FACTS AND MYTHS ABOUT THE SARS-COV-2 SEROLOGY TEST: WHAT DO RESULTS MEAN?

BACKGROUND

Serologic assays are commonly used to assess whether a specific antibody response has been developed to an infectious agent. For SARS-CoV-2, the FDA has developed two distinct pathways for marketing such tests. One involves registration without data submission and without verification of the performance of the assay. The second is through an Emergency Use Authorization (EUA) which requires that performance data on sensitivity and specificity be submitted to and reviewed by the FDA. Reliable serologic assays will play a very important role in assessing how widespread SARS-CoV-2 infections are in the US and globally and are a key component of the White House "Opening up America Again Testing Blueprint".

CLINICAL UTILITY

- The primary clinical utility of a serologic test is to document immune response to infection as evidence that a past infection has occurred. It is NOT a diagnostic test to establish a current, active infection.
- The tests may also be useful in patients with a high clinical suspicion for infection for whom molecular tests have not yielded positive findings due to low sensitivities of single sample results related to a number of variables. If clinical suspicion remains high, these tests may help qualify a patient for a specific therapeutic trial.
- Potential plasma donors may also be screened for presence of specific SARS-CoV-2 antibodies as a condition for convalescent serum donation. However, it is not known which antigen specific antibodies are most useful for this purpose.
- Serologic testing is an important sero-surveillance tool of infection in the community.

INTERPRETATION OF A POSITIVE RESULT

A positive (or reactive) result indicates that anti-SARS-CoV-2 IgM, IgG, or both were detected in the specimen of tested individual and s/he is likely to have had a recent SARS-CoV-2 infection. Note that positive test results are not definitive for diagnosis of SARS-CoV-2 virus infection. Diagnosis is generally made by molecular testing using an FDA EUA approved molecular diagnostic assay for SARS-CoV-2. It is also possible that an antibody test can give a positive result that is wrong, i.e., a "false positive" result. Individuals with a history of infections by closely related coronavirus strains, such as human coronavirus OC43, HKU1, 229E, or NL63, may harbor antibodies against these related viruses that are potentially cross-reactive with the anti-SARS-CoV-2 antibody test.

NOTE: Quantitative reporting of results that indicate the level of antibody response may be useful in the future to assess protective immunity or vaccine efficacy but are not yet widely used.

INTERPRETATION OF A NEGATIVE RESULT

A negative (or non-reactive) test result indicates that anti-SARS-CoV-2 IgM, IgA, IgG, or all 3 were not detected in the specimen of tested individual. However, a negative test result does not rule out SARS-CoV-2 virus infection. It is known that not all SARS-CoV-2-infected individuals will make antibodies against the viral antigens detected in a given assay. It is also

possible that the specimen was collected too early after infection/exposure and the individual had not yet had sufficient time to produce antibodies for the test to measure. Initial testing using an FDA-EUA approved molecular diagnostic specifically for SARS-CoV-2 should be performed to rule out acute infection by the virus.

FACTORS AFFECTING RESULTS

Pre-analytic issues

- Samples for antibody testing must be obtained after a sufficient period of time has elapsed for development agent-specific antibodies. It is generally accepted that at least a week must pass before early antibodies are detected, and ideally at least 14 days for other antibodies to be detected.
- Specimens used for the tests include whole blood, serum, or plasma, depending on the test format and assay developer. Most assays use either venipuncture collected blood samples or fingerstick collected blood samples.

Analytic issues

- The antigens selected for detection of SARS-CoV-2 antibodies is a very important parameter in design of serologic assays. The antigens selected vary among assays currently available (See Table), and it is not known which antigen specific antibodies are predictive of protective immunity. Protective immunity is generally associated with antibodies that neutralize infectivity in cell culture, a technique that is difficult for laboratories to perform for validation purposes.
- Antibody specificity is another extremely important parameter for SARS-CoV-2 serologic testing. If not designed properly, ***individuals with a history of infections by closely related coronavirus strains such as human coronaviruses causing common colds (OC43, HKU1, 229E, or NL63) may be potentially cross-reactive in the anti-SARS-CoV-2 antibody test. It is equally important to demonstrate that other infections do not provoke antibody responses to SARS-CoV-2, a finding that is known to occur with other viral infections***.
- Recently, FDA has advocated for a 2-step serologic assay format to improve overall sensitivity and specificity for SARS-CoV-2 sero-surveillance. This would entail initial testing for a more broadly reactive antibody followed by a more specific antibody. Such 2-tier testing is commonly employed in laboratories as for syphilis or Lyme Disease serologic testing.
- Formats that have been developed for SARS-CoV-2 serology are of two main types. One is a rapid membrane bound assay that is often performed near patients. These assays may provide results in 15-30 minutes but are single unit tests and not amenable to large scale population testing. The other format is a conventional ELISA (enzyme-linked immunosorbent assay) which is performed in multiple sample testing devices and is amenable to high throughput testing.
- Antibody type is the last analytical parameter. Assays may be designed to detect **IgM** (early antibodies), **IgA** (mucosal pathogen related antibodies), or **IgG** (late antibodies); or they may be configured to detect more than 1 (“pan”) antibody type. While IgM detection may indicate a more recent infection, it is not definitive for a current or ongoing infection.

- **NOTE**: The **Abbott Architect IgG assay** against the SARS-CoV-2 nucleocapsid protein has been granted an EUA and has been rapidly adopted as an automated high throughput assay in Memphis area laboratories.

Post-analytic issues

- **Serologic assays are NOT intended for diagnostic purposes**. They complement molecular assays that directly detect the SARS-CoV-2 virus but are not a substitute for such diagnostic assays.
- Results generated are primarily **qualitative**, that is positive or negative, and simply indicate whether exposure to an infectious agent has occurred.

ANTIBODY AND IMMUNITY

A positive test result is indicative of some sort of immunity. However, it is not known whether or which antibodies detected are protective and have neutralizing activity against the virus, and if so, how long they last.

UNANSWERED QUESTIONS AND MISCONCEPTIONS

- Specific antibodies that indicate protection from future infection with the same or a closely related virus has not been determined, thus the use of these assays to generate an **"immunity certificate"** is not recommended at this time. Similarly, which specific antibodies are likely to demonstrate evidence of adequate protective immunity post-vaccination are unknown as an effective vaccine has not yet been developed.
- The clinical utility of serologic tests as a "return to work" test is not known and if performed should be used with caution.
- It is also not known how long or even if protective immunity persists after a natural infection or vaccination. Coronaviruses are somewhat notorious for short-lived antibody responses.
- Finally, a role for Point-of-care (POC) testing or consumer-directed testing has not been established. In fact, at this point in time FDA requires serologic assays for SARS-CoV-2 to be performed strictly in a CLIA high complexity laboratory setting, and to be specifically ordered by and reported to an authorized healthcare provider.

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