

COVID-19 Antibody Testing Overview and Potential

Overview of antibody testing for COVID-19: An analysis of capabilities, platforms, strategies, and risks for healthcare executives and laboratory leaders

Created
April 22nd, 2020

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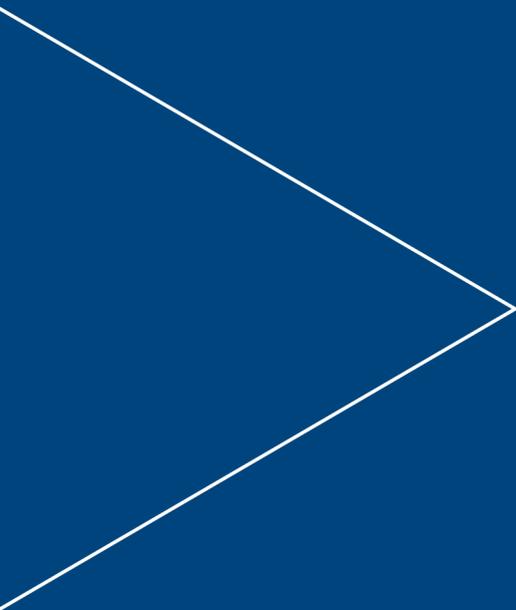
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Introduction

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Context

- The COVID-19 pandemic has created a responsibility for laboratories to provide appropriate and accurate testing information in an emotional environment of rapidly evolving, untested approaches and claims.
- Serology of coronaviruses in previous SARS and MERS epidemics predicted IgG can become protective; the methods for detecting and quantifying an immune response include classical immunochemistry tests. Such a response in COVID-19 is yet to be verified.
- WHO and FDA Guidance provides recommendations regarding test performance, but to date has accepted incomplete standardization and product validation, in many cases leading to inconsistent results and epidemiological quandaries.
- The pathway to Best Practices requires understanding and experience. Each clinician must determine the needs of its patients and community before deciding what testing to implement and how.

Intended Audience

- This document is intended for PDL clients and clinicians.
- Adjacent audiences such as those creating policy surrounding COVID-19 will find details regarding the potential of antibody testing in order to develop down-stream strategies.

Introduction

Important Terminology

Molecular Diagnostics (MDx)

- NAT: Nucleic Acid Test (either RNA or DNA)
- PCR: polymerase chain reaction
- Isothermal amplification
- Sequencing

Serological Methods

- IA: Immunoassay
- ELISA: Enzyme Linked Immuno Sorbent Assay
- LP: Lateral Flow
 - LPO: LF Optically read
 - LPM: LF Manually read

Assay Types

- FDA-cleared
 - Waived, Moderate and High Complexity
- LDT: Laboratory Developed Test
- POCT: Point of Care Test

Miscellaneous

- COVID-19: Infectious Disease caused by Coronavirus (SARS CoV-2)
- SARS CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2 (causative agent of COVID-19)
- FDA: Food & Drug Administration
- IFU: Instructions for Use (package insert)
- EUA: Emergency Use Authorization
- CMS: Center for Medicare & Medicaid Services
- WHO: World Health Organization
- CLIA: Clinical Laboratory Improvement Act
- IVD: In-Vitro Diagnostics
- CPT code: Current Procedural Terminology

Serology Markers

- Ab: Antibody
 - IgM: Immunoglobulin M
 - IgG: Immunoglobulin G
 - IgA: Immunoglobulin A
- Ag: Antigen

COVID-19 Serologic Overview

Typical Viral Serologic Profile

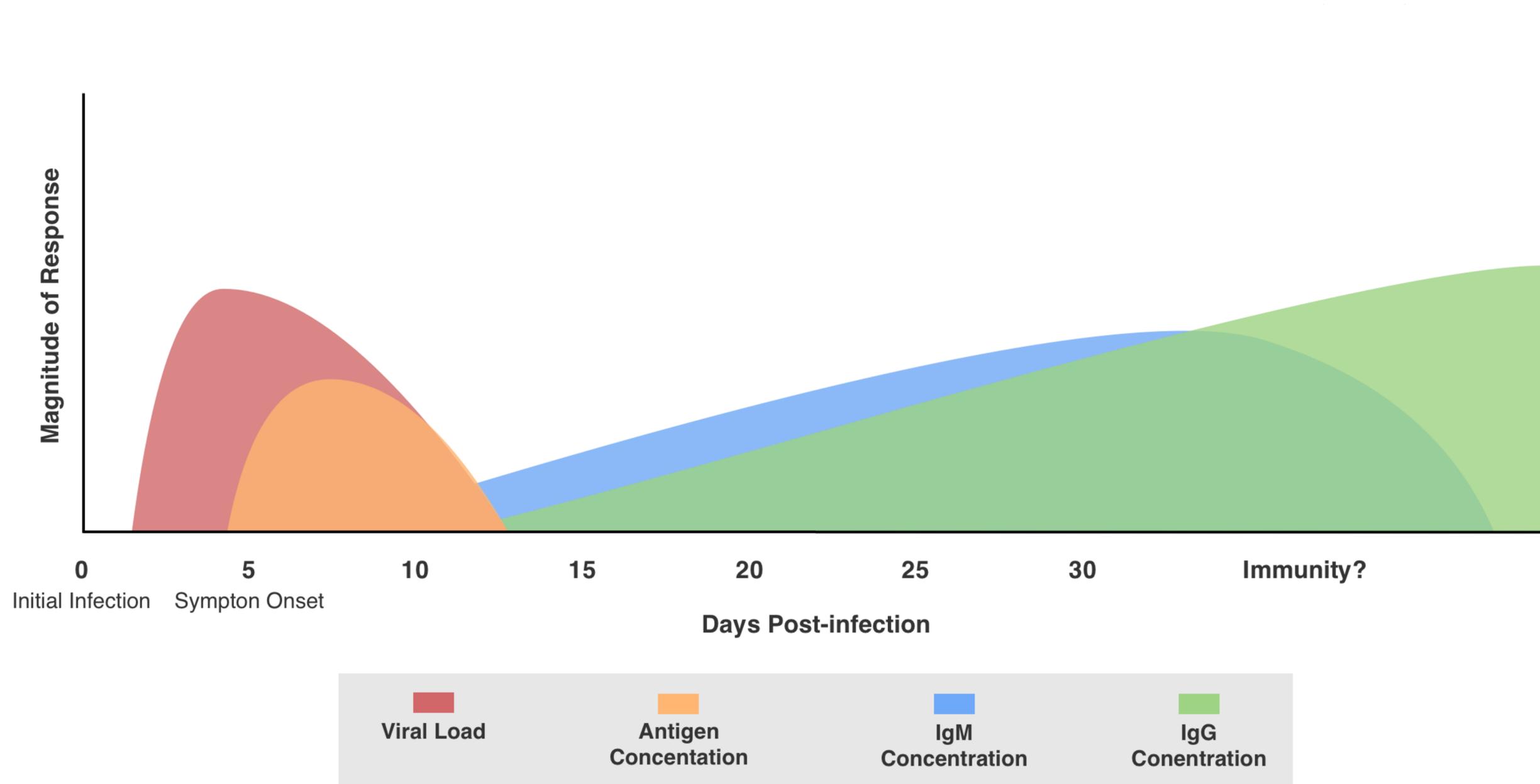
Serologic Markers of Infection

How the COVID-19 Response Differs

Types of COVID-19 Antibody Tests

COVID-19 Serologic Overview

Typical Viral Serologic Profile



Different quantitative measurements are informative at different time intervals post-infection. The quantity of viral antigen or classes of immunoglobulins also change over time. It remains to be seen if COVID-19 elicits a similar profile.

For Illustrative Purposes Only

COVID-19 Serologic Overview

Serologic Markers of Infection

The target pathogen, or viral particle, can be detected in two ways:

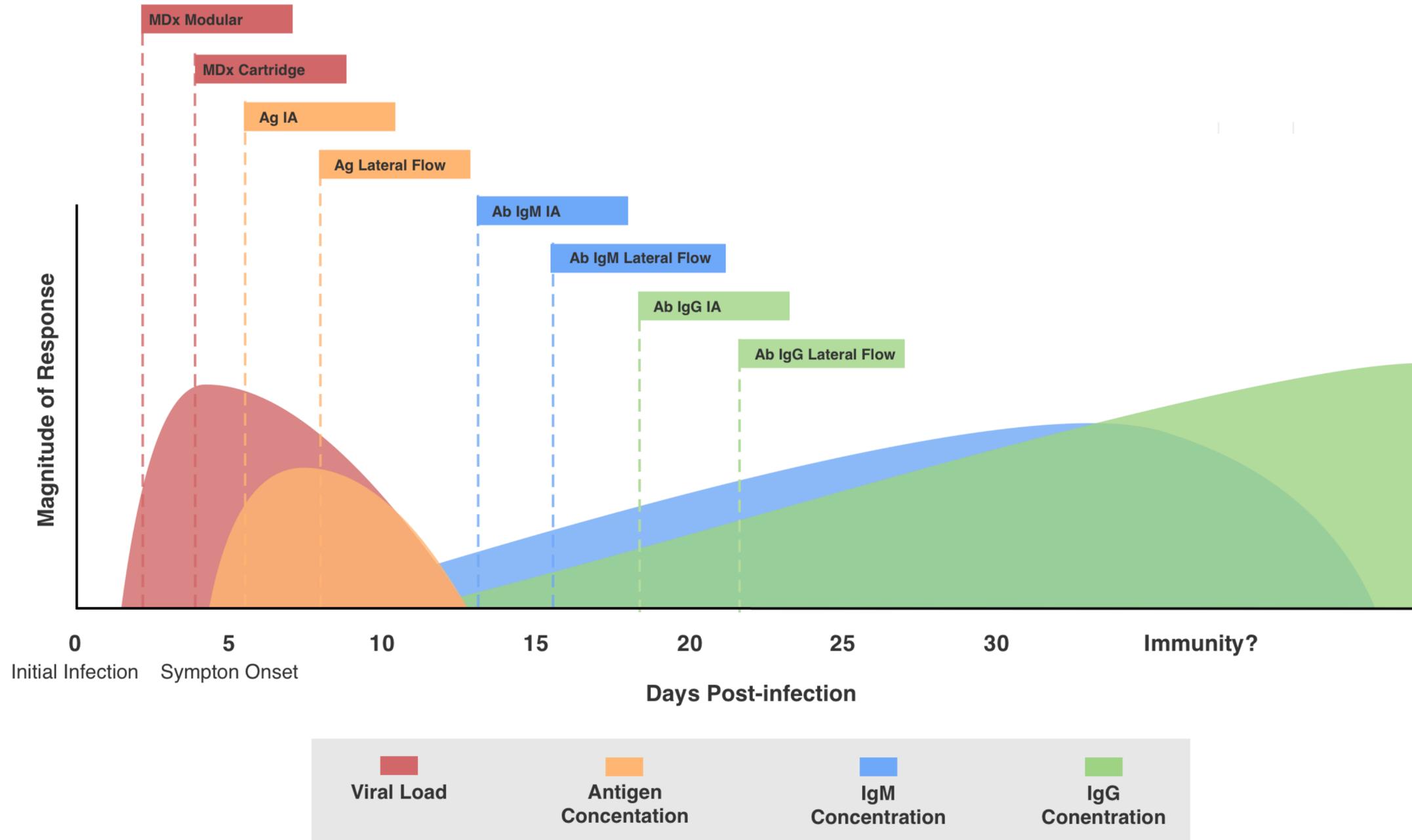
- **Nucleic Acid Test (NAT):** Viral nucleic acid (RNA) can be extracted from the specimen, amplified using PCR (or other amplification techniques), then detected using various molecular diagnostic (MDx) techniques. RNA is unstable unless processed appropriately. These methods are very sensitive and specific for the target virus, but require expensive dedicated instrumentation and reagents, and often require “high complexity” CLIA licensure to perform.
- **Direct Antigen (Ag):** utilizes standard immunoassay procedures usually targeting either viral surface or nuclear proteins. These methods are analytically less sensitive than MDx assays (longer time from symptoms to detection) but are more easily automated on standard immunoassay instruments used by clinical labs. Assays can usually be performed under CLIA’s “moderate complexity” licensure.

Antibody Response to Viruses

- **IgM:** Typically the earliest Ab type produced in response to infection. IgM responses elicited by different pathogens are variable in time to appearance and overall duration. Many IgM responses are present within 5-10 days and can last for months subsequent to the resolution of infection. IgM Abs are usually of low affinity and function to hold the pathogen in-check until a more specific immune response can be produced.
- **IgG:** Similar to IgM, IgG responses elicited by different pathogens are also variable in context of their time to appearance and overall duration. IgG responses are usually present within 8-14 days post infection, with the vast majority of infected individuals having substantial IgG responses after 14 days. IgG Ab responses are very specific and of high affinity, and function to neutralize the pathogen.
- **IgA:** IgA: plays a role in the immune function of mucous membranes, and as such, may be a useful diagnostic marker for pathogens whose primary portal of entry is a mucosal membrane surface.

COVID-19 Serologic Overview

Serologic Markers of Infection (cont'd)



Timeline of Markers

Detection of viral serologic markers (Nucleic Acid, Ag, or Ab) as early as possible requires highly sensitive methods to provide greatest clinical utility. Ab responses take time to develop. As such, serological testing is usually not conducted until 10+ days after the onset of symptoms.

For Illustrative Purposes Only

COVID-19 Serologic Overview

How Does the COVID-19 Response Differ From Typical Serologic Profiles?

Since COVID-19 is a newly described coronavirus (SARS-CoV-2), it is unclear whether patients with IgG responses will demonstrate long-lasting immunity to the virus. Until longitudinal studies are completed over the next several years, the only guidance we have is based on similar coronaviruses such as SARS and MERS. Immunity to these coronaviruses has persisted from 1 to 8 years. Comprehensive understanding of the Ab responses elicited by SARS-CoV-2 is critical to the efficacy of vaccination and immune treatment strategies.

Methods Aren't Fully Developed

Methods for direct detection of viral antigens are not yet commercially available except for poorly performing insensitive dipstick methods. "High-sensitivity" immunoassays are in development, potentially detecting levels of viral antigens approaching the sensitivity of current MDx methods on the market, with higher throughput at a lower cost;

There Are Two Primary Ab Targets

Like most immunological responses to viruses, IgM is expected to rise first. IgG Ab to COVID-19 is present earlier than in other viral infections, often concomitantly or shortly after IgM is detected. Several commercial methods target total Ab rather than measuring IgM and IgG individually;

IgA Ab Could Be More Important

IgA Ab may be important in COVID-19, since the primary portal of entry for SARS Cov-2 are the mucous membranes of the respiratory tract. What is unclear is whether IgA detection is best in circulating blood (similar to other antibodies), or is optimal if measured in respiratory secretions (similar to RNA methods).

COVID-19 Serologic Overview

Types of COVID-19 Antibody Test Methods

Immunoassay

Immunoassays are tests that measure the presence or concentration of an immunogenic molecule in a solution. Immunoassays may use specific binding properties of an antibody to detect and quantify an antigen. They can also detect and quantify antibodies. The molecule detected by the immunoassay is often referred to as an "analyte" and is in many cases a protein.

Immunoassays come in many different formats and variations and are often run in multiple steps with reagents being added and washed away or separated at different points in the assay. Multi-step assays are often called separation immunoassays or heterogeneous immunoassays. Some immunoassays can be carried out simply by mixing the reagents and sample in a single step and making a physical measurement. Such assays are called homogeneous immunoassays, or less frequently non-separation immunoassays.

ELISA

The enzyme-linked immunosorbent assay uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a ligand (commonly a protein) in a liquid sample using antibodies directed against the protein to be measured.

In the simplest form of an ELISA, antigens from the sample are attached to a surface. Then, a matching antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change.

PDL will refer immunoassay testing to Lab Corp until our In-house Siemens' methodology is brought online in late June.

Lateral Flow

These tests, also known as lateral flow immunochromatographic assays, are simple devices intended to detect the presence of a target substance in a liquid sample without the need for specialized and costly equipment. These tests are widely used in medical diagnostics for home testing, point of care testing, or rapid laboratory use. For instance, the home pregnancy test is a lateral flow test that detects a certain hormone. These tests are simple, economic and generally show results in around 5-30 minutes. Many lab-based applications increase the sensitivity of simple lateral flow tests by employing additional dedicated equipment to objectively read the reaction.

Antibody Testing Uses and Strategies

What Can Antibody Testing Tell Us?

Use-case Scenarios

Testing Strategies

Antibody Testing Uses and Strategies

What Can Antibody Testing Tell Us about COVID-19?

Does Not Indicate Infection

Ab testing does not indicate whether the patient is currently infected; therefore, a negative result does not rule out whether a patient is contagious.

Evidence That an Individual has Immunity

Ab testing can be used to identify if an individual has been exposed to the virus and may indicate immune status. Serology may differentiate individuals protected from future infection from individuals who remain susceptible. The usefulness of Ab testing hinges on whether everyone who is infected with SARS CoV-2 actually develops antibodies, whether those antibodies protect against secondary infections, and if so, how long the antibodies stay in the body and remain protective

Data for Epidemiological Research

Ab test results can also be useful for widespread disease surveillance and epidemiological research.

Identify Potential Blood Donors to Treat COVID-19

Ab levels can help identify individuals qualified to donate blood for convalescent plasma, an investigational product potentially useful in treating patients infected with SARS CoV-2.

“Serological Testing potentially identifies individuals who have been exposed to SARS CoV-2, may be immune, and potential immune plasma donors”. It may also identify those individuals who successfully defended against the virus and who may also successfully defend again if re-infected

Antibody Testing Uses and Strategies

Use Case Scenarios for Antibody Testing

Clients should develop use case scenarios of who, where and when to test before implementing testing platforms. As with MDx testing, it's advisable to identify multiple platforms for each use case to ensure an adequate supply of test kits.

Who

Identify the population to be tested: Healthcare Workers, First Responders, Patients? Then, prioritize testing by group to control any limited supply of inventory. Consider the acuity of and impact on patient care before implementing your testing strategy.

Where

Map out the key locations required to perform testing and understand their constraints: where will specimens be collected, by whom, where will the test be performed, and how will specimens be transported?

When

Based on currently available information, it is estimated that the optimal time to test for immunity is 10+ days after the onset of symptoms (or lab confirmation of presence of the virus).

Antibody Testing Uses and Strategies

Antibody Testing Strategies

Dual testing may be on the horizon

- Infectivity: Viral RNA/Ag testing requires a 'respiratory' specimen (nasal, throat, nasopharyngeal, saliva).
- Exposure: Ab testing requires whole blood or serum.
- Combining these two tests can offer a more complete clinical picture
 - Both specimen types (respiratory and whole blood) can be collected by the provider at the same time and potentially performed on the same instrument.

Multiplex viral panel could be in the future

- Since Coronavirus and Influenza clinically present with similar symptoms, there is a potential need to test for both viruses once the 'flu season' starts again in late fall. This could be accomplished in several ways which PDL is actively researching:
 - Separate tests for Flu A/B and for SARS Cov-2 on separate specimens using multiple platforms.
 - Multiplex testing for Flu A/B and SARS Cov-2 on the same specimen on the same platform.
 - Viral panels encompassing multiple respiratory pathogens including Flu A/B and SARS Cov-2 .

Immunity Passport has limited utility

- Some countries (including the US) are contemplating proof of immunity as a pre-requisite for relaxing social distancing and allowing re-entry into the workforce.
 - Termed an "Immunity Passport", antibody testing could be a component of action plans for returning to work, especially in health care settings. Unfortunately, this differentiation also presents a risk of labeling those without the passport as 'inferior'.

WHO and FDA Guidance

WHO and FDA Guidance

WHO Guidance

The WHO has issued several warnings over the past few weeks regarding antibody testing for SARS Cov-2, primarily due to inconsistent performance of many kits on the market, lack of correlation with viral detection, and the lack of evidence linking the presence of antibodies to immunity.

April 8th, 2020

- “Molecular (e.g. PCR) testing of respiratory tract samples is the recommended method for the identification and laboratory confirmation of COVID-19 cases. However, based on current data, WHO does not recommend the use of antibody-detecting rapid diagnostic tests for patient care (but encourages the continuation of work to establish their usefulness in disease surveillance and epidemiologic research).”

April 13th, 2020

- “Not all people who recover from COVID-19 have the antibodies to fight a second infection” based on a study of patients in Shanghai where some patients had “no detectable antibody response” while others had a very high response.”

April 17th, 2020

- **“There’s no evidence serological tests can show whether a person has immunity (and if so for how long) or is no longer at risk of becoming re-infected.”**

WHO and FDA Guidance

FDA Guidance

The FDA has tried to balance the risks associated with the quick release of as many diagnostic kits as possible for COVID-19 with the need to meet increasing demand for testing yet ensuring public safety.

EUA vs Registration

- Initial strategy was to support releasing as many kits as possible via vendor **registration** under “Pathway D option” **rather than** subject those kits to the **EUA** review and approval process. As of mid-April, of the ~90 assays listed in the FDA’s registry, only 4 were granted EUA approval.
- Most of these tests were based on lateral flow (LF) immunochromatography, which utilizes cellulose-based cartridges to qualitatively detect the presence of a target analyte without the need for instrumentation. These assays are simple and economic, with results available within 15-30 minutes.
- Many of these assays were manufactured in Asia or Europe, where the use of antibody testing has been more prevalent than RNA tests. There have, however, been reports of poor analytical (and therefore clinical) sensitivity, exacerbated by the lack of standardization on how to measure performance.

Buyer Beware

- While the FDA had published a template for IVD manufacturers to follow for EUA approval of MDx kits measuring RNA, they have not yet released a template for measurement of antibodies using either Immunoassay (lateral flow) or ELISA techniques.
- Performance studies have not standardized basic components of testing, including time from symptoms or RNA detection to measure for the presence of antibodies, nor what potentially interfering respiratory viruses should be evaluated for cross-reactivity. Since a patient’s antibody levels increase over time, vendors can ‘manipulate’ their sensitivity by using specimens from patients drawn much later in the progression of disease.
- Some test developers have misused the serology test kit registration list to falsely claim their serological tests are FDA approved or authorized (via an EUA designation). Others have falsely claimed that their tests can diagnose COVID-19.

WHO and FDA Guidance

FDA Guidance (cont'd)

List of Disclaimers

- The FDA provided a list of disclaimers to be included by **manufacturers** of registered Ab assays in their IFU instructing **laboratories** to include these statements on their lab report:
 - The method was not reviewed by the FDA; Negative results do not rule out COVID-19 Infection particularly in those who have been in contact with the virus; Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS CoV-2; Positive results may be due to past or present infection with non-SARS CoV-p2 coronavirus strains such as coronavirus HKU1, NL63, OC43, or 229E or past or present infection with SARS virus (no. 6).
- If the test is cleared as an EUA by the FDA, Manufacturers should state that the test:
 - Has been authorized by FDA under an EUA for use by authorized laboratories; has been authorized only for the presence of IgM and IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens; and is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 .

Latest FDA Updates

April 10th, 2020

- The FDA clarified that, when it grants an EUA for a point-of-care test, that test is deemed to be CLIA-waived. For the duration of the national emergency declaration for COVID-19, such tests can be performed in any patient care setting that operates under a CLIA Certificate of Waiver or Certificate of Compliance.
- FDA clarified that tests for SARS-CoV-2 that are offered prior to or without an EUA have not been reviewed by FDA, are not FDA authorized, and have not received a CLIA categorization. Thus, those tests are considered high complexity by default.

April 18th, 2020

- FDA released their approach to expanding access to serology tests:
- Encourage developers to submit EUA requests for their tests (rather than registration only); to date only 3 commercially available antibody assays (Cellex, Chembio, Ortho) have been granted EUA status. (Note-a 4th test developed by Mt Sinai in NYC has also been approved, but it is not commercially available).
- Working on EUA Templates that laboratories and commercial manufacturers may use to facilitate the preparation and submission of an EUA request for antibody testing.
- Establish a capability at NIH to evaluate serological tests for developers. This effort includes tests already available for use, as well as tests not yet on the market where additional validation data is needed for an EUA.

Risks and Considerations

CPT Codes and Reimbursement

Availability of Equipment and Supplies

Off-label Use and IFUs

Accuracy of Testing

In-house Testing vs Reference Labs

Risks and Considerations

CPT Codes and Reimbursement

Antibody Testing

- **Current (general codes)**

- 86318: Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step method (eg, reagent strip);
 - Add 59 modifier if 2 Abs tested (ie, IgG, IgM).
 - CMS Reimbursement \$12.88 each Ab
- 86760: Antibody; virus, not elsewhere specified (Qualitative or Semiquantitative Immunoassays)
 - Add 59 modifier if 2 Abs tested (ie, IgG, IgM).
 - CMS Reimbursement \$12.88 each Ab

- **New Codes (specific for COVID-19)**

- 86328: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]) single step: This code applies to a POC assay (i.e., Cellex) that uses a single step lateral flow cartridge.
 - Add 59 modifier if 2 Abs tested (ie, IgG, IgM).
 - CMS Reimbursement TBD
- 86769: Antibody; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]) multiple steps. This applies to more traditional immunoassay instruments that utilize multiple steps (i.e., Ortho Vitros):
 - Add 59 modifier if 2 Abs tested (ie, IgG, IgM).
 - CMS Reimbursement TBD

Risks and Considerations

Availability of Equipment and Supplies

While many labs did not have MDx platforms to leverage, most labs have high throughput immunoassay analyzers, often coupled with chemistry analyzers on a track-based system. Each of the “big 5” IVD vendors selling automated chemistry/Immunoassay platforms have indicated their intent to commercialize Ab assays:

“Big 5” IVD vendors who sell automated IA platforms intend to sell Ab assays:

- **Ortho Clinical Diagnostics:** The first to achieve EUA approval for their total Ab test on their Vitros analyzers and are already delivering product to users.
- **Abbott:** Has announced a total Ab test for their Architect series analyzer, with production climbing from 1M at launch to ~4M tests/week by May. Although not yet EUA approved, Abbott claims to have already submitted validation data to the FDA.
- **Roche:** Has announced the availability of an 18-minute total Ab test on its e411 analyzer, with separate IgG and IgM assays forthcoming.
- **Siemens:** Has announced a total Ab test on their ADVIA Centaur® XP and XPT and high-throughput Atellica® Solution IA Analyzers targeting a late May release. **PDL has chosen Siemens for their quality, consistency and immediate availability.**
- **Beckman Coulter:** Has announced development of their assays targeting IgM and IgG antibodies for use on any of its high- throughput Access family of IA systems, but has not indicated a release date.

In addition to the ‘big 5’:

Other vendors have or will release Ab assays from mid-sized immunoassay/ELISA platforms (i.e., Diazyme, EuroImmun) to point of care platforms using nano based high sensitivity immunoassay (i.e., LumiraDx) or lateral flow based immunoassay cartridges (i.e. Cellex, Chembio).

Risks and Considerations

Accuracy of Testing

Antibody Test Accuracy is Dependent on Multiple Factors That are Difficult to Control

- Absent an FDA template defining validation requirements, Ab assays do not carry standardized performance specifications, yet most claim sensitivities and specificities above 90%.
- A key indicator of performance is the # of days blood is drawn post symptoms, but this information is often not included in the sensitivity data in the Instructions for Use (IFU).
- The longer the window between symptoms and blood collection, the higher the likelihood of Ab presence, so higher claims of sensitivity may be more reflective of the timing of testing rather than actual analytical performance.
- There have been numerous reports in the literature indicating that the actual performance of several Ab kits does not meet performance claims, resulting in a high # of false negatives.
- Even though several COVID-19 antibody tests claim 95+% specificity, testing a low prevalence population will result in a significant # of false positives. One mechanism to increase the PPV of the tests is to run two different Ab tests in series, provided that the two tests target different antigenic sites (i.e., Spike vs. Nuclear proteins).

Accuracy by Methodology

- Assay performance, especially sensitivity, is dependent on several factors, especially methodology (as listed below in order of decreasing sensitivity):
 - Automated Immunoassays – chosen by PDL
 - Automated ELISA
 - Lateral Flow instrument read assays
 - Lateral Flow manually read assays
 - Dipstick assays

Note: there can be a 10-20x difference in sensitivity between the most vs. least sensitive methods.

Summary

Outlook

Executive Takeaway

Outlook and Executive Takeaway

Outlook

- Harvard researchers estimate states need to conduct 152 tests per 100,000 residents daily. But 34 states were conducting fewer than 50 tests per 100,000 people during the week ended April 15.
- Currently, the US is producing ~150,000 test results per day, but most plans to re-open the country indicate that at least 500,000 test results per day are necessary, with some recent models projecting the need at 1-10M tests/day!
- The idea that ‘everyone who gets sick is going to get a test and we’ll be able to count every case with a lab diagnosis’ is probably not feasible until the production and inventory of test kits catches up to and exceeds the demand for testing, which may not occur for months.
- Most infectious disease epidemiologists speak about the pandemic in terms of years, not months. Most medical and industry experts are projecting the need for testing will remain in effect for at least 2 years, and potentially longer if a vaccine is either delayed or immunity is transient (as it is for influenza), in which case testing for Covid-19 becomes part of the annual respiratory viruses affecting the population.
- CDC Director warns “that second wave of coronavirus is likely to be even more devastating.....because it is likely to coincide with the start of the flu season!”

Outlook and Executive Takeaway

Executive Takeaway

- Antibody testing will be essential for epidemiologists and valuable for clinical diagnostics. Unlike molecular tests for COVID-19 infectivity, antibody tests are better suited for public health surveillance and vaccine development.
- The current antibody testing landscape is varied and unverified. With new kits and equipment being introduced and subject to expedited approval via the FDA's EUA process, the importance of hybrid approaches and phased roll-outs is heightened.
- Like antigen testing, population strategy, collection methods, and data reporting will be as important as achieving technical competency.
- Antibody testing should be used in conjunction with antigen testing to provide a comprehensive clinical picture and be effective in combating and monitoring spread.
- It is paramount to understand the prevalence of infection to COVID-19 in your target population to select an effective platform that optimizes the positive predictive value (PPV) of the Ab test.
- Knowing the EUA status of a platform, its accuracy and deficiencies, availability of supplies, and complexity are essential for understanding if it is appropriate for your health system and/or lab.
- PDL has COVID-19 antibody testing available now through our close reference laboratory relationship with LabCorp. We expect to have the testing available in house on the preferred Siemens platform in June.
- For any questions, please contact your PDL Sales/Service Representative or call Client Services at 866-591-4610.