LETTER TO THE EDITOR

What to Expect from Antibody Assays of SARS-CoV-2?

TO THE EDITOR:
The COVID-19 pandemic has resulted in nearly 7 million confirmed infections and >400 000 deaths as of June 7, 2020, with 3 billion people under lockdown worldwide. Nucleic acid tests of the disease-causing SARS-CoV-2 have been the mainstream diagnostic method in the early phase of outbreak, but the abundance of virus is reported to fade 3–5 weeks after infection (1). Furthermore, with growing concern about halted social activity and intention to reopen, serological assays detecting SARS-CoV-2 antibodies for population surveillance have gained increasing interest. To have reasonable expectations of the antibody tests, better understanding of their properties is warranted.

KEY PROTEINS IN SARS-COV-2
SARS-CoV-2 is an enveloped, single-stranded, and positive-sense RNA virus that belongs to the family of Coronaviridae. It has 4 major structural proteins—spike (S), envelope, membrane, and nucleocapsid (N)—and a number of other nonstructural proteins. The S protein is essential to viral attachment, fusion, entry, and transmission. Its N-terminal S1 subunit, composed of N-terminal domain and receptor-binding domain (RBD), is responsible for virus–receptor binding, and the C-terminal S2 subunit is responsible for virus–cell membrane fusion. The S1-RBD interacts with host cells expressing angiotensin-converting enzyme 2 as receptors. The receptor binding triggers conformational changes in the S2 subunit that result in virus fusion and entry into the target cell (2).

HUMORAL RESPONSE TO SARS-COV-2
The time course of SARS-CoV-2 seroconversion is not fully understood at this time, but studies are rapidly emerging. The seroconversion window may range from a few days to 3 weeks after symptom onset (3). The median duration of the IgM and IgA detection period was reported to be 5 days, whereas IgG was detected at a median of 14 days after symptom onset (4). Within 2 weeks after symptom onset, the positive rate of total antibody usually increased to nearly 100%.

FORMAT OF SEROLOGICAL ANTIBODY TESTS
The development of antibody tests starts from selecting antigen epitopes in the viral protein to allow antibodies (IgM, IgA, IgG) present in infected individuals to bind to recombinant viral proteins. S protein tends to be the a more desirable target given its specificity and high immunogenicity, with the RBD in S protein being the target of many neutralizing antibodies; however, other strategies use both S and N proteins. Several formats of antibody assays for SARS-CoV-2 have been produced, including qualitative lateral flow assay, qualitative or quantitative ELISA, or chemiluminescent assay and neutralizing assays. The first 2 types test for either total antibodies or for specific IgG, IgM, or IgA antibodies. Neutralizing assays test for active antibodies that can effectively block the virus. The antibody assay also needs to demonstrate minimal cross-reactivity with other coronaviruses (2).

As of June 7, 2020, the US Food and Drug Administration has issued emergency use authorizations for 17 serological tests, and we summarized their technical features in Table 1. The Vitros assay, for example, uses a SARS-CoV-2 S protein antigen to immobilize antibodies in the blood sample and murine monoclonal antihuman IgM/IgG antibodies for detection. The Diasorin assay uses recombinant S1 and S2.
Table 1. Antibody assays with US Food and Drug Administration emergency use authorizations as of June 7, 2020.

| Test name                           | Manufacturer | Approval date | Format                        | Antigen target                          | Antibody classes |
|-------------------------------------|--------------|---------------|-------------------------------|-----------------------------------------|------------------|
| qSARS-CoV-2 IgG/IgM Rapid Test      | Cellex       | 4/1/2020      | Lateral flow immunoassay       | SARS-CoV-2 S and N proteins             | IgM, IgG         |
| DPP COVID-19 IgM/IgG               | Cymbio Diagnostics System | 4/14/2020 | Lateral flow with reader       | SARS-CoV-2 N protein                    | IgM, IgG         |
| VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack | Ortho Clinical Diagnostics | 4/14/2020 | Qualitative chemiluminescent assay | SARS-CoV-2 S protein and RBD protein    | IgG              |
| COVID-19 ELISA IgG Antibody Test   | Mount Sinai Laboratory | 4/15/2020 | Antibody-titer ELISA           | SARS-CoV-2 S protein and RBD protein    | IgG, IgG         |
| Anti-SARS-CoV-2 IgM/IgG Rapid Test | Autobio Diagnostics | 4/24/2020 | Lateral flow immunoassay       | SARS-CoV-2 S protein                    | IgM, IgG         |
| LIAISON SARS-CoV-2 S1/S2 IgG       | DiaSorin     | 4/24/2020      | Qualitative chemiluminescent microparticle immunoassay | SARS-CoV-2 S1 and S2 proteins         | IgG              |
| SARS-CoV-2 IgG assay               | Abbott Laboratories | 4/26/2020 | Antibody-titer ELISA           | SARS-CoV-2 N protein                    | IgG              |
| Platelia SARS-CoV-2 Total Ab assay | Bio-Rad Laboratories | 4/29/2020 | Antibody-titer ELISA           | SARS-CoV-2 N protein                    | IgG              |

Continued
| Test name | Manufacturer | Approval date | Format | Antigen target | Antibody class(es) |
|-----------|--------------|---------------|--------|----------------|-------------------|
| New York SARS-CoV Microsphere Immunoassay for Antibody Detection | Wadsworth Center, New York State Department of Health | 4/30/2020 | Microsphere immunoassay | SARS-CoV-2 N protein | Total Antibody |
| Elecsys Anti-SARS-CoV-2 | Roche Diagnostics | 5/2/2020 | Qualitative chemiluminescent immunoassay | SARS-CoV-2 N protein | Total Antibody |
| Anti-SARS-CoV-2 ELISA (IgG) | EUROIMMUN US | 5/4/2020 | ELISA | SARS-CoV-2 S protein | IgG |
| Atellica IM SARS-CoV-2 Total (COV2T) | Siemens Healthcare Diagnostics | 5/29/2020 | Qualitative chemiluminescent microparticle immunoassay | SARS-CoV-2 S protein | Total Antibody |
| ADVIA Centaur SARS-CoV-2 Total (COV2T) | Siemens Healthcare Diagnostics | 5/29/2020 | Qualitative chemiluminescent microparticle immunoassay | SARS-CoV-2 S protein | Total Antibody |
| COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) | Healgen Scientific | 5/29/2020 | Lateral flow immunoassay | SARS-CoV-2 S protein | IgM, IgG |
| RightSign COVID-19 IgG/IgM Rapid Test Cassette | Hangzhou Biotest Biotech | 6/4/2020 | Lateral flow immunoassay | SARS-CoV-2 S protein RBD domain | IgM, IgG |
| Vibrant COVID-19 Ab Assay | Vibrant America Clinical Labs | 6/4/2020 | Qualitative chemiluminescent immunoassay | SARS-CoV-2 S (S1, S2, RBD) and N proteins | IgM, IgG |
antigens to capture sample antibodies on the solid-phase, and mouse monoclonal antibodies to human IgG for detection. The majority of the assays claimed positive agreement with PCR between 80% and 90% and close to 100% negative agreement.

NEUTRALIZING ANTIBODIES IN CONVALESCENT PATIENTS

Neutralizing antibody from a convalescent patient is believed to block SARS-CoV-2 from entering into target cells. Lessons from the 2003 SARS-CoV-1 showed that the specific IgG antibodies and neutralizing antibodies were highly correlated, peaking at month 4 after the onset of disease and decreasing gradually thereafter. Long-lasting specific IgG and neutralizing antibody were reported at least 2 years and as long as 17 years after infection (5). Because SARS-CoV-2 S protein is responsible for interacting with host receptors, antibody assays that target the S protein with titer results that correlate well with neutralizing assay would be of great value in assessing patients’ immunity and potentially managing plasma donation from convalescent patients.

In summary, antibody assays of SARS-CoV-2 are mainly developed against the viral S or N protein and are available in multiple formats. Their clinical utility is for the identification of individuals with past exposure history to SARS-CoV-2 in the previous 2 weeks or longer. Presence of SARS-CoV-2 IgG potentially correlates with a virus-blocking effect (i.e., immunity), but further direct evidence needs to be collected.

Nonstandard abbreviations: S, spike; N, nucleocapsid; RBD, receptor-binding domain.

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Xin Yi
Jing Cao
Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, TX
Department of Pathology, Baylor College of Medicine, Texas Children’s Hospital, Houston, TX

Address correspondence to this author at: Baylor College of Medicine, Texas Children’s Hospital, Pathology, 6621 Fannin St., West Tower, Room BB100.39, Houston, TX 77030. Fax 832-605-5110; e-mail jeac@texaschildrens.org.

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