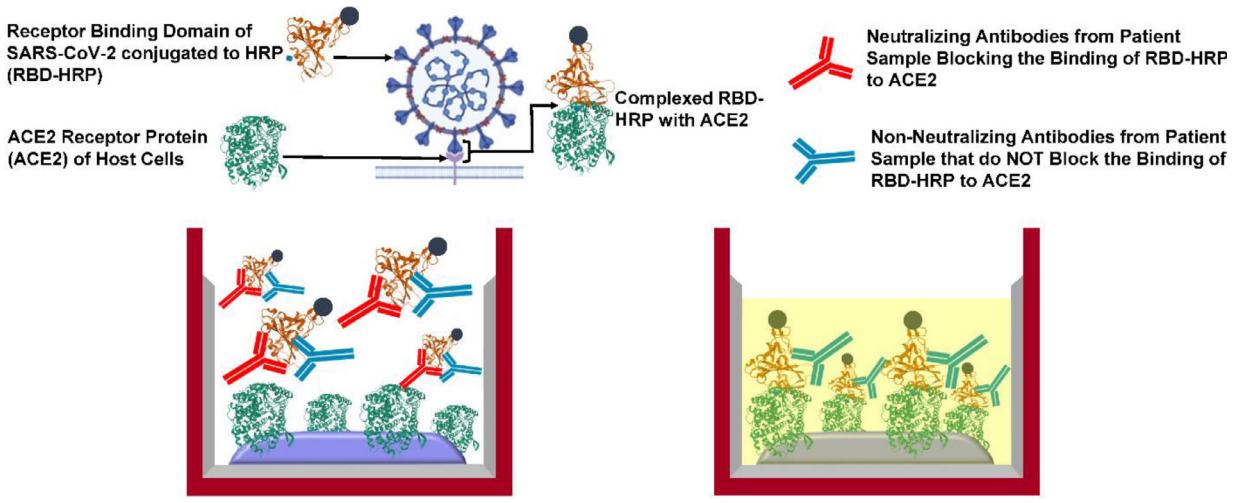

Fig. 1. *cPass Design and Description. A. cPass Design.* The test consists of purified RBD-HRP conjugate (brown) in solution and ELISA plates coated with hACE2 receptor (green) which form a strong complex. When mixed with a sample containing proteins, small molecules or antibodies that block the interaction between the RBD and hACE2 receptor, a low OD450 will be measured after incubation with TMB and stop solution. *B. Performing cPass.* Sample dilutions are initially mixed with the RBD-HRP solution with incubation for 30 min at 37 °C to permit binding of components to the RBD. If the sample does not contain constituents that bind and block the RBD-hACE2 interaction after a 15 min incubation at 37 °C (bottom four wells) the RBD-HRP will bind to the hACE2-coated wells giving a yellow color after incubation with TMB for 15 min at 37 °C followed by stop solution. If the sample does contain blocking constituents, they will bind to the RBD during the initial 30 min and inhibit the interaction with hACE (top four wells) giving a light yellow or clear color after addition of stop solution.
(Fig. 1: is from our recently published, open access article (Taylor, S. C. et al. A New SARS CoV-2 Dual Purpose Serology Test: Highly Accurate Infection Tracing and Neutralizing Antibody Response Detection. J Clin Microbiol, doi:10.1128/jcm.02438–20 (2021)) [2].

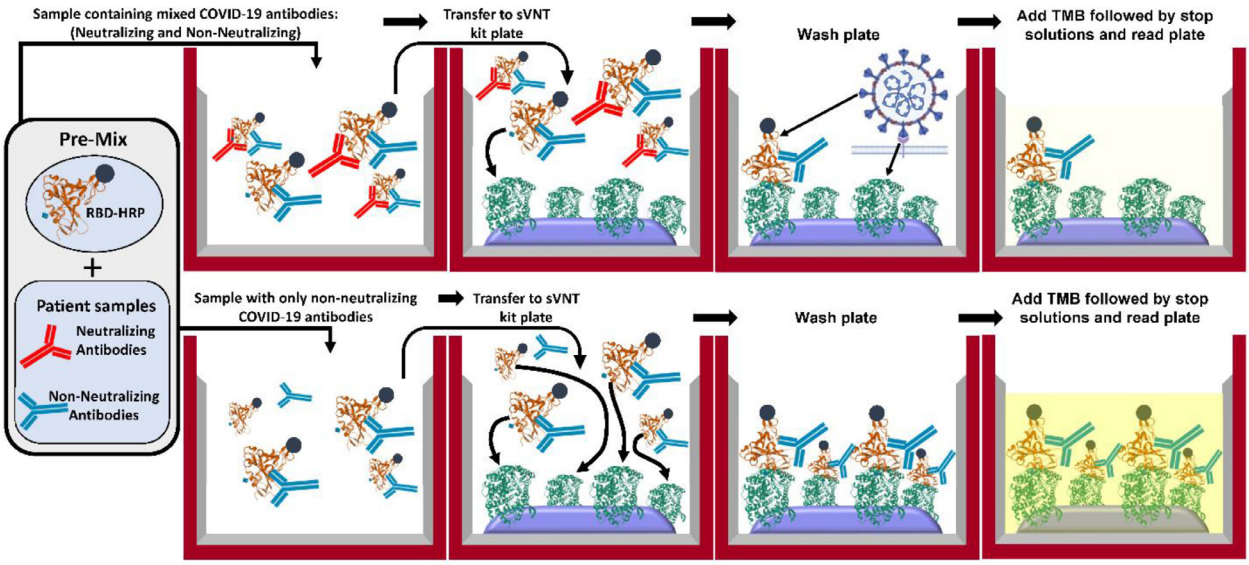

Fig. 1. Continued

ies detecting both total IgG as well as neutralizing-specific antibodies support a gradual decline in their levels over time and animal studies have shown a direct correlation to neutralizing antibody levels and susceptibility to SARS-CoV-2 reinfection [32,39,40,50–53,56–58,60,63]. Although these data have not yet been fully verified in humans, some recent work strongly supports neutralizing antibody titers as a protective correlate to SARS-CoV-2 infection that can be used as potential measurable molecular determinants that trigger the requirement for a booster shot [21–23]. The recently initiated human challenge studies should substantiate the current body of data [65,66]. nAbs will likely play a role and can be measured semi-quantitatively to investigate the correlation of their levels with protection post-SARS-CoV-2 infection/recovery and/or post-vaccination [55]. Therefore, to mitigate continued pandemic waves of infection globally, the periodic measurement of nAb titer levels may reveal a minimal titer required for protection that would trigger the need for booster vaccinations.

5. Conclusion

SARS-CoV-2 pandemic control continues to be a challenge globally and is further complicated by the distribution of different vaccines with varying efficacies towards the variant strains [14–16]. The question around the longevity of immunity post-vaccination remains unanswered but the periodic measurement of nAb titers may give a good indication. To minimize the potential for future outbreaks, a revised testing strategy to assure initial and continued protection post-vaccination will likely be required. This could include a combination of qPCR (for non-vaccinated and susceptible people) and nAb (for previously infected and vaccinated people) assays. cPass sVNT could be a useful tool for substantiating individual immunity in a SARS-CoV-2 post-vaccination era.

6. Regulatory status

The cPass SARS-CoV-2 Neutralization Antibody Test is CE Marked for diagnostic use in European Union and authorized for emergency use only (EUA) by Health Sciences Authority in Singapore and the US Food and Drug Administration (FDA) for qualitative delineation between positive and negative patient samples. The semi-quantitative cPass sVNT EUA by FDA is pending. The semi-quantitative and automation cPass sVNT protocols have not yet been authorized by FDA, European Union or Singapore and are currently Research Use Only. Brazil's National Health Surveillance Agency (ANVISA) (Agência Nacional de Vigilância Sanitária) has authorized the use of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit for both qualitative and semi-quantitative detection of neutralizing antibodies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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