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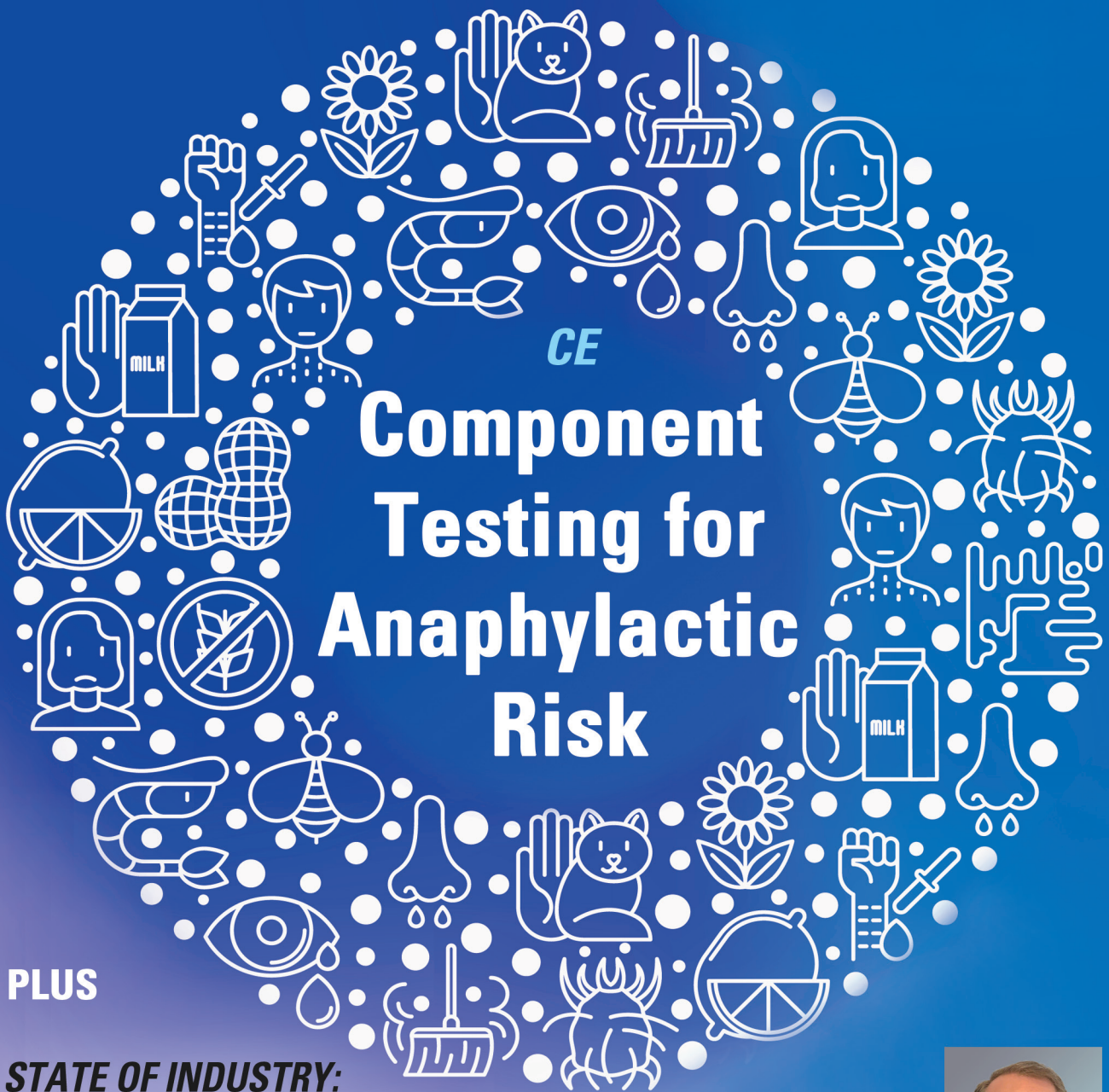

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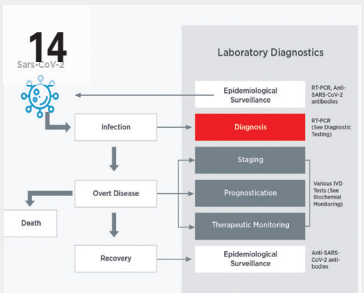

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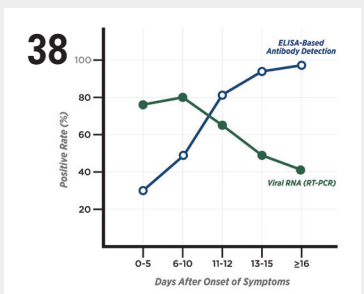
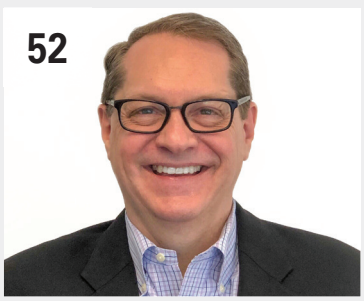
STATE OF THE INDUSTRY





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Labs conquer many COVID-19 challenges

By Linda Wilson
Managing Editor

Amid the chaos and suffering SARS-CoV-2 unleashes as it infiltrates mega-metropolitan areas and rural hamlets alike, lab managers work diligently to ramp up testing to detect both active infections and the antibodies the body develops to fight off the disease.

We all know that testing is a frontline activity in the fight against the pandemic. Testing reveals people who have an active infection, allowing them to isolate and minimize community spread. It also shows providers which patients warrant careful follow-up and care. And in the aggregate, data from testing helps public health officials track the trajectory of the disease and develop public policy.

But for many laboratorians, the planning and execution of testing services has been frustrating and exhausting, as well as (sometimes) quietly rewarding.

As *Medical Laboratory Observer* has followed laboratorians efforts over the past six months to ramp up testing by overcoming daunting challenges, I have been inspired by the level of dedication *MLO*'s readers demonstrate for SARS-CoV-2 testing. They plow ahead every day at work even as the number of healthcare workers infected with and dying from SARS-CoV-2 increases continually, according to information tracked by the U.S. Centers for Disease Control and Prevention (CDC).

Most recently, our readers have told us about their work helping patients by responding to *MLO*'s third State of the Industry Survey, focusing on COVID-19 testing, which we report on in this issue.

What we found is that lab managers have worked doggedly and creatively to source analyzers, testing kits, supplies and personal protective equipment (PPE), so they can test their community members for COVID-19. Despite those efforts, some have not been able to provide as much in-house testing as they would like, while others have not been able to launch a service at all.

Sourcing the components of a new testing service is just the beginning. In their survey responses, *MLO* readers also talked about efforts to refine workflows, train staff, modify laboratory information systems (LIS), and figure out how to break-even financially on testing.

Now, the next phase of this work begins. As the United States reopens its economy and residents venture outside in the warm summer sunshine, laboratorians and their peers throughout the healthcare delivery system wait to learn what this increased community activity portends for future COVID-19 caseloads. Experts say this is the time to refine current COVID-19 workflows to bring down the cost-per-test, source more supplies, and improve coding and billing.

And maybe take a few minutes to reflect on the state of the laboratory industry. As Sonya Engle, Chief Operating Officer at Sonora Quest Laboratories, told me while describing COVID-19 testing, "It is such an inspiring story of people who went into the laboratory business because they want to serve, and they care. Each person delivered in ways that are unprecedented."

I welcome your comments, questions and opinions — please send them to me at lwilson@mlo-online.com.

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• These tests are 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 under Section 564(b)(1) of the Act, 21 U.S.C.§ 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

† The Panther Fusion SARS-CoV-2 assay is available for EUA use in the United States, Australia, New Zealand, and Canada. EUA does not apply to the European Union. The Aptima SARS-CoV-2 assay is available for EUA use in the United States.

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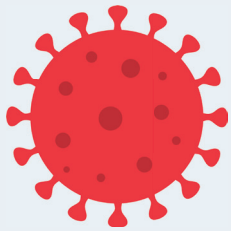
¶ In development and not for sale.
§ Seeking dual claim for the HIV-1 Quant assay.



Fast Facts COVID-19

Labs across the country are offering testing services to detect SARS-CoV-2, the novel coronavirus that causes COVID-19, overcoming significant challenges.

85%

of lab managers say supply chain disruptions have delayed or decreased testing.

55%

of lab manager say manufacturers/ suppliers have said they cannot purchase testing kits or reagents because of government restrictions/allocations for these products; 60% of academic medical centers and community hospitals and 30% of commercial reference labs agree with this statement.

60%

of labs are running full staffing seven days a week to run SARS-CoV-2 testing.

34%

of commercial reference labs process more than 500 SARS-CoV-2 tests per day, compared with 18% of labs at community hospitals, health systems or academic medical centers.

90%

of labs plan to increase testing capacity over the next three months.

84%

of labs will increase testing capacity by adding platforms. Other methods: increase staff (42%), increase lab shifts (39%), and add testing locations (22%).

88%

of labs said they are not experiencing significant numbers of false negatives on SARS-CoV-2 tests.

43%

of labs report turnaround times of 12-24 hours for their primary test method and 34% report turnaround times of 24-48 hours.

- **Source:** https://www.amp.org/AMP/assets/AMP_SARS-CoV-2_Survey_Report_FINAL.pdf

Multinational consortium reports on COVID-19 impact on cancer patients

People with cancer sickened by COVID-19 have a crude death rate of 13 percent, according to the largest series of data released thus far from a multinational perspective. The data on more than 900 patients, published in *The Lancet* and simultaneously presented at ASCO20 Virtual, also revealed cancer-specific factors associated with increased mortality.

The information is the first report from an ongoing international initiative by the COVID-19 and Cancer Consortium (CCC19) to track outcomes within this vulnerable population. The CCC19 registry was built and is maintained as an electronic REDCap database housed at Vanderbilt University Medical Center. The data in this first report from CCC19 was gathered from 928 patients in Spain, Canada and the United States.

"While older patients and those with major comorbid conditions are at substantially increased risk of dying from COVID-19, our early findings are encouraging news for patients without major medical conditions who receive their cancer therapy within four weeks of their infection. However, more data are needed to reliably assess individual higher risk therapies," said Nicole Kuderer, MD, with the Advanced Cancer Research Group in Seattle, one of the study's lead authors.

These early data showed no statistical association between 30-day mortality and cancer treatments, suggesting that surgery, adjuvant chemotherapy and maintenance chemotherapy could continue during the pandemic with "extreme caution."

The cancer-specific factors associated with increased mortality included having an Eastern Cooperative Oncology Group (ECOG) performance status of two or worse. ECOG is a grading scale for measuring how cancer impacts a patient's daily living abilities. A score of two designates a patient who is capable of self-care but unable to work and who is up and about more than 50 percent during waking hours. Another factor associated with increased mortality was an active cancer status, particularly progressive cancer.

The mortality risk also increased with the number of comorbidities, such as hypertension or diabetes, particularly with two or more comorbidities. As is the case with the non-cancer population, mortality increased with age. Mortality was 6 percent for cancer patients younger than 65, 11 percent for those 65-74 and 25 percent for those older than 75. Males also had a higher death rate than females, 17 percent compared to 9 percent.

CCC19 was formed to rapidly collect data as part of an effort to understand the unique effects the coronavirus has on people with cancer.

Study: Remdesivir benefits some COVID-19 patients

A study published in the *New England Journal of Medicine* found that hospitalized COVID-19 patients who received Gilead Sciences' antiviral drug remdesivir recovered a median of four days earlier than those who received a placebo.

Preliminary findings from the double-blind Adaptive COVID-19 Treatment Trial (ACTT-1), sponsored by the U.S. National Institute of Allergy and Infectious Diseases (NIAID), showed that 538 patients randomly assigned to receive remdesivir had a median recovery time of 11 days, compared with 15 days in the 521 patients who received a placebo.

The patients were enrolled from February 21 to April 19 at 60 study sites and 13 subsites in the United States and Mexico (79.8 percent), Europe (15.3 percent), and Asia (4.9 percent), on the basis of the evolving epidemiology of the pandemic. Patients received either a placebo for 10 days or remdesivir intravenously as a 200-milligram (mg) loading dose followed by 100-mg maintenance doses for the next nine days or until release from the hospital or death. The analysis includes only patients with at least some post-baseline data available.

The most common disease severity score, rated from 1 (not requiring hospitalization) to 8 (dead), was 5 (needing oxygen). In patients with a score of 5, remdesivir was associated with a 47 percent speedier recovery, versus 20 percent in patients with a score of 6 (needing high-flow ventilation), and only 0.05 percent in patients with a score of 7 (needing intubation or extracorporeal membrane oxygenation [oxygen added to the blood outside the body]).

The authors noted that patients requiring supplemental oxygen derived the most benefit from remdesivir, while it did not benefit those requiring more intense treatments. After the NIAID made the results public late last month, the U.S. Food and Drug Administration on May 1 issued an emergency use authorization (EUA) for remdesivir for the treatment of seriously ill COVID-19 patients. The authors also suggested further study to evaluate outcomes from antivirals combined with other therapeutic agents.

No improvement in death rate for COVID-19 patients who received hydroxychloroquine

A research team led by investigators from Brigham and Women's Hospital has evaluated real-world evidence related to outcomes for COVID-19 patients who were treated with hydroxychloroquine or chloroquine analogues (with or without a macrolide). Investigators found no evidence that either drug regimen reduced the death rate among patients. Patients treated with hydroxychloroquine or chloroquine regimens were far more likely to experience abnormal, rapid heart rhythms (known as ventricular arrhythmias) than their counterparts who had not received the drugs. The team's findings are published in *The Lancet*.

"No matter which way you examine the data, use of these drug regimens did not help," said corresponding author Mandeep R. Mehra, MD, executive director of the Brigham's Center for Advanced Heart Disease. "If anything, patients had a higher likelihood of death. We also saw a quadrupling in the rate of significant ventricular arrhythmias in patients with COVID-19 who had been treated with hydroxychloroquine or chloroquine regimens."

Mehra and colleagues conducted their study using the Surgical Outcomes Collaborative database, an international registry comprised of de-identified data from 671 hospitals across six continents. The analysis included data on more than 96,000 patients hospitalized with COVID-19. This included almost 15,000 patients who had received the anti-malarial drug chloroquine or its analog hydroxyquinone with or without an antibiotic (macrolides such as azithromycin and clarithromycin) early after COVID-19 diagnosis. The study's primary endpoint was death or discharge from the hospital.

Mehra and colleagues found that 10,698 patients died in the hospital (11.1 percent) and 85,334 survived to discharge. The team compared death rates for those taking one of the drug regimens to that of a control group, after accounting for confounding variables, such as age, sex, and underlying risk factors. The death rate among the control group was 9.3 percent. Each of the drug regimens of chloroquine or hydroxychloroquine alone, or in combination with a macrolide, was associated with an increased risk of in-hospital death with COVID-19.

In addition, each of the drug regimens was associated with an increase in the risk of ventricular arrhythmia. Among the treatment groups, between 4 and 8 percent of patients experienced a new ventricular arrhythmia, compared to 0.3 percent of patients in the control group.

Chloroquine and hydroxychloroquine have been known to cause cardiovascular toxicity and previous studies have shown that macrolides can increase the risk of sudden cardiac death. A preliminary analysis of patients in Brazil treated with chloroquine and an antibiotic has suggested a high dose of chloroquine may be a safety hazard. Results from randomized, controlled clinical trials are not expected until the summer.

The authors caution that the current study is observational in nature – this means that it cannot absolutely answer the question of whether the drug regimens were solely responsible for the changes in survival. Randomized clinical trials will be required before any conclusion can be reached regarding harm.

"These findings suggest that these drug regimens should not be used outside of the realm of clinical trials and urgent confirmation from randomized clinical trials is needed," the authors conclude.

NIH launches study to investigate pregnancy outcomes resulting from COVID-19

The National Institutes of Health (NIH) has launched a multipronged study to understand the effects of the COVID-19 pandemic during and after pregnancy. Researchers will analyze the medical records of up to 21,000 women to evaluate whether changes to healthcare delivery that were implemented as a result of the pandemic have led to higher rates of pregnancy-related complications and cesarean delivery. They also seek to establish the risk of pregnant women with COVID-19 infection transmitting the virus to their fetus. Newborns will be monitored and assessed until they are discharged from the hospital.

In addition, the study will track more than 1,500 pregnant women confirmed with COVID-19 infection, monitoring their health for six weeks after childbirth.

The study will be conducted by researchers in the Maternal-Fetal Medicine Units (MFMU) Network, a group of 12 U.S. clinical centers funded by NIH's Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). MFMU Network sites cover more than 160,000 deliveries a year, and their racial, ethnic, and geographic diversity allows researchers to generalize their study findings to the U.S. population.

MFMU Network investigators plan to contribute data collected from the current study to a larger registry to help inform future studies of how COVID-19 affects maternal health and pregnancy.

No benefit of convalescent plasma in COVID-19 patients, study finds

Convalescent plasma therapy did not help 52 seriously ill COVID-19 patients recover in the first known randomized study of its kind, although it was stopped early because of low enrollment. The study, published in JAMA, involved coronavirus patients with severe illness (respiratory distress and/or low oxygen levels) or life-threatening disease (shock, organ failure, or the need for mechanical ventilation) in seven medical centers in Wuhan, China, from February 14 to April 1, with final follow-up on April 28.

The trial was stopped early because, owing to containment of the Wuhan outbreak, researchers were able to recruit only 103 of 200 patients needed to generate a clinically meaningful result. One patient withdrew from the study, and one in the control group received convalescent plasma, a protocol violation, because of a life-threatening infection.

Convalescent plasma therapy, which dates back to the early 20th century, involves the transfusion of blood plasma collected from patients recovered from infection to improve immune response in the recipient. Although evidence supporting its use in COVID-19 patients is scarce, the U.S. Food and Drug Administration (FDA) recently approved its emergency use in patients who have severe or life-threatening coronavirus infection.

The authors noted that most previous studies on convalescent plasma lacked standardization and procedure control in donor selection and the type or level of antibodies in the convalescent plasma. The researchers called for further studies on optimal patient selection and timing of convalescent plasma therapy in COVID-19 patients.

In a commentary in the same journal, Arturo Casadevall, MD, PhD, of the Johns Hopkins Bloomberg School of Public Health in Baltimore and colleagues said that while convalescent plasma didn't lead to significant improvements in clinical status or death rate, the study generated "potentially hopeful signals," adding that convalescent plasma may be useful in combination with antiviral drugs.

Component testing emerging as critical indicator for anaphylactic risk

By Lakiea Wright, MD, MAT, MPH

Most allergic reactions elicit mild to moderate symptoms, such as sneezing, watery eyes, or a rash. Sometimes, though, the immune system overreacts to an allergen, creating a serious, life-threatening condition called anaphylaxis. During anaphylaxis, a person can experience an almost instantaneous rush of symptoms, which may include a rash (typically hives), a high pulse, and even shock (called anaphylactic shock). An anaphylactic reaction like this needs to be treated immediately with an epinephrine (adrenaline) injection; without immediate treatment, anaphylaxis can be fatal.

What causes anaphylaxis?

Researchers estimate that between 1.6 percent and 5.1 percent of Americans have experienced anaphylaxis.[1] In many cases, medications are to blame. However, anaphylaxis can also be caused by other more ordinary occurrences, such as being stung by an insect or by eating foods that are known to cause allergies, such as peanuts or tree nuts. Studies have shown that food-related anaphylaxis is relatively common, although fatalities remain rare with a reported range of approximately 0.03 to 0.3 deaths per million person years in the general population.[2]

Likewise, stinging insect allergy is fairly prevalent, affecting up to 5 percent of the population during their lifetime,[6] and one of the most frequently associated with anaphylaxis in both adults and children.[12] Anaphylaxis to insect stings has occurred in 3 percent of adults and can be fatal even on the first reaction,[3] and researchers estimate that there are between 90 to 100 deaths per year caused by insect sting anaphylaxis.[4]

At a molecular level within the body, anaphylaxis is the result of a rapid and complex series of chemical reactions, some of which remain poorly understood, in part because they can be quite individualistic. For example, people who have experienced an allergic reaction to an insect sting have a 60 percent chance of a similar or worse reaction if stung again.[4] That is why new diagnostics that gauge a patient's sensitization to certain proteins can play a critical role in helping clinicians determine who is most at risk of anaphylaxis.

The recent evolution of allergy testing

Historically, standard clinical management for allergies involved an assessment of a patient's symptoms and history, followed by skin prick testing and/or immunoassays of specific whole allergen immunoglobulin E (IgE). But the emerging field of molecular allergology is changing all that with innovative, enhanced methods that help healthcare providers refine the diagnosis and treatment of allergies.

Today, a routine blood test coupled with advanced diagnostics can allow healthcare providers to identify, on a molecular level and with great specificity, which component proteins a patient is sensitized to. These new specific IgE blood tests, which are also called component tests, quantify IgE antibodies to single, pure allergen components, and they can be used to help pinpoint the cause of an allergy. In addition, these tests can help gauge where a patient falls on the spectrum of possible reactions – and identify who is most at risk of anaphylaxis.

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Recall the causes and symptoms of anaphylaxis response in humans.
2. Recall the types of allergic sensitivities that most commonly are involved in an anaphylaxis response.
3. Discuss specific IgE blood tests on the market today and the types of allergic sensitivities they identify.
4. Discuss how the specific IgE blood tests help healthcare practitioners make informed decisions about their patients' allergy management plans.

Specific IgE component blood tests enable more proactive allergy diagnosis and management and are now available to identify allergic sensitization to:

- environmental allergens, including seasonal and perennial triggers, both outdoor and indoor
- common food allergens, such as peanut, tree nut, egg, and milk
- pet allergens, including dog, cat, and horse
- stinging insect allergens, including up to eight different proteins found in the venom of bees and wasps

It is also important to note here that specific IgE component blood tests, which are available in most major U.S. laboratories, can be ordered for patients of any age, regardless of skin condition, current medication, disease activity or pregnancy status. Since patients often react to more than one allergen, they can also be quite helpful for distinguishing primary allergic sensitization from cross reactivity. Cross-reactivity occurs when the proteins in one substance (e.g., pollen) are similar to the proteins in another (e.g., fruit and vegetables). Then, when a patient with an allergy to one protein comes in contact with a similar protein, their immune system can react in the same way, even though they may not be truly allergic to the similar protein.

Specific IgE blood tests should be ordered with reflex

Clearly, results from specific IgE blood tests can have significant diagnostic implications. The fastest, easiest way to get these results is for healthcare professionals to order specific IgE blood tests with reflex. That way, if the initial testing results to a whole allergen test falls outside of the normal range, an additional test – the reflex to component specific IgE blood tests – will be performed to gather additional information to aid in a proper diagnosis. Because the reflex test happens automatically, the patient does not have to go back to the lab for a second blood draw. That saves the patient time and potential pain.

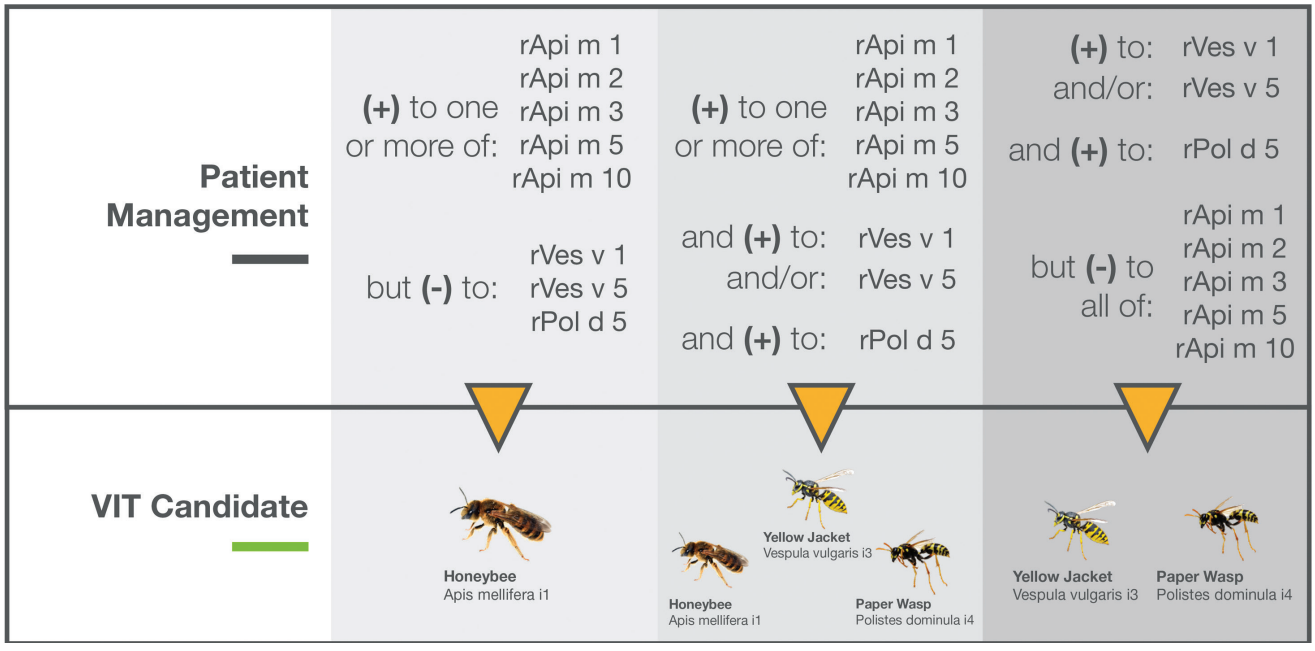
On the downside, reflex testing does involve additional expense, although the cost is not exorbitant. Remember, reflex testing only occurs if a whole allergen test indicates sensitization, and as a proxy for what an insured patient could reasonably expect to be billed, the Centers for Medicare & Medicaid Services (CMS) reimburses $22.14 per component allergen.

Allergy component testing helps improve the diagnosis of peanut allergy

Among all the possible food allergies, allergen component testing has been the most extensively studied for the diagnosis of peanut allergy – and for good reason. Peanut allergic reactions are generally the most common culprit of fatal food-induced anaphylaxis, with the highest-risk groups being adolescents with asthma.[13] In fact, studies show that people allergic to peanuts have a higher risk of anaphylaxis compared with people who are allergic to other foods, like milk or egg.[13] In addition to all that, peanuts and peanut products are quite commonplace, making it especially difficult to manage the loss of safety and spontaneity that comes with a peanut allergy diagnosis.

Diagnostic testing for peanut allergy typically starts with blood or skin testing for the whole allergen, i.e., all of the proteins found in a peanut; however, once these tests produce a positive result showing that a patient is sensitized to peanuts, the next questions become, "Is this patient at risk for a systemic reaction like anaphylaxis, or can they actually tolerate peanuts, and therefore be a good candidate to proceed with an oral food challenge?"

Specific IgE component blood tests can help provide those answers. To better diagnose peanut allergies,



Venom Interpretation Guide

Table 1. Risk Factors for Severe Insect Venom Reactions

Clinical Markers		Laboratory Markers
• Very severe previous reaction	• Frequent exposure	• Positive venom sIgE blood test or skin test
• Insect species (honeybee)	• Multiple or sequential stings	• High basal serum tryptase
• No urticaria or angioedema	• Mastocytosis	• Basophil activation test (not commonly available)
• >45 years old, male	• Medications (angiotensin-converting enxyme [ACE] inhibitors)	• Platelet activating factor – acetylhydrolase (not commonly available
		• ACE

Adapted from: Golden, et al. *Ann Allergy Asthma Immunol.* 2017;118:32. 98856.AL.US1.EN.v1.19

Clinical and Laboratory Markers for Risk Factors for Severe Insect Venom Reactions

component blood testing can assess sensitization to several peanut proteins, including Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9. Sensitization to Ara h 1, 2, 3, and 6 indicates a higher likelihood of having a systemic reaction to peanuts.[14] Sensitization to Ara h 8 is mainly associated with either no clinical reactivity or with isolated local reactions, for example, oral allergy syndrome.[15]

These specific IgE blood tests enable labs to provide a more complete view of a patient's overall peanut sensitization, including whether a patient is at risk for cross-reactivity. The results can play a critical role in understanding the likelihood of a life-threatening reaction, and help clinicians make more informed decisions regarding their patients' allergy management plans.

Allergy component testing improves diagnosis of tree nut allergy

Tree nut allergies are another common type of food allergy for both children and adults. Tree nuts come in a wide range of varieties, including almonds, cashews, and walnuts, to name just as few. As with the allergic reactions to peanuts described above, tree nut allergic reactions can run the gamut – from less severe, localized reactions, such as itching and tingling of the mouth and lips, to widespread, systemic reactions, including anaphylaxis. Where someone falls on this spectrum of reactions may depend on which protein in different tree nuts is causing the reaction, and knowing which protein is causing the reaction is important because different proteins can cause different allergic reactions.

Specific IgE blood testing is currently available for tree nut components of hazelnuts, walnuts, brazil nuts, and cashews. That means that component testing can help healthcare providers refine the diagnosis to determine if a patient is allergic to one tree nut or more than one and assess the likelihood of a systemic reaction such as anaphylaxis. As with specific IgE testing for peanut allergies, testing with allergen components specific to tree nuts can help healthcare providers determine when to recommend oral food challenges.

Allergy component testing helps improve the diagnosis of allergies to venom from stinging insects.

Allergies to the venom of stinging insects are also fairly prevalent, and in some cases, may result in severe allergic reactions, including anaphylaxis. Over 16.5 million Americans have a history of systemic anaphylactic reactions to insect stings,[6] and researchers estimate that between 90 to 100 deaths per year are caused by insect-sting anaphylaxis.[4] The insects involved are from the Hymenoptera order and include bees, wasps, hornets, yellow jackets, and fire ants – all of which release venom when they sting.

Recently, the U.S. Food and Drug Administration (FDA) approved diagnostic specific IgE blood testing for a number of allergenic components associated with honeybees and wasps. To facilitate an even more precise diagnosis, several honeybee and wasp allergens have been precisely characterized and are now available as recombinant antigens for component-resolved diagnostics. Results from these specific IgE tests with component-resolved diagnostics can help specialists and other healthcare providers discriminate between true sensitization and cross reactivity. They can also help identify culprit insect(s) in patients with inconclusive patient history and guide the selection of future therapy, including prescription of venom immunotherapy.

Here are just two examples to illustrate the point: 68 percent of patients with a history of reactions to honeybee venom are sensitized to protein components Api m 3 or Api m 10, and 4.8 percent are sensitized to these components exclusively.[7] However, because these allergen components are under-represented or absent from some of the licensed preparations used for honeybee venom immunotherapy, patients with Api m 3 or Api m 10 sensitivity exclusively may not always be getting the treatment that is most effective for them.[7]

Similarly, up to 50 percent of venom allergic patients test positive for both bee and wasp venom.[8] For them, specific IgE blood testing with recombinant protein allergens rApi m 1, rVes v 1, and rVes v 5 can discriminate double sensitization from cross reactions and nonspecific sensitization related to carbohydrate determinants frequently found in Hymenoptera venom.[16] As these examples show, specific IgE tests with component-resolved diagnostics using recombinant venom allergens can improve the specificity of results, leading to more successful venom immunotherapy.

Research on allergies to venom from stinging insects has also revealed that tryptase tests can aid in the diagnosis of other medical conditions. More specifically, researchers have learned that mastocytosis occurs in approximately 2 percent of patients with insect sting allergy, and that insect sting allergy occurs in approximately 25 percent of patients with mastocytosis.[9]

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Mastocytosis is a condition that occurs when mast cells, which play a fundamental role in immune responses, accumulate in skin and/or internal organs.

Studies indicate that mast cell activation may also initiate certain allergic reactions, although the exact mechanism remains unclear. Because a severe insect sting reaction may be the first symptom of mastocytosis, some experts recommend that patients with severe reactions be evaluated for mastocytosis.[10] For people with diagnosed mastocytosis, experts recommend testing for Hymenoptera venom sensitivy, and testing basal serum tryptase for patients who have had a systemic reaction to an insect sting or before starting venom immunotherapy.[9]

Improved testing means better allergy risk management

If someone's immune system overreacts to an allergen, the serious, life-threatening condition called anaphylaxis can result. Given that 30 percent of adults and 40 percent of children in the U.S. suffer from allergies – and that those percentages are continuing to climb – it is growing increasingly critical to be able to assess which allergy sufferers are at the greatest risk of anaphylaxis.[11]

Fortunately, scientists have developed a new generation of advanced diagnostics that can precisely identify, at the molecular level, specific proteins a patient is sensitized to. These new specific IgE allergen component blood tests, are now available for many of the most common allergic triggers, including food allergens, pet allergens, and stinging insect allergens. The tests work by quantifying IgE antibodies to single, pure allergen components, based on clinical studies. They have been designed to help identify the allergic sensitizations and help clarify the options for effective allergy management. Specific IgE blood tests can also help gauge where a patient falls on the spectrum of possible allergic reactions, ranging from a local reaction such as oral itching to a systemic reaction during anaphylaxis.

Once identified, those at risk of anaphylaxis need to take precautions. First and foremost, they need to avoid coming into contact with their allergic triggers. However, since that is not always possible, someone who is at risk for anaphylaxis, should always carry autoinjectable epinephrine and learn how and when to self-administer it. The hormone epinephrine (also known as adrenalin) can reverse the symptoms of anaphylaxis, for example, raising blood pressure, increasing heart rate, and opening constricted airways to improve breathing.

But epinephrine is a rescue medication only, and anyone who has had a severe allergic reaction still needs to be seen immediately by a medical professional. Because anaphylaxis is so serious and can be life-threatening, many people who are at risk of it also wear a bracelet or necklace that alerts others of their allergies. That way, in an emergency, caregivers or medical professionals are able to administer the appropriate treatments as quickly as possible.

Assessing someone's risk of anaphylaxis is enormously beneficial. On the one hand, learning that they are at low risk can ease a patient's mind; on the other, learning that they are at high risk can help a patient take the proper precautions. Combining specific IgE allergen component blood tests with whole allergen testing and a comprehensive clinical history allows specialists and other healthcare providers to better assess their patients' sensitizations to common allergens. This comprehensive approach allows clinicians to identify allergy triggers more precisely. Then, they can discriminate between true sensitization and cross reactivity, determine where a patient falls on the spectrum of possible allergic reactions, and create optimal allergy management protocols.

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TEST QUESTIONS

Circles must be filled in, or test will not be graded. Shade circles like this: ● Not like this: Ⓧ

1. **Between ____ percent and ___ percent of Americans have experienced anaphylaxis.**
   - A. 1.2 percent and 4.6 percent
   - B. 1.6 percent and 5.1 percent
   - C. 2 percent and 5 percent
   - D. 3 percent and 6 percent
2. **Food-related anaphylaxis is relatively common, but fatalities remain rare.**
   - A. True
   - B. False
3. **Stinging insect allergy is fairly prevalent, affecting up to ___ of the population during their lifetime.**
   - A. 2 percent
   - B. 3.5 percent
   - C. 5 percent
   - D. 10 percent
4. **The number of deaths per year caused by insect-sting anaphylaxis is between ___ and____.**
   - A. 50; 75
   - B. 90;100
   - C. 200; 250
   - D. 250;300
5. **Diagnostics that gauge a patient's sensitization to certain _____ help clinicians determine who is most at risk of anaphylaxis.**
   - A. histiocytes
   - B. proteins
   - C. carbohydrates
   - D. lipids
6. New component tests quantify ___ antibodies to single, pure allergen components, and they can be used to help pinpoint the cause of an allergy.
   - A. IgE
   - B. M (IgM)
   - C. G (IgG)
   - D. IgA
7. **Specific IgE component blood tests enable more proactive allergy diagnosis and management and are now available to identify allergic sensitization to:**
   - A. pet allergens, including dog, cat, and horse.
   - B. common food allergens, such as peanut, tree nut, egg, and milk.
   - C. environmental allergens, including seasonal and perennial triggers, both outdoor and indoor.
   - D. All of the above.
8. **Cross-reactivity occurs when the proteins in one substance are very different from the proteins in another.**
   - A. True
   - B. False
9. **If the initial testing results to a whole allergen test panel fall outside of the normal range, which additional test will be performed to gather additional information to aid in a proper diagnosis?**
   - A. Total IgA
   - B. IgM specific antigen testing
   - C. Component specific IgE
   - D. All of the above
10. **The Centers for Medicare & Medicaid Services (CMS) reimburses___ per component allergen.**
    - A. $18.75
    - B. $22.14
    - C. $25.15
    - D. $30.00
11. **Among all the possible food allergies, allergen component testing has been the most extensively studied for the diagnosis of _____ allergy.**
    - A. gluten
    - B. dairy
    - C. peanut
    - D. None of the above
12. **Sensitization to the peanut proteins_____ indicate a higher likelihood of having a systemic reaction to peanuts.**
    - A. Ara h 1, 2, 3, and 6
    - B. Ara h 1,5 and 6
    - C. Ara h 8 and 9
    - D. All of the above
13. **Component-specific IgE blood tests enable labs to provide a more complete view of a patient's overall peanut sensitization, including whether a patient is at risk for cross-reactivity.**
    - A. True
    - B. False
14. **Which IgE specific testing for tree nuts is available that helps physicians to determine which oral food challenges to recommend?**
    - A. Hazelnuts, walnuts, brazil nuts and cashews
    - B. Hazelnuts, walnuts, brazil nuts and peanuts
    - C. Hazelnuts and brazil nuts
    - D. Hazelnuts, walnuts, brazil nuts, cashews and peanuts
15. **The insects involved in causing systemic anaphylactic reactions to insect strings are from the Hymenoptera order and include bees, wasps, hornets, ___ and ____.**
    - A. Yellow jackets, and fire ants
    - B. Yellow jackets and spiders
    - C. Sawflies and spiders
    - D. All of the above
16. **Which venom-associated insect allergies has the U.S. Food and Drug Administration (FDA) recently approved for diagnostic specific IgE blood testing for a number of allergenic components associated with hornets and yellow jackets?**
    - A. Yellow jackets and hornets
    - B. Mosquitoes and fire ants
    - C. Bumble bees and carpenter bees
    - D. Honeybees and wasps
17. **While large commercial labs now offer nucleic acid amplifn of the test, other testing methods are still lacking.**
    - a. True
    - b. False
18. **Sixty-eight percent of patients with a history of reactions to honeybee venom are sensitized to protein components ___ or ___, and 4.8 percent are sensitized to these components exclusively.**
    - A. rApi m 1; rVes v
    - B. Api m 3 or Api m 10
    - C. rVes v 1, and rVes v 5
    - D. All of the above
19. **Mastocytosis is a condition that occurs when mast cells, which play a fundamental role in immune responses, accumulate in skin and/ or internal organs.**
    - A. True
    - B. False
20. **For people with diagnosed mastocytosis, experts recommend testing for _____ sensitivity.**
    - A. peanut
    - B. peanut and tree nut
    - C. hymenoptera venom
    - D. All of the above

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Navigating through crisis in a pandemic event

By Shamiram Feinglass MD, MPH, and Joseph Chiweshe MD, MPH

The 1918 H1N1 pandemic illustrates one of the worst scenarios for preparedness, as it produced the greatest influenza mortality in recorded history. It is estimated that about 500 million people or one-third of the world's population at the time became infected with the virus and at least 50 million died.[1] Since that time, disease outbreaks, natural disasters and other mass casualty events have pushed healthcare systems to identify and refine emergency preparedness protocols.[2]

In 2009, the Institute of Medicine issued the "crisis standards of care" to define the level of health and medical care capable of being delivered during a catastrophic event. An event that would most likely stem from a pervasive (e.g., pandemic influenza) or catastrophic (e.g., earthquake, hurricane) disaster by which healthcare resources become overwhelmed.[3] Today, the world is currently facing such an event due to SARS-Cov-2; the virus behind the COVID-19 pandemic.

Given the growing disease burden caused by SARS-CoV-2 and the ensuing COVID-19 infections across the globe, health officials are facing ethical dilemmas and difficult decisions governing the provision of care with limited resource supplies. In vitro diagnostic companies play a crucial role within the healthcare ecosystem, and as such, it is vital that we work to support the continuous improvement of our ability to better respond to pandemics and provide support to those on the front lines as they navigate through crisis.

Clinical laboratories are often the first line of defense in response as they perform diagnostic testing and oftentimes may be the first to identify the causes of illnesses in communities. It is thus important that laboratories update and maintain their pandemic preparedness protocols and training throughout the year.

Evolution of a novel pandemic

This is the third serious coronavirus outbreak in less than 20 years, following SARS in 2002-2003 and MERS in 2012.[4] As SARS-CoV-2 appears to be transmitted person-to-person through respiratory droplets and close contact, a key component of risk mitigation is prevention of SARS-CoV-2 infection in at-risk populations. Current evidence suggests these populations include older adults, those with serious chronic medical conditions, immunosuppressed patients, and those with prior or active cancer.[5] Globally, as we continue to see younger people being affected, we should remain fully cognizant that the young and healthy are not free of risk of death.[6]

Additionally, health disparities and global health inequities represent some of the greatest barriers to pandemic preparedness.[7] For example, in New York city, the Hispanic coronavirus victims comprised 34 percent for all COVID-19-related deaths as of April 2020, while making up only 29 percent of the city's population. Similarly, In Chicago, African Americans constituted 71 percent of deaths, whilst making up 29 percent of the population.[8]

Poverty and health are connected in that minority communities may have generations of poverty and higher preexisting health conditions such as hypertension, diabetes and asthma that put them at greater risk during a pandemic. Crowding, another established risk factor which has been associated with Hispanic and Asian households, can also increase the likelihood of pathogen transmission.[9]

There is some reassurance that perhaps over 80 percent of symptomatic subjects will experience only mild flu-like symptoms. However, it is concerning that perhaps 15 percent of affected patients will become seriously ill and 5 percent will need critical care. Respiratory viruses are known to respond to seasonal variation, and we might expect that increasing temperatures in the summer could reduce the transmissibility of the novel coronavirus to some extent. As warmer weather may slow down the spread, it will continue to be prudent to interrupt community transmission via social distancing strategies.[10] Furthermore, In conjunction with public health efforts, health systems can dramatically expand access to testing through commercial, hospital and public health laboratories.[11,12]

Standard testing for acute infection entails reverse transcription polymerase chain reaction (RT-PCR) amplification of reverse-transcribed viral RNA from respiratory specimens, most commonly nasopharyngeal swabs, but also oropharyngeal swabs, sputum, and bronchoalveolar lavage fluid.[13]

Serological testing will also be valuable for evaluating the extent of the pandemic. With the ability to assess a patient's immune response to SARS-CoV-2, this testing modality may enable clinicians to clear hospital staff, emergency responders and others to get back to work with an indication that they have had prior exposure and therefore, may have some level of immunity to the disease. This test also could allow those without immunity to be identified and kept safe until the pandemic subsides.

Building a strong foundation for preparedness

Preparing for a potential infectious disease pandemic from influenza or a novel coronavirus is an essential component of a business continuity plan, especially for businesses that provide critical healthcare and infrastructure services. Pandemics can not only interrupt an organization's operations and compromise long-term viability of an enterprise, but also disrupt the provision of critical functions. Businesses that regularly test and update their pandemic plan can significantly reduce harmful impacts to the business, play a key role in protecting associates' and customers' health and safety, and limit the negative impact of a pandemic on the community and economy.[14] It is important to lay a foundation for regularly training staff, especially those within the lab, about business continuity in the event of a pandemic.

In order to keep associates healthy, everyone must do their part. Hand hygiene with soap and water, washing for 20 seconds, or alcohol-based hand rub (ABHR) are the most effective, simple and low-cost measures against COVID-19 cross-transmission. By denaturing proteins, alcohol inactivates enveloped viruses, including coronaviruses, and thus, ABHR

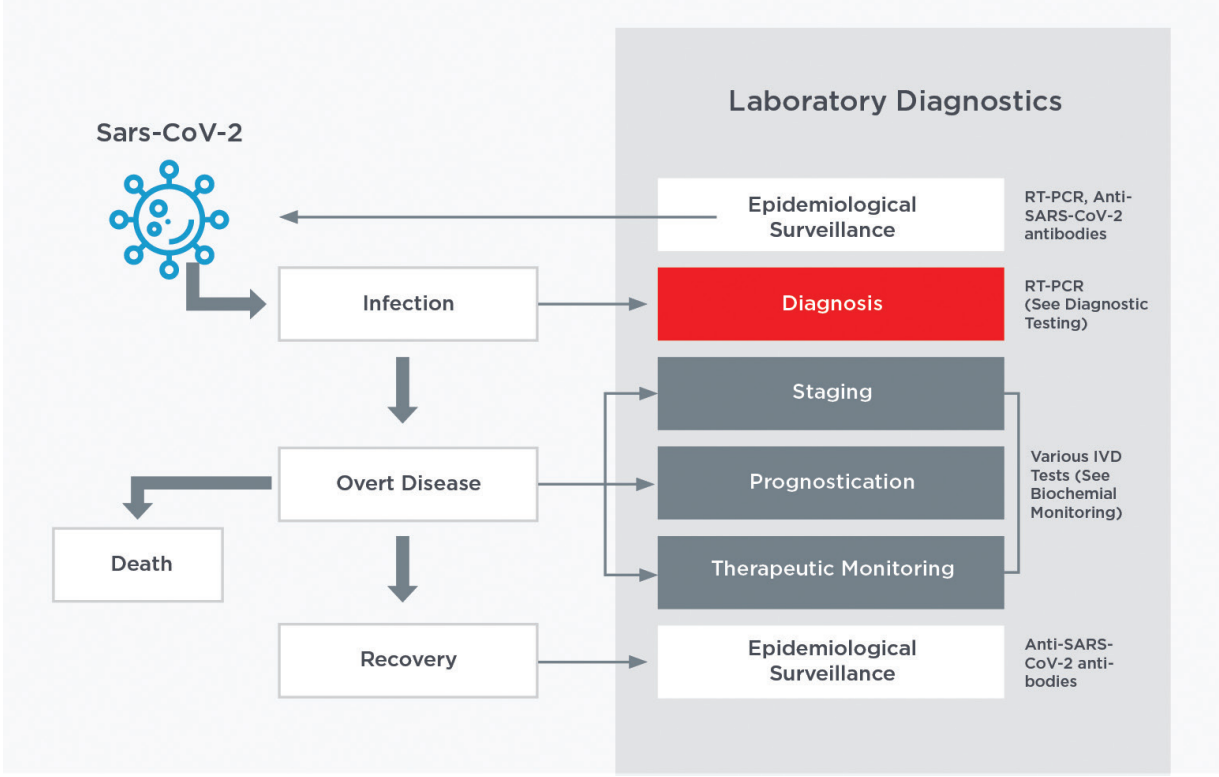

Figure 1. The critical role of laboratory medicine in COVID-19 (Modified from Lipi et al, PMIC: 32191623).

formulations with at least 60 percent ethanol have been proven effective for hand hygiene.[15] Soap contains fat-like substances known as amphiphiles, and the soap molecules "compete" with the lipids in the virus membrane. The chemical bonds holding the virus together are not very strong, so competition is enough to break the virus's coat as well as any grease or dirt they may be clinging to.[16]

At this time, there are no vaccines available and there is little evidence on the effectiveness of potential therapeutic agents. In addition, there is presumably no pre-existing immunity in the population against the new coronavirus and everyone in the population is assumed to be susceptible.[15] When a novel virus with pandemic potential emerges, nonpharmaceutical interventions, are often the most readily available interventions to help slow transmission of the virus in communities. Community mitigation is a set of actions that persons and communities can take to help slow the spread of respiratory virus infections. Community mitigation is especially important before a vaccine or drug becomes widely available.[16]

During this time, it is important to establish clear roles and responsibilities. This can be coordinated through the formation of a crisis management team. This team provides guidance, and support regarding priorities and direction for response and recovery from an enterprise perspective. Establishing clear roles and communication should take a multi-tiered organizational structure to maximize protection of life and minimize potential interruptions to business continuity. To ensure readiness, the importance of training and tabletop exercises cannot be understated and should be incorporated into overall disaster preparedness efforts.

The Centers for Disease Control and Prevention (CDC) has described four levels of mitigation strategies by level of community transmission: none to minimal, minimal to moderate, substantial and post-pandemic.[18]

None to minimal:

- Know where to find local information on COVID-19 and local trends of COVID-19 cases.
- Know the signs and symptoms of COVID-19 and what to do if staff become symptomatic at the worksite.
- Review, update, or develop workplace plans to include:
  - Liberal leave and telework policies
  - Consider 7-day leave policies for people with COVID-19 symptoms
  - Consider alternate team approaches for work schedules
  - Encourage employees to stay home and notify workplace administrators when sick (workplaces should provide non-punitive sick leave options to allow staff to stay home when ill).
  - Encourage personal protective measures among staff (e.g., stay home when sick, handwashing, respiratory etiquette).
  - Clean and disinfect frequently touched surfaces daily.
  - Ensure hand hygiene supplies are readily available in building.

Minimal to Moderate:

- Encourage staff to telework (when feasible), particularly individuals at increased risk of severe illness.
- Implement social distancing measures:
  - Increasing physical space between workers at the worksite
  - Staggering work schedules
  - Decreasing social contacts in the workplace (e.g., limit in-person meetings, meeting for lunch in a break room, etc.)
- Limit large work-related gatherings (e.g., staff meetings, after-work functions).
- Limit non-essential work travel

• Consider regular health check (e.g., temperature and respiratory symptom screening) of staff and visitors entering buildings (if feasible).

Substantial:

• Implement extended telework arrangements (when feasible).
• Ensure flexible leave policies for staff who need to stay home due to school/childcare dismissals.
• Cancel non-essential work travel.
• Cancel work-sponsored conferences, tradeshows, etc.

Post-pandemic:

• Guided by surveillance data, implement a phased approach to returning to work and full operations.
• Gradually ease physical distancing measures in a concerted and careful manner and continue to control SARS-CoV-2 transmission so we do not revert back.
• To ultimately move away from future reliance on physical distancing as our primary tool for controlling future spread, we need:
• Better data to identify areas of spread and the rate of exposure and immunity in the population.
• Improvements in state and local healthcare system capabilities, public-health infrastructure for early outbreak identification, case containment, and adequate medical supplies.

In conclusion

The rapid spread of COVID-19 across the world has exposed major gaps in the abilities of most countries to respond to a virulent new pathogen. Moving forward, as we work to control the COVID-19 pandemic and as we plan for future pandemics, a key lesson is that early availability of diagnostic testing is of great value for patient management and public health. Thus, the development, validation, scale-up in manufacture, and distribution of diagnostic tests should be a key priority in early preparation during an emerging infectious disease outbreak.

The examples of Singapore, Taiwan and Hong Kong in limiting the impact of the sudden acute respiratory syndrome, SARS-CoV-2, demonstrates that it is possible to mount an effective response to an outbreak via major investment in pandemic preparedness.[19]

Early in an outbreak, we need to understand and define the risk factors for infection, the role of asymptomatic or mild infection and the nature of 'super-spreaders.' We must improve response rates and estimates of death rates by age.[20] This will help forward looking viral pandemic preparedness.

Last, businesses that regularly test and update their pandemic plan can significantly reduce harmful impacts to the business, play a key role in protecting associates' and customers' health and safety, and limit the negative impact of a pandemic on the community and economy. For healthcare organizations, regular training of staff for preparedness and readiness inclusive of revised workflows – should they be needed – is crucial, as these frontline healthcare workers are key to protecting and treatment of the population at large.

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The expanding role of biomarker testing in non-small cell lung cancer

By Bharathi Vennapusa, MD

Less than a decade since the American Society of Clinical Oncology (ASCO) endorsed routine testing for epidermal growth factor receptor (EGFR) mutations in all patients with lung adenocarcinoma, and the approval of crizotinib for the treatment of anaplastic lymphoma kinase (ALK)-rearranged non-small cell lung cancer (NSCLC) – both in 2011 – biomarker testing has become integral in day-to-day patient decisions.[1] The rapid advances in precision medicine are evidenced in the continuing expansion of targeted therapeutics available and, in parallel, the elucidation of specific underlying mutations in biomarkers and the development of technology for identifying biomarkers and subtyping patients.

Non-small cell lung cancer (NSCLC) represents 85 percent of all lung cancer and has a slightly more favorable survival rate than small-cell lung cancer. During initial evaluation and diagnosis, NSCLC is further subtyped into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Adenocarcinoma is the most common, representing 60 percent of all NSCLC. In addition to histological subtyping, an NSCLC diagnosis also includes staging information.[2] According to the NCCN, 55 percent of cases from 2009 to 2015 were stage IV (metastasized) at diagnosis. Many current guidelines for biomarker testing are focused on patients with metastatic NSCLC.[3]

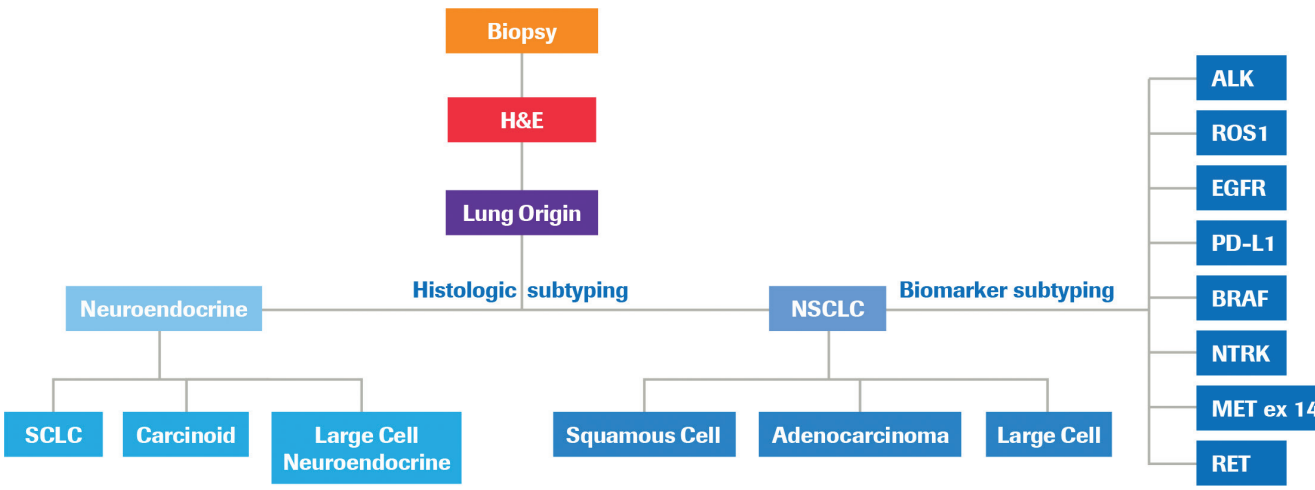

Figure 1. Diagnosis and subtyping of lung cancer

These new developments have improved patient survival in this leading cause of cancer death in the United States, while bringing with them the challenge of implementation in day-to-day patient care. Today, biomarker testing is vital to NSCLC subtyping and therapy selection, as organizations including the National Comprehensive Cancer Network (NCCN) and the International Association for the Study of Lung Cancer (IASLC) devote significant resources to maintaining up-to-date guidelines to help translate advances into clinical practice. This article provides an overview of how biomarker testing is used today in NSCLC, with a focus on predictive biomarkers that can guide targeted therapy.

Lung cancer classification

Lung cancer is the leading cause of cancer death and the most common cancer worldwide. In the U.S., the National Comprehensive Cancer Network (NCCN) estimates 228,820 new cases and 135,720 deaths in 2020. The advent of targeted therapeutics, powered by the discovery of biomarkers and underlying molecular pathways, has opened the door to improved survival. All of this requires the accurate classification of patients not only by histological subtypes but also biomarkers of oncogenic mutations and fusions and immune response to the cancer.

NSCLC biomarkers

NSCLC biomarkers fall into two categories. Prognostic biomarkers are indicative of patient survival regardless of treatment. KRAS mutations represent one of the best-known prognostic biomarkers and are associated with shorter survival. For patients with KRAS mutations, targeted therapy is not available. Further biomarker testing may not be beneficial, with the exception of PD-L1, as immune checkpoint inhibitors (ICIs) appear to be effective.

Predictive biomarkers are associated with effectiveness of the corresponding targeted therapy and, thus, are used to identify patients who are likely to benefit from a specific therapy. This discussion will focus on predictive biomarkers.

Anaplastic lymphoma kinase (ALK) gene fusions have been identified in a small subset (5 percent) of NSCLC patients who will benefit from targeted therapy such as crizotinib or ceritinib. ALK testing is commonly performed using immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). ALK fusions can also be detected using next-generation sequencing (NGS).

Current guidelines recommend all patients diagnosed with lung adenocarcinoma be tested for ROS proto-oncogene 1 (ROS1) rearrangements. Patients with ROS1 mutations respond to crizotinib or ceritinib. Given the rarity of ROS1

rearrangements (1 percent to 2 percent of NSCLC), the use of an IHC assay to detect ROS1 protein expression prior to confirmatory testing may decrease the overall cost of ROS1 testing.

In addition, current guidelines also recommend that patients diagnosed with adenocarcinoma be tested for epidermal growth factor receptor (EGFR) mutations. Deletions in exon 19 and a point mutation in exon 21 are the most common, and both are associated with sensitivity to small-molecule EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib, gefitinib, afatinib, osimertinib and dacomitinib. EGFR mutations are detected using NGS.

MET exon 14 skipping mutations occur in 3 percent to 4 percent of patients with adenocarcinomas and 1 percent to 2 percent of patients with other NSCLC histologies. Targeted therapy includes capmatinib and crizotinib. NGS and reverse transcription polymerase chain reaction (RT-PCR) assays can be used to detect METex14 skipping mutations.

RET is a tyrosine kinase receptor that affects cell proliferation and differentiation. RET rearrangements (fusions) may occur in 1 percent to 2 percent of patients in NSCLC, more frequently in patients with adenocarcinoma. RET rearrangement positive patients respond to selpercatinib, cabozantinib and vandetanib. FISH, RT-PCR and NGS assays can be used to detect RET rearrangements.

B-Raf proto-oncogene (BRAF) point mutations, specifically one associated with a change in amino acid position 600 (V600E), are found in 1 percent to 2 percent of patients with adenocarcinoma. Guidelines recommend testing for BRAF mutations in patients with metastatic nonsquamous NSCLC based on data showing efficacy of dabrafenib plus trametinib for patients with BRAF V600E mutations. BRAF mutations are tested using NGS and PCR assays.

Neurotrophic tyrosine receptor kinase (NTRK) gene fusions (NTRK1, NTRK2, NTRK3) occur in 0.2 percent to 3.3 percent of patients with NSCLC and resulting tropomyosin receptor kinase (TRK) fusion proteins have been identified as oncogenic drivers. With the availability of selective TRK inhibitors larotrectinib and entrectinib, NCCN guidelines recommend NTRK gene fusion testing in patients with metastatic NSCLC patients, who are negative for main oncogenic driver mutations like ALK, ROS1, EGFR and BRAF. A pan-TRK IHC assay is available in addition to FISH and NGS.

Programmed death ligand 1 (PD-L1) is a protein expressed by tumor cells and tumor infiltrating immune cells to inhibit T-cell mediated cell death by binding to PD-1, a receptor expressed on activated cytotoxic T-cells. PD-L1/PD-1 binding weakens the immune system's response to cancer. By blocking PD-L1/PD-1 interaction, immune checkpoint inhibitors (ICIs) restore T-cell activation.[4] Approved ICIs include atezolizumab, nivolumab, pembrolizumab and durvalumab. In May, 2020, NCCN Guidelines were updated to specify that only patients who test positive for PD-L1 expression should be administered first-line ICI therapy, by deleting "or unknown" in its statement about PD-L1 expression levels and ICI therapy.[5]

Biomarker	Alteration/ Expression	Targeted Therapeutic	Test Methodologies	Comments
ALK	Gene fusion	Crizotinib, ceritinib	IHC, NGS, RT-PCR	
ROS1	Rearrangement	Crizotinib, ceritinib	IHC, FISH, RT-PCR	Rare (1–2% of NSCLC)
EGFR	Exon 19, exon 21	Osimertinib, erlotinib, gefitinib, afatinib, dacomitinib	NGS	Absence of EGFR mutations precludes treating with EGFR TKIs
MET	Exon 14	Capmatinib, crizotinib	NGS, RT-PCR	
RET	Rearrangement	Selpercatinib, cabozantinib, vandetanib	FISH, NGS, RT-PCR	
BRAF	V600E	Dabrafenib plus trametinib	NGS, IHC, FISH	
NTRK	Gene fusions (NTRK1, NTRK2, NTRK3)	Larotrectinib and entrectinib	IHC, RT-PCR, NGS	DNA based NGS may under-detect NTRK fusions; IHC may yield false positives
PD-L1	Expression inhibits antitumor activity	Checkpoint inhibitors atezolizumab, nivolumab, pembrolizumab, durvalumab	IHC	

Table 1. Overview of NSCLC Predictive Biomarkers

Lastly, it should be noted that ALK and ROS1 fusions, BRAF mutations and sensitizing EGFR mutations are generally mutually exclusive. Thus, for patients who relapse on initial therapy based on testing positive for ALK or ROS1 fusions, subsequent targeted therapy for the other mutations listed here is not recommended.[5]

Selecting biomarker testing methodologies

IHC, FISH and NGS are currently the most commonly used platforms for biomarker testing. PCR is also used, but to a lesser degree. Several factors should be considered in assessing the options, with the overall goal of elevating the standard of patient care by supporting timely decisions. With this in mind, first and foremost is the quality of the diagnostic information, driven not only by the quality of test results but also by the extent to which a test result is actionable (e.g., availability of a targeted therapy). Turnaround time is important, as a test result can not only drive the treatment decision but also inform next steps in the subtyping process. Maximizing diagnostic information from limited specimen is critical, as are cost considerations.

Because of the amount of information available, NGS is a commonly used diagnostic tool for NSCLC. At 10 days to two weeks, NGS turnaround time is a challenge, but the level of information (e.g., with up to 500 markers) is unmatched, often justifying not only the turnaround time but also the cost. However, the large amount of information also demands careful interpretation before it is used to make treatment decisions.

At the opposite end of this continuum is IHC, which requires a small sample (a single tissue section), with results available generally within 24 hours and at a minimal cost. Improvements in technology and quality of results further add to the usefulness of IHC by reducing the need for confirmatory testing in some cases (e.g., in ALK testing). FISH is also a staple for biomarker testing.

The optimal use of cell-free DNA in the clinic has been a topic of interest. Proponents suggest its utility in patients who

lack sufficient tissue for biomarker testing, and those who are progressing on their initial therapy and for whom a repeat biopsy is not possible.[1] Another recent development is the use of tumor mutational burden (TMB) as a biomarker for patients with metastatic NSCLC and in particular for predicting response to immune checkpoint drugs independent of PD-L1 expression. However, there is no consensus on how to measure TMB.[5]

An additional consideration for biomarker testing is its use for therapy selection. In the U.S, approved tests are designated as either a companion diagnostic, which is required for the patient to receive the therapy, or a complementary diagnostic, which allows the clinician more discretion in prescribing the corresponding therapeutic. The designation may change as additional clinical data becomes available.

The laboratory's role

The rapidly changing landscape of targeted therapies in NSCLC presents the laboratory with several opportunities to contribute to improved patient outcomes. As new biomarkers are identified or as guidelines are updated on the use of specific biomarkers, the laboratory and pathology can be an important resource for the oncologist in highlighting new developments and offering guidance on how they can be incorporated into day-to-day practice.

At the same time, the laboratory has the responsibility to bring new tests online as quickly as possible – not only in implementing the tests but also considering how they fit into current clinical workflow and the institution's testing algorithms. A strategic perspective of the laboratory's test offerings – menu, methodologies, workflow – will guide conversations with the clinical team in determining the best solutions for the patient.

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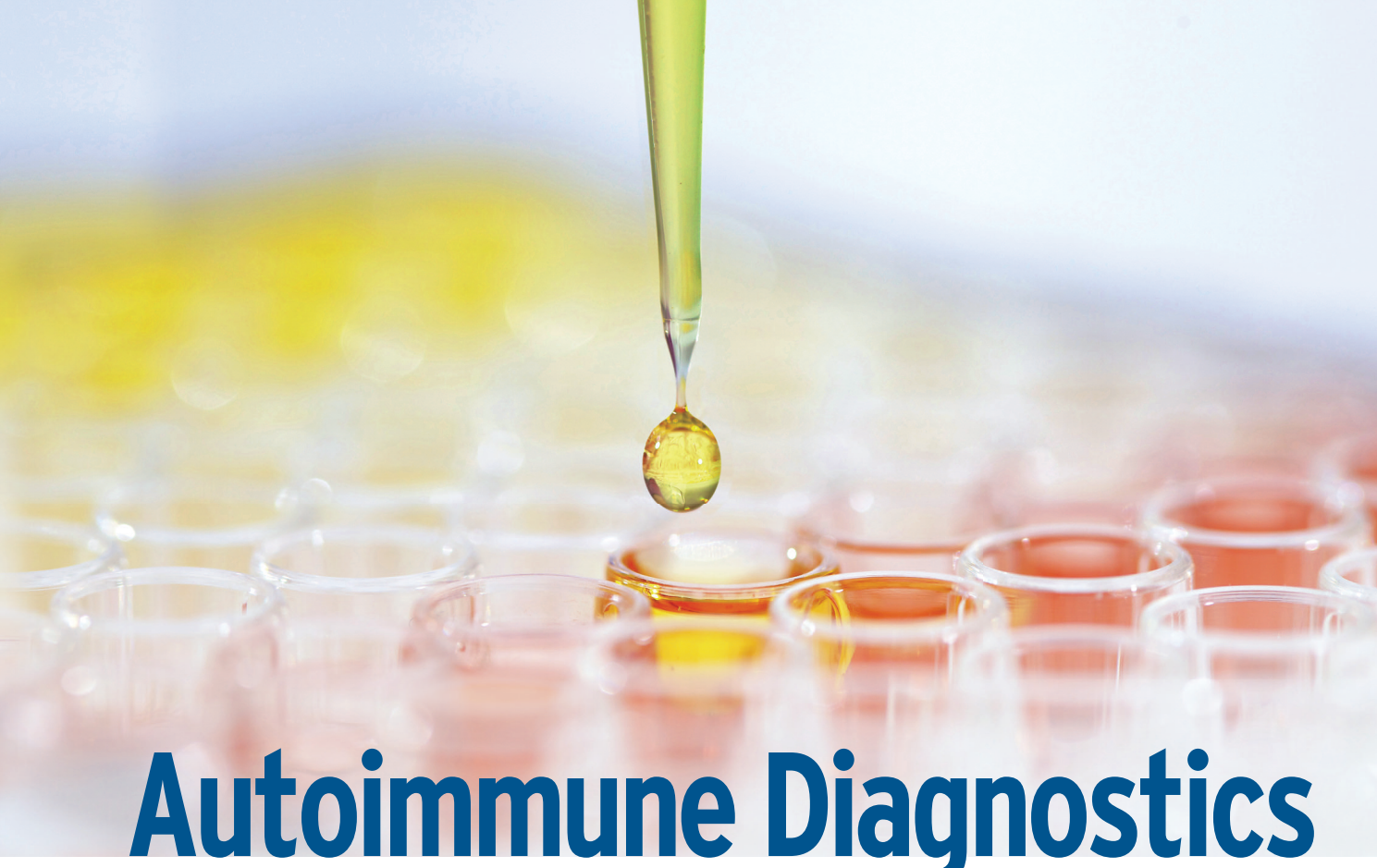

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Choosing a test method to measure HbA1c

By Priya Sivaraman, PhD

Diabetes is a significant public health issue in the United States. According to the U.S. Centers for Disease Control and Prevention (CDC), 34.2 million people – or 10.5 percent of the U.S. population – have diabetes, which is the 7th leading cause of death in the United States.[1]

Clinicians typically diagnose diabetes using either fasting plasma glucose (FPG) value, 2-h plasma glucose (2-h PG) during a 75-g oral glucose tolerance test (OGTT), or Hemoglobin A1c (A1c or HbA1c).

When using an A1c method, laboratorians should use a method that has been certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay.[2] This article examines some of the methods used to measure A1c that meet both of those criteria.

High-Performance Liquid Chromatography (HPLC)

HPLC is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. Pressurized liquid solvent containing the sample mixture passes through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

The components of the sample mixture separate from each other due to their different degrees of interaction with the adsorbent particles. There are various types of HPLC such as ion exchange, reverse phase, size exclusion, partition, normal phase and affinity chromatography.[3]

Both ion-exchange and affinity chromatography are used to measure A1c. Although both fall in the category of HPLC, they use different separation theories. In ion exchange-HPLC, separation is based on charge, while in affinity chromatography, separation is based on structure.

Cation-Exchange HPLC

In cation-exchange HPLC, a type of ion-exchange-HPLC, separation of hemoglobin molecules is based on charge, and hemoglobin molecules are positively charged.

Red blood cells are lysed and passed through a negatively charged resin packed in a column. Positively charged hemoglobin molecules interact with the negatively charged resin, so the negatively charged molecules move at a faster rate. The bound hemoglobins are released by varying solvent conditions injected into the column (e.g., increasing the ion effect of the solvent system by increasing the salt concentration of the solution, increasing the column temperature, changing the pH of the solvent, etc.).[3]

A chromatogram shows the eluted components in the form of quantifiable peaks. The advantage of using cation-exchange HPLC is that it not only provides an A1c value but also provides presumptive identification of commonly occurring hemoglobin variant traits that can potentially interfere with A1c results.

Table 1 provides a quick snapshot of these commonly occurring variants.[4]

Cation-exchange HPLC is considered an artistic technology because of the finesse with which each column is packed and the level of detail a chromatogram offers. To keep up with the rising number of A1c tests, this method has grown faster over the years but has also preserved the advantages of ion-exchange HPLC. The enhancements in the analyzers allow for automated chromatogram review and compatibility with track-line systems to accommodate the needs of higher-volume laboratories.

Type of Hemoglobin	Type of population affected and frequency
HbS	African American population (83%) Hispanic American (1%) Mediterranean, Indian, Saudi Arabian
HbC	African American, West African descent (2.3%)
HbD	India (Specially Punjab)
HbE	Asian American (South East Asians (upto 30%); China, India, Philippines, Turkey)
HbF	Circulating Fetal Hb in adults (1.5% of the US population)

Table 1. Commonly occurring variants

In this method, particular attention is given to the quality, type and size of resin used in packing a column (non-porous resin materials have been shown to be an efficient tool in A1c analysis),[5] and in consistency measures adopted in the column packing process. The quality of resolution of peaks in the chromatogram is equally important in this method. A well resolved A1c peak yields an accurate result. Partiular attention is also given the the simplicity of the A1c result. Some manufacturers use a direct measure of area under the peak, eliminating the need for complex calculations.

Boronate affinity chromatography

As mentioned earlier, this technique is an HPLC method that separates each component in a mixture based on structure. The high specificity of boronate makes it suitable for the separation of compounds, like glycated hemoglobins, that have a cis-diol in their molecular structure. The basic interaction for boronate chromatography is esterification between boronate ligands and cis-diols configuration formed by stable glucose attachments to Hb. As the glycated and non-glycated products elute through a column, the hemoglobin interacts with boronic acid giving 2 peaks: glycated (GHb) and non-glycated hemoglobin.[6,7] This method is less prone to interferences with variants; however, it lacks the ability to presumptively identify variant hemoglobins. Interference with A1c results in the presence of HbF above 15 percent is seen in this method.[8] The method does not directly measure A1c but calculates A1c from the glycated fraction.

Capillary electrophoresis (CE)

Capillary Electrophoresis (CE) separates Hb molecules based on charge and mass. The charged molecules (in this context,

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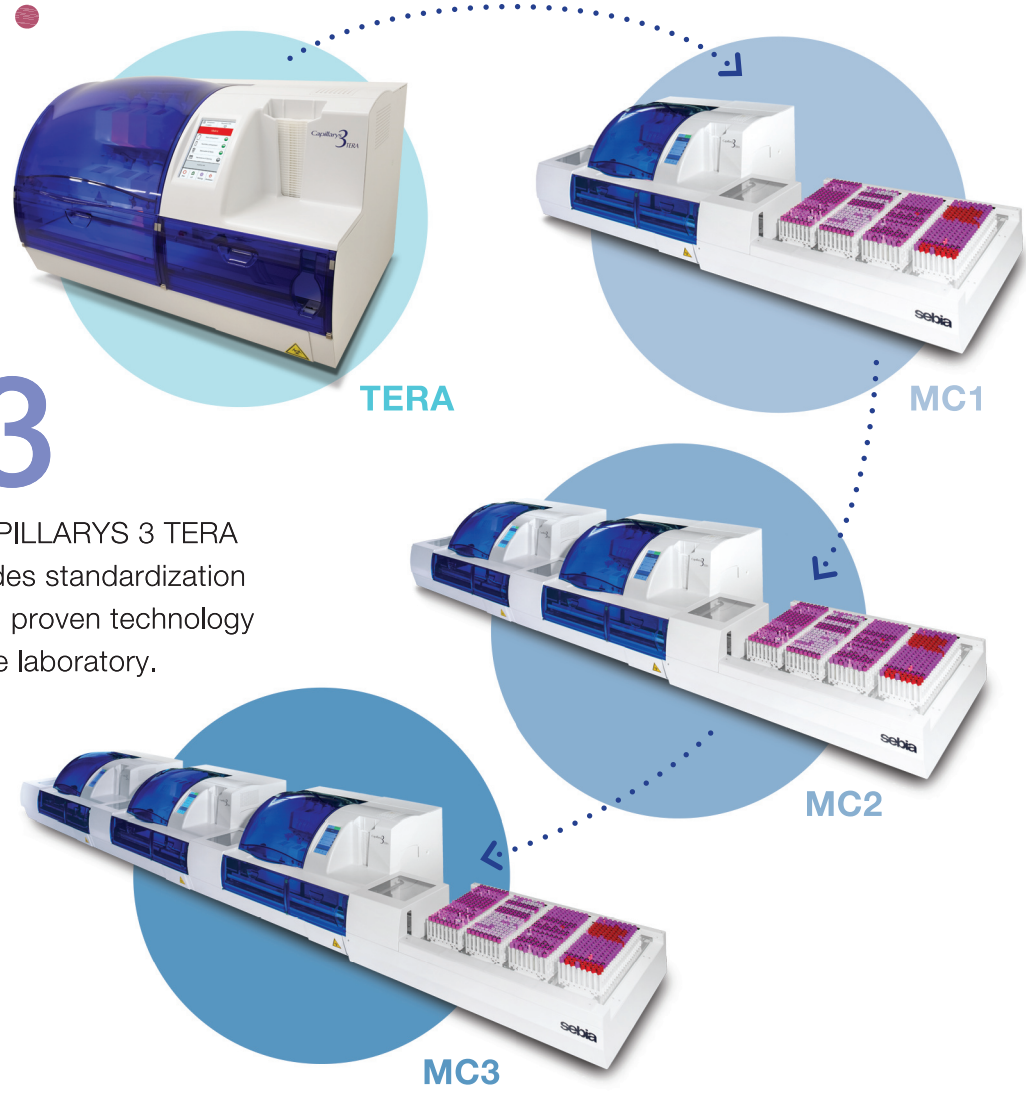

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the hemoglobins) are resolved by their electrophoretic mobility. The technique uses capillary tubes through which high-voltage electricity is used to generate an electric field that facilitates the migration of hemoglobins from anode to cathode.

The separation of the hemoglobin species occurs in decreasing order of their charge-to-mass ratio, with the positively charged hemoglobins eluting first. If there are two or more molecules that have the same charge, the system will further resolve these by size, and the large molecules will elute first. Positively charged molecules are followed by neutral species and, finally, by negatively charged Hb species.

Capillary electrophoresis is a more recent technology when it comes to HbA1c detection, compared with HPLC and immunoassay. Just as in cation-exchange HPLC, this method allows for presumptive identification of hemoglobin variants that can be seen on an electropherogram. The most common heterozygous Hb variants (HbS, HbC, HbD, and HbE) do not interfere with the method.[9]

However, analyzers using this method may need more maintenance because of the number of small-diameter capillaries necessary to carry out testing. High-volume laboratories will benefit from such analyzers only if multiple analyzers can be connected to a track-line.

Immunoassays

Immunoassays are bioanalytical methods in which the quantitation of the analyte depends on the reaction of an antigen with an antibody where the antigen is usually the analyte tested for. The competitive (inhibition) immunoassay is based on a competitive binding reaction between a fixed amount of a labeled form of an analyte and a variable amount of unlabeled sample analyte for a limited amount of binding sites on a highly specific anti-analyte antibody. When these immunoanalytical reagents are mixed and incubated, the analyte is bound to the antibody forming an immune complex.

This complex is separated from the unbound reagent fraction by a physical or chemical separation technique. Analysis is achieved by measuring the label activity (e.g. radiation, fluorescence, or enzyme) in either of the bound or free fraction. A standard curve, which represents the measured signal as a function of the concentration of the unlabeled analyte in the sample, is constructed. Unknown analyte concentration is determined from this calibration curve.[10]

In the context of A1c, immunoassays use antibodies that target N-terminal glycated amino acids on the ß chain to quantify A1c. The quality of an immunoassay very much depends on the specificity of the antibody to the first few amino acids on ß chain of HbA. Point mutations for HbS and HbC occur at ß6 and care must be taken to choose a method where the antibody epitope does not span that area. There are other uncommonly occurring variants that span this epitope. Further, the point mutations for HbE and HbD (Los Angeles) occur at ß26 and ß121 respectively.

Immunoassay analyzers are known for their speed of analysis and satisfy the needs of laboratories running a high volume of A1c samples. For laboratories running a menu of analytes, the ability to consolidate assays on one single analyzer becomes an important decision-maker in choosing an analyzer. The biggest argument against selecting an immunoassay method is the inability of the method to presumptively identify variant hemoglobins. The A1c result is merely a number.[1] Another important consideration is the physical space constraints of a laboratory in accommodating an analyzer with a large footprint. Laboratories must be aware of the trade-offs in selecting an immunoassay analyzer for A1c.

Enzymatic methods

In this method, whole blood samples are subject to breakdown of protein into amino acids and small peptides (proteolytic digestion). HbA1c is a glycated hemoglobin in which glucose is bound specifically to the N-terminal valine of the Hbβ chain. Proteolytic digestion releases the glycated N-terminal valine from the Hbβ chains. Glycated valine serves as a substrate for a specific enzyme that cleaves the N-terminal valine and produces hydrogen peroxide that is subjected to a chromogenic reaction. The signal produced is directly proportional to the amount of A1c in the sample. Just as in immunoassay, this method provides only an A1c value and no additional information about the presence of hemoglobin variants or labile and carbamylated Hb.[9]

This overview suggests that a number of factors are involved in choosing the right analyzer for a laboratory's needs. In addition to the accuracy and precision of the test results, other common considerations include sample throughput, workflow efficiencies, frequency of instrument maintenance, sample workload, environmentally friendly disposal of reagents, and compatibility with track-line automation.

Variant interferences play an equally important role. In the United States, where the population is ethnically and racially diverse, the ability for a method to presumptively identify commonly occurring hemoglobin traits provides an additional level of certainty in the result.

In conclusion, every laboratory has different needs. When choosing an analyzer, it is important to meet the economic needs of the laboratory without compromising on the quality of an A1c result, which is becoming an ever-more-important tool for diagnosing and monitoring diabetes.

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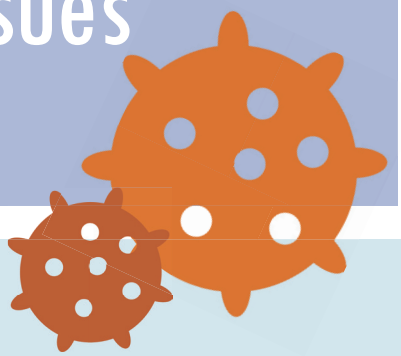
COVID-19 testing, despite supply chain issues

By Linda Wilson



Once the novel coronavirus (SARS-CoV-2) hit the United States, Central Ohio Primary Care, with more than 75 locations, wanted to launch polymerase chain reaction (PCR) testing to detect the virus.

And it did – eventually.

But not without a protracted effort to overcome stumbling blocks. "We've had a lot of trouble getting test reagents," said Cynthia Roberts, MBA, MLT (ASCP), Laboratory Manager at Central Ohio Primary Care, based in Westerville. The lab also has struggled to source nasopharyngeal swabs, she said.

Central Ohio Primary Care – which performs four million tests annually and operates three patient draw centers – already had the Cepheid GeneXpert in-house, but it also purchased the DiaSorin Molecular Liaison MDX and the Abbott ID Now, Roberts said. With a combination of the three platforms, the lab ramped up its capacity to 90 tests per day.

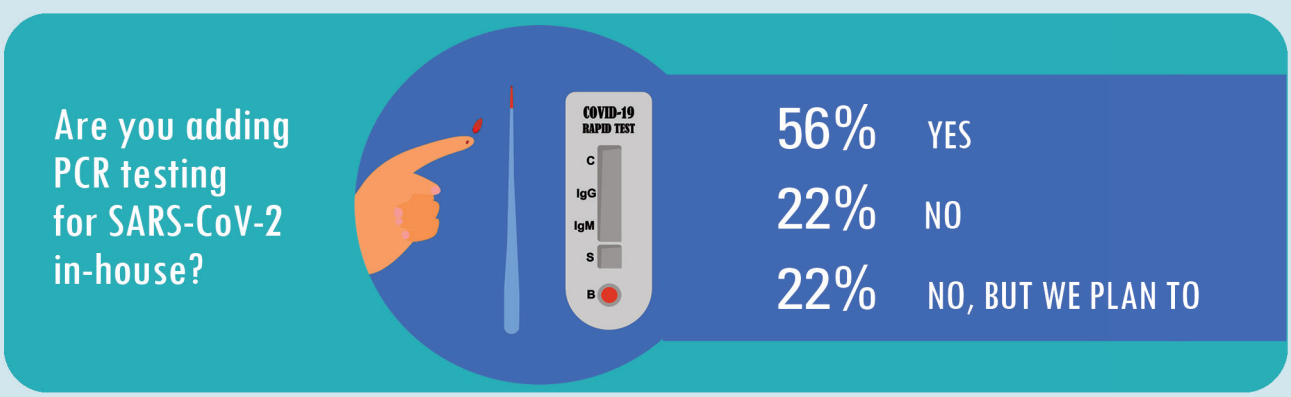
The healthcare provider is not alone. Many of Roberts' peers at other laboratories described similar issues in *Medical Laboratory Observer's (MLO)* third State of the Industry survey, which focuses on disease management and testing for COVID-19.

Most of the labs with in-house PCR testing to detect SARS-CoV-2 (63 percent) are conducting a modest number of tests per day, or between one and 50 tests.

Among the other labs that responded to *MLO's* survey, the breakdown of daily test volume is as follows:

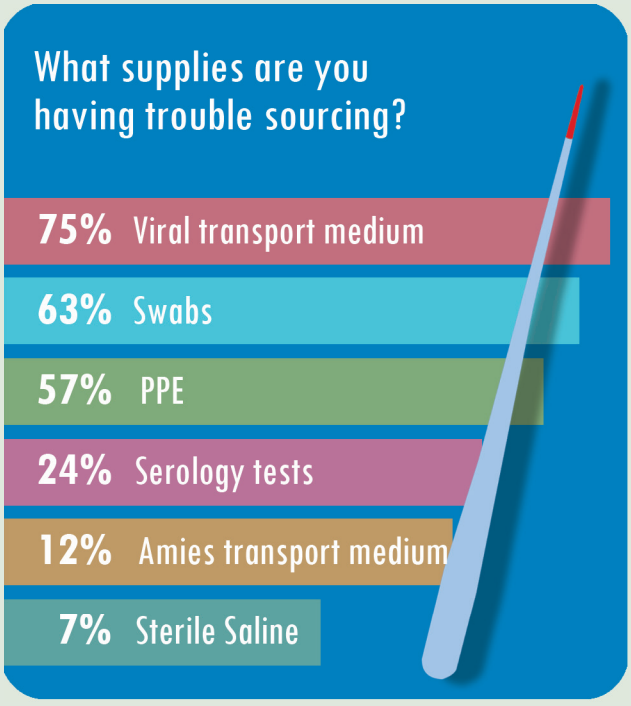
- 51-200 tests for 18 percent
- 201-500 for 10 percent
- 501-1,000 for 6 percent
- 1,001-and 2,000 for 2 percent
- More than 2,000 tests for 1 percent

The majority, or 72 percent, of survey respondents work at hospital labs, while 12 percent work in physician's office labs, 7 percent work in integrated clinical labs, 3 percent work in government labs, 2 percent work in independent labs, and 1 percent work in reference labs. Three percent of survey respondents selected the "other" category when asked what type of lab they work in.



SOURCING SUPPLIES

Nearly half of respondents, or 49 percent, said sourcing supplies has been the single biggest challenge during the pandemic. Other respondents noted other challenges, such as communicating effectively (16 percent), sourcing PPE (14 percent), scaling up to the volume of testing necessary (13 percent) and scheduling personnel (8 percent).



Roberts said Central Ohio Primary Care has had trouble getting vendors' attention. "Companies are prioritizing who gets supplies, and we weren't made a priority. Then it took weeks to months to be able to purchase new platforms because supply couldn't meet demand," Roberts said. "Many things are allocated based on prior usage – if you weren't using NP (nasopharyngeal) swabs or VTM (viral transport media) prior to this crisis, they wouldn't sell you any," she said.

SwedishAmerican Hospital in Rockford, IL, collects about 120 specimens per day but sends out more than 100 of those to reference labs. The hospital, a division of University of Wisconsin Health, simply has not been able to source supplies or new testing platforms, according to Nicole Radford, FACHE, MS, MT(ASCP), laboratory director at SwedishAmerican.

Finding enough testing capacity at reference labs also has been difficult, she said.

"For the various reference labs that we've used, we've noted that, even if they don't discuss it, there are only so many that they can do within the established turnaround time. Some of the labs we've used have accepted anything sent their way, but the turnaround time increases to (at times) unreasonable levels. Other labs will have put a cap on the amount that will be accepted per client in order to protect the turnaround time. Either way, this has presented a problem for our lab's offering of COVID-19 testing," she said.

As a result, she said SwedishAmerican has switched reference labs a number of times. While this led the lab managers to rework their process multiple times, it also has allowed the hospital to meet the demand for testing.

Finding new analyzers during the pandemic, which would allow SwedishAmerican to perform more tests in-house, also has not been easy. They are "very few and far between at this point," Radford said. The hospital inked a contract for a high-throughput machine before the pandemic but still has not taken delivery of it. "I've heard time and again from a number of vendors that precedence and priority are given to clients who are obtaining the equipment with capital funds (outright purchase) versus leasing or reagent rental options," Radford said.

For their part, vendors have also had to react to the havoc caused by the pandemic.

Timothy Templet, executive vice president of sales at Puritan Medical Products, Guilford, ME, said, "During these unprecedented times, Puritan is managing its current manufacturing capabilities while working diligently to increase capacities over three product categories with additional manufacturing locations all based in the USA. Puritan continues to receive multiple requests for COVID-19 testing swabs from new potential customers and we are doing our best to accommodate everyone now and in the future."

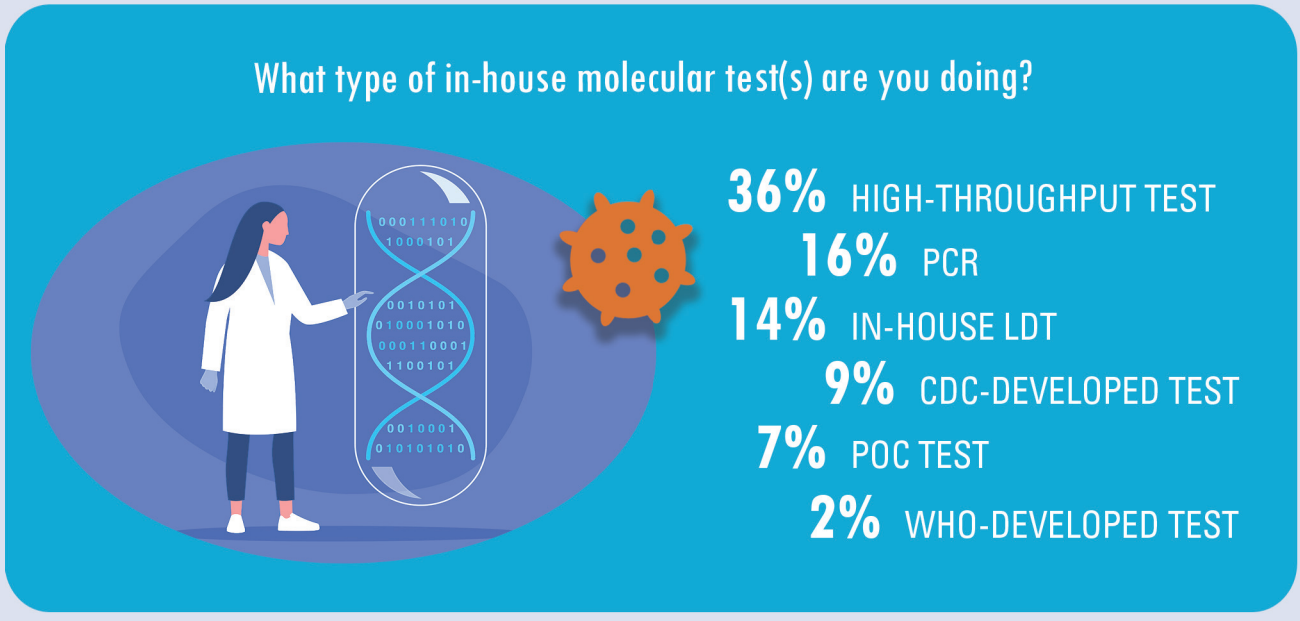
The company makes foam, spun polyester and flock swabs and primarily sells them to vendors of testing platforms and distributors.

SUPPLY CHAIN SOLUTIONS

Because of the shortages of supplies, such as swabs, many labs are conducting SARS-CoV-2 tests on multiple vendors' platforms. "We encourage our clients to develop two, three or even four different platforms for testing. That's our short-term solution," said David Nichols, President and Founder of Nichols Management Group, which specializes in consulting services to laboratories.

lowering their cost-per-test. "We believe that the coronavirus will come and go in peaks and valleys, and there will be COVID-19 related testing for an extended period of time," he said.

"I would urge organizations to migrate to the lowest cost platform that is appropriate and secure the supplies. If you are working diligently on it now, it is possible to get them in place by late fall," he said.



Nichols said he thinks supply shortages, particularly for PPE, have leveled out somewhat since *MLO* conducted its survey in April and May. "I have spoken to a number of health systems in the last couple of days who have said they are 100 percent back in inventory for their PPE. It has really eased up," Nichols said. However, he also added that "it is hard to generalize for the country. It is back to inventory levels, but there still are pockets of need."

He urges clients to move from a short-term crisis response to medium- and long-term planning, so they will be prepared when subsequent waves of COVID-19 potentially crash through their doors. He suggests labs evaluate their current automation platforms and lease commitments, with a goal of

Taking a long-term approach to purchase anlyzers is the route Radford from SwedishAmerican has followed since the beginning. She said, "We have to consider the future post-COVID. Will this be a platform that we'll be 'stuck' with later? Will we be able to use the other tests on the menu when and if the number of COVID test requests are no longer the number one ordered test?"

"There's an example of a vendor whose equipment I strongly considered. While the analyzer would certainly be helpful for COVID testing, it would not be needed later because we already have more cost-effective and reliable methods of performing all of the other tests on the instrument's menu," she explained.

REIMBURSEMENT WOES

Nichols said his clients are spending between $40 and $150 per test, compared to reimbursement rates from the Centers for Medicare & Medicaid Services (CMS) of about $51 for a standard PCR assay and $100 for a high-throughput test.

As Roberts notes, that margin hardly covers test kits – not to mention overhead such as rent, salaries and PPE.

At SwedishAmerican, the expenses for the in-house tests are not "far below" the Medicare reimbursement rate of $100 per test, and the cost of each test sent to outside labs "is very close to or more than $100," Radford said.

Nichols recommends that labs form a committee comprised of employees from operations, billing and information technology to make sure they are capturing the appropriate revenue for the testing services. This involves such steps as capturing accurate demographic information, utilizing the proper billing codes and closely tracking payments from government and commercial payers.

"This needs to be a robust effort because guidelines are changing and practices are changing. If that is not done, the lab will find that COVID-19 testing will lead to a very significant reduction in their operating margin," he said.

MLO also asked survey respondents if the revenue generated from SARS-CoV-2 tests plus other tests performed on patients with a confirmed diagnosis of COVID-19 was enough to offset the reduced test volumes associated with elective procedures. A total of 58 percent said "no," 4 percent said "yes," and 38 percent said the question did not apply to their situation.

When asked what other types of tests clinicians are ordering for patients with confirmed or suspected cases of COVID-19, 44 percent said D-dimer value, 35 percent said cardiac enzymes, 28 percent said glucose, 8 percent said ferritin, and 28 percent selected the "other" category. Slightly more than one-third, or 39 percent, said they are not processing other tests for COVID-19 patients.

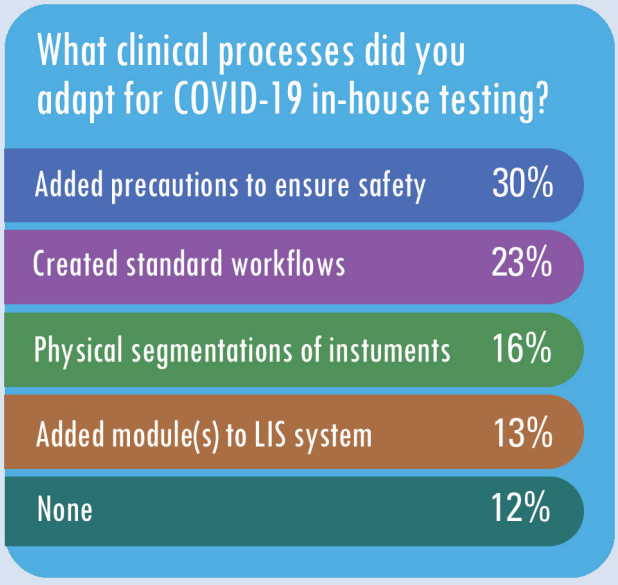
POC TESTING

In addition to lab-based testing, Central Ohio Primary Care also added POC testing with the Abbott ID Now system, which uses dry swabs. However, Roberts said she has assigned a laboratory staff member to oversee the testing process at the patient draw center, ensuring the appropriate procedures are followed and test results are accurate.

venues, according to survey respondents, including emergency departments (72 percent of respondents), drive-up testing (56 percent of respondents), physician's offices (40 percent of respondents) and urgent care centers (39 percent of respondents).

WORKFLOWS AND TRAINING

MLO also asked respondents what changes they made to their work processes to make in-house testing for SARS-CoV-2 possible.



While a majority of respondents, said they did not have to modify their existing guidelines for infectious disease testing to accommodate testing for SARS-CoV-2, Radford said SwedishAmerican has made some modifications to its infectious-disease processes for COVID-19.

For example, she said, "we are testing all patients within 72 hours prior to a planned procedure that fit specific criteria of risk. While this is not unlike other surveillance that we may do (i.e. MRSA screening), what is different is that the patient must self-isolate for the 72 hours prior to the procedure," Radford said.

For labs using the high-throughput testing apparatus, they used a variety of approaches to train staff on the machines. Nearly two-thirds, or 62 percent, used a train-the-trainer approach, while 31 percent used training provided by the vendors, 13 percent used online training modules, and 7 percent used lunch-and-learn sessions.

SEROLOGY TESTING

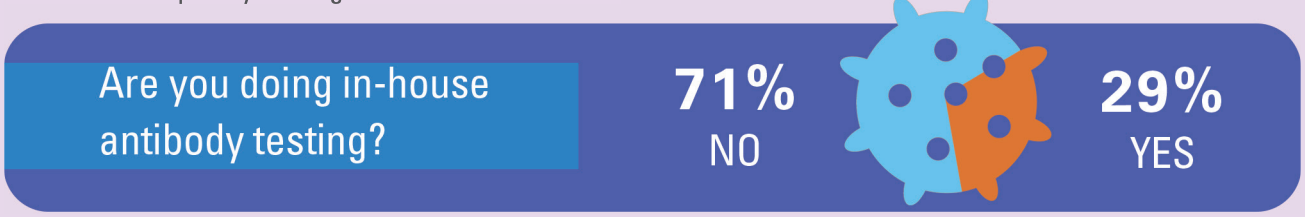
In addition to molecular testing to diagnose active cases of COVID-19, some providers have added serology testing to detect the antibodies the body produces to fight off viruses such as SARS-CoV-2, known as immunoglobulin M (IgM) and immunoglobulin G (IgG).

Indeed, Central Ohio Primary Care launched serology testing in May. But the Ohio provider is in the minority. According to the *MLO* survey, less than a third of survey respondents said they have added serology testing.

Nichols thinks that all labs certified to provide moderate complexity testing and above should add serology testing. It is not only a valuable service in high demand but also yields an acceptable margin benefiting lab budgets.

"The good news is it seems that the reimbursement for the antibody testing, which is largely going to be performed on large-scale automated equipment, has a significantly higher margin than the antigen testing," Nichols said. "I would think every one of the top vendors will have this serology capability on an automated platform within three months," he said.

After all, he notes, "the demand for this kind of testing may not be going to go away."



Sonora Quest takes on COVID-19 in Arizona

By Linda Wilson

Sonora Quest Laboratories processes about 7,000 tests daily related to COVID-19 – and that number continues to grow.

The lab – a joint venture between Banner Health and Quest Diagnostics – rolled out its testing program in phases. It launched an in-house molecular test to detect SARS-CoV-2 in March and a serology test to detect the antibodies the body produces in response to the virus in May.

In May it also launched a program with the state of Arizona to test staff and residents at all 147 nursing homes in the state, or the equivalent of about 125,000 tests. Sonora Quest plans to use its mobile diagnostic services, which already service long-term care facilities for routine blood draws, to collect specimens for both the molecular and serology tests. Administrators at the individual facilities will decide whether they want molecular tests, serology tests, or a combination of both.

But getting to this point has not been easy. It involved long hours, creativity, and teamwork.

On the molecular front, there were numerous challenges to overcome.

The lab uses high-throughput analyzers from Roche and Hologic for molecular testing. To meet the expected demand for testing in Arizona, Sonora Quest purchased additional machines from those vendors. Once the machines arrived, staff from both Sonora Quest and the vendors worked to get the machines installed, validated and in production in just days rather than weeks, which is typically the case under normal circumstances.

Then they hustled to find enough test kits, including both the reagents and swabs.

When it became clear that Sonora Quest would not be able to get enough test kits from the manufacturers, the lab sourced the materials separately in bulk. "Volunteers from every functional team came in and met in a warehouse and built kits together," explained Sonya Engle, chief operating officer at Sonora Quest.

The molecular department also validated alternatives to nasopharyngeal swabs – such as those made of flock and spun polyester – and viral transport media – such as sterile saline.

Training was another significant challenge. With a slow-down in elective procedures and visits to doctor's offices, Sonora Quest redeployed some employees from other functional departments to molecular testing to accommodate the ramping up process and the 24/7 operation of the high-throughput machines. "The training has to be thorough. Somebody has to be there working the bench and training somebody at the same time. That takes a lot of resources," said Brian Koeneman, PhD, scientific medical director of molecular diagnostics at Sonora Quest.

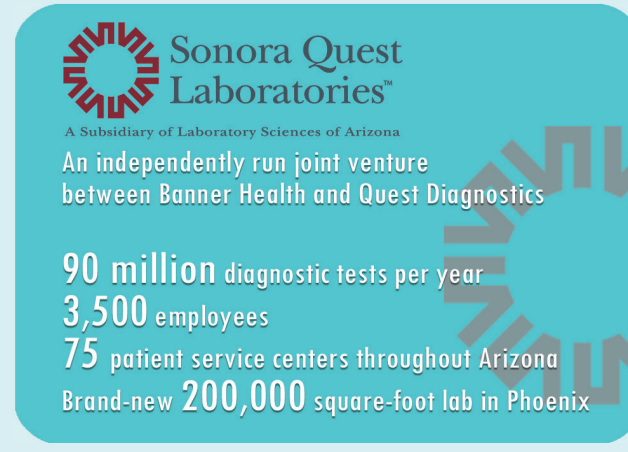
In addition to the challenges of sourcing supplies and equipment, there also were challenges for the lab's information systems (LIS) department to overcome.

For example, Sonora Quest developed a process so it could send out specimens for the molecular tests to Quest facilities in other states when demand exceeded its in-house capacity. And, for the first time, Sonora Quest committed to processing specimens from Quest in other states on days when the reverse was true.

That meant the information systems department at Sonora Quest had to create an electronic interface between its laboratory information system (LIS) and the IT system at Quest Diagnostics.

The IT staff also had to modify its LIS, NeTLIMS, to accommodate new equipment for both molecular and serology testing. The goal was to have testing "flex" behind-the-scenes at Sonora Quest to accommodate the multiple instrument platforms, but keep that process invisible to customers who send in specimens, such as hospitals and doctor's offices, as well as patients, who are able to order the serology test directly from Sonora Quest without an order from a provider.

"The bigger challenge was because everything was changing so much. We had to do some very creative building on our side with our NeTLIMS partner to make that work," Jackie Carlisle, director of laboratory information systems at Sonora Quest, said. "I think it has really challenged us to think outside the box and look for solutions that we didn't even know we could do," she said.



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Lab testing for GBS screening unchanged with new guidelines

By Frances C. Esteve

Group B Streptococcus (GBS) remains the leading cause of early-onset sepsis and meningitis among newborns in the United States, with a diseases fatality rate of 4 percent to 6 percent for infants that become infected. The primary risk factor for GBS early onset disease (EOD) is maternal colonization. Vertical transmission to the newborn infant usually occurs during labor or after rupture of the membranes. It is estimated that 25 percent of all pregnant women in the United States are colonized with GBS bacteria, with the majority being asymptomatic.

The rate of transmission to the baby is approximately 50 percent and in the absence of prophylactic intervention with antibiotics, one in 200 of colonized newborns will develop GBS EOD. However, if the mother is treated with antibiotics during labor, the risk of developing GBS EOD drops to one in 4,000 babies. It has been documented that the implementation of the guidelines for antepartum screening and the use of intrapartum antibiotic prophylaxis (IAP), have had a positive impact in the reduction of GBS EOD incidence by decreasing from 1.8 cases per 1000 live births in the early 1990s to 0.22 cases for every 1,000 live births in 2017, a decline of over 80 percent.[1,2]

GBS guidelines

It has been over 25 years since the publication of the first guidelines for the prevention of GBS infection in newborns. It was not until 2002 that the Centers for Disease Control and Prevention (CDC) first recommended universal screening for all pregnant women (antenatal screening), which remains as the current standard. Universal culture-based screening at 35-37 weeks gestation for identifying candidates for GBS intrapartum antibiotic prophylaxis was demonstrated to be greater than 50 percent more effective at preventing disease than using a risk-factors-based approach. The effectiveness of the intrapartum antibiotic prophylaxis is associated with the temporary decrease in maternal vaginal GBS colonization, the prevention of surface and mucus membrane colonization of the newborn, and the ability to reach levels of the antibiotic in the newborn bloodstream above the minimum inhibitory concentration for killing GBS.[3,4,5]

In 2010, the CDC issued a revision to the Prevention of Perinatal Group B Streptococcal Disease guidelines in alignment with the American College of Obstetricians and Gynecologists (ACOG), the American Academy of Pediatrics (AAP), the American Academy of Family Physicians (AAFP) and several other groups. Among other recommendations, the 2010 revision of the guidelines introduced new algorithms for neonatal management and IAP usage, but for the laboratories the most notable change came with the inclusion of the use of nucleic acid amplification tests (NAATs) after broth enrichment of vaginal-rectal swabs to identify maternal GBS colonization during antepartum screening.[3]

Most recently, in a coordinated effort in 2019, ACOG and AAP published their respective revised guidelines for preventing/managing infections caused by GBS in infants. ACOG's and AAP's new guidelines align with the CDC's 2010 publication in supporting universal maternal screening and when appropriate, the use of IAP to prevent transmission of GBS from mother to infant during labor. One noted change is the timing for the antepartum screen at 36-38 weeks gestation instead of 35-37 weeks as previously recommended.

This new recommended timing for screening provides a five-week window which includes births that occur up to a gestational age of 41 weeks. These changes have minimal to no impact to the laboratory with regards to antepartum testing for expecting mothers. ACOG recognizes the use of NAATs after broth enrichment for the identification of GBS from vaginal-rectal swabs provides the best opportunity to identify a colonized mother, even when sample collection is not performed properly. Ultimately these new guidelines will replace the current CDC's 2010 Perinatal GBS Infection Prevention guidelines.[4,5]

NAAT testing for GBS

While the overall rate of GBS infection has significantly decreased in the United States, a concern remains with the number of GBS EOD cases coming from mothers that were appropriately screened, but GBS colonization was not detected. A recent study that used data collected from 2006-2015 through the CDC Active Bacterial Core (ABC) Surveillance program (covering 10 states across the U.S.) specifically looked for infants younger than 90 days with invasive GBS disease, and found that there were missed opportunities for prevention of EOD. Of the total number of GBS EOD cases (1,277), 48.3 percent (617) were missed. Of the missed cases, 82.9 percent (512/617) were mothers with screening histories negative for GBS and the remaining 17.1 percent (105/617) had unknown status.[6] Two additional studies have reported similar results, where women who screened negative for GBS had babies with GBS EOD.[4]

False negative GBS screening results can be attributed to several factors including suboptimal specimen collection, timing of the collection, transport and processing of the sample, and the method used for testing.

Incorrect sample collection (mainly vaginal collection without rectal sampling) is one of the most common errors made when collecting samples. In order to maximize GBS recovery, it is recommended that a single swab be obtained first from the lower vagina and then the rectum. The combined sample provides higher culture yield than single source samples. The laboratory has no way of knowing if the sample was collected properly, and since incorrectly collected samples can lead to false negative results, the use of a NAAT test after the enrichment broth step has demonstrated to be the most appropriate testing approach to maximize detection.[4,5]

ACOG's new recommended timing for sample collection for universal screening between 36-38 weeks of gestation, allows for a valid culture result that covers births up to 41 weeks. Studies have shown that the predictive ability of the prenatal cultures decreases significantly when the culture-to-birth interval is longer than five weeks. Testing at 36-38 weeks also helps minimize repeat testing to confirm any changes in the colonization status of the mother.[5]

Transportation of the sample from the collection site to the laboratory can also negatively impact test results. Placing the swabs in an appropriate transport medium can help sustain viability of the organism. However, the recovery of the isolates declines within one to four days, especially if the samples are subjected to elevated temperatures. The best recovery is obtained when the sample is stored at 4°C and processed within four hours of collection. Samples should undergo 18-24 hours of incubation at 35-37°C in broth enrichment medium to enhance the recovery of GBS. Studies have shown that when direct plating is used without broth enrichment,

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as many as 50 percent of women who are GBS carriers have false negative culture results. In a situation where microbial load is low due to unfavorable transport conditions, even with broth enrichment, the use of a NAAT test can help enhance detection.[3,5]

When it comes to testing methods, the combination of broth enrichment followed by a nucleic acid amplification test has shown in repeated studies to outperform culture-based tests. Yet the CDC and ACOG recognize that there is still a high percent of GBS EOD cases occurring from mothers with a negative antenatal screen.

This observation can be attributed in part to the fact that NAAT testing has not been widely adopted. The results from a recent study that surveyed 544 laboratories (93 percent participation) as part of the 2016 CDC ABC Surveillance program, reported that only 18.7 percent of the laboratories used NAAT for GBS testing (7.3 percent used NAAT for antepartum testing only, 4.1 percent for intrapartum testing, and 3.4 percent for both). Of the labs using NAAT for antepartum testing, 82 percent reported using an enrichment step. Of the participating laboratories the vast majority (97.4 percent) were hospitals or clinical laboratories and 12.6 percent were private or commercial laboratories. Even though the use of NAAT has increased from 2010 to 2016, its use remains limited, with room for improvement.[3,5,7]

Proponents of NAAT tests recognize other benefits associated with molecular tests, most notably faster turnaround time compared to culture and the ability to report results back to physicians more quickly. While it is widely accepted that accuracy is more important than turnaround time for antenatal GBS screening, it's worth taking into consideration that faster results can bring peace of mind to anxious expectant mothers. Also, the move from culture-based methods to NAAT allows for enhanced workflow, labor efficiencies and decreased hands-on time.

Additionally, molecular tests remove subjectivity from results interpretation, which in turn helps minimize errors. The accuracy with which GBS is diagnosed can a have significant impact and long-term consequence on the life of a newborn. Let's hope that the trend continues with more laboratories making the move from their GBS cultures to molecular assays for antenatal screening of pregnant mothers.[3,4,7]

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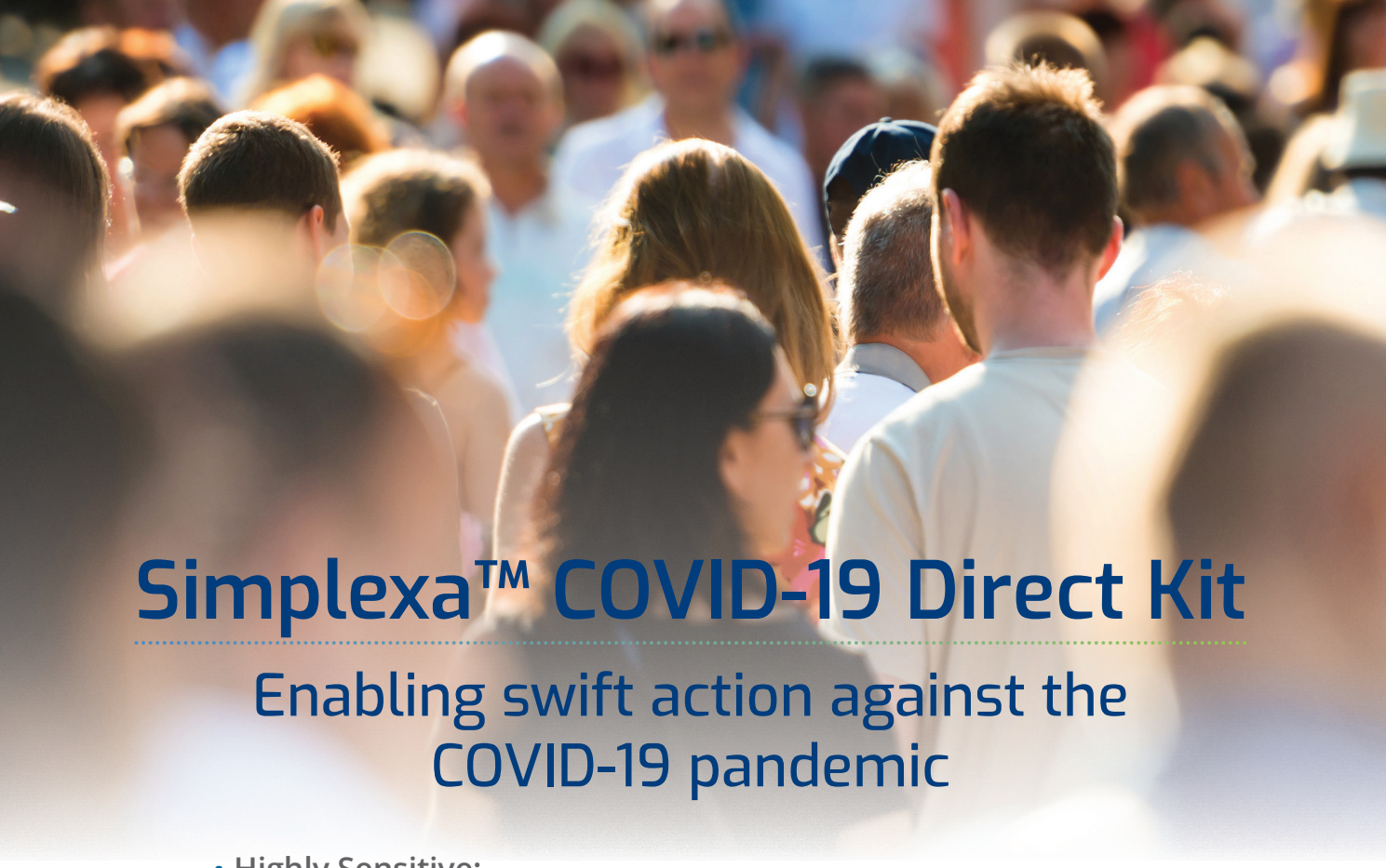
With more than 35 years of experience in IVD at **Meridian Bioscience**, Frances C, Esteve is currently a Marketing Product Manager. She has held positions in product research and development, manufacturing, ASQC Certified Quality Auditor, and international sales. She received her Bachelor of Biological Sciences from the University of Cincinnati.



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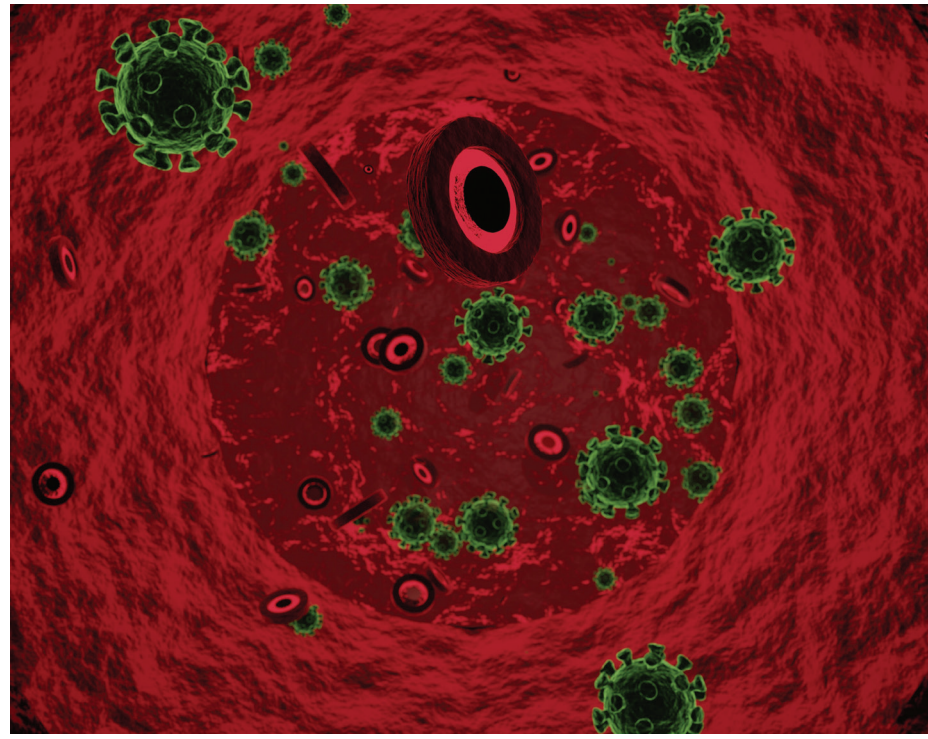
The significance of serology antibody testing for SARS-CoV-2

By Iswariya Venkataraman, PhD, Maite Sabalza, PhD, Stanley J. Naides, MD

The World Health Organization (WHO) declared the coronavirus SARS-CoV-2 disease (COVID-19) outbreak a global pandemic on March 11, 2020.[1] This is the third coronavirus to cross species, following the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012, and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1) in 2003.[2] However, SARS-CoV-2 has had a more rapid spread with over 7.2 million confirmed cases and over 400,000 deaths worldwide.[3] The United States has nearly 2 million confirmed COVID-19, cases and above 100,000 related deaths.[3]

As of June 9, 2020, the U.S. Food and Drug Administration (FDA) had provided Emergency Use Authorizations (EUAs) for molecular-based detection and/or diagnosis of COVID-19 to 74 manufacturers and 36 high-complexity laboratories who had developed molecular-based laboratory developed tests.[4] Also as of June 9, 2020, the FDA has issued only 19 EUAs for antibody-based detection tests. The FDA has not objected to the development and distribution of serology tests in identifying antibodies for SARS-CoV-2 as long as manufacturers apply for EUA within 10 days of notifying the agency of their intent to market the tests.[5]



Serological tests aid in understanding the immune responses to SARS-CoV-2

Why is serology testing important for SARS-CoV-2?

Currently, SARS-CoV-2 infection is diagnosed by the detection of the virus via molecular-based techniques, such as real-time reverse transcriptase polymerase chain reaction (RT-PCR) or deep sequencing. However, these tests have a high rate of false negativity. [6-8] These detection systems rely on adequate viral load at the sample collection site.[6] SARS-CoV-2 can cause both upper, but predominantly lower, respiratory tract infections. The positivity rate for the different samples ranges from 74.4 percent to 88.9 percent for sputum, 53.6 percent to 73.3 percent for nasal swabs, and a much lower rate in throat swabs collected ≥8 days post-disease onset.[9]

The highest positivity rate has been shown in bronchoalveolar lavage fluid specimens (93 percent).[10] However, lower respiratory tract sampling requires both a skilled technician and a suction device. Additionally, false-negative results are observed with RT-PCR methods when the viral replication time-window is missed.[6] Liu et al. 2020 showed 11 days' post symptom onset (PSO) is a transitional time point, when infected patients produce anti-viral antibodies, allowing confirmation of viral infection. The positivity rate for detection of IgM and/or IgG antibodies was >80 percent at this time point and was close to 100 percent after 14 days PSO. In contrast, the positivity rate of RT-PCR was about 60 percent 11 days PSO and began to rapidly decline afterwards **(Figure 1).**

The overall positivity rate was significantly higher by antibody testing (81.5 percent) compared with RT-PCR (64.3 percent) at day 11 PSO.[7] In another study, only 51.9 percent of patients were positive by a single RT-PCR test, but the positivity rate increased to 98.6 percent following and antibody assay on PCR- negative individuals.[6] At this stage of the pandemic, a molecular false-negative result could pose a serious threat by allowing infectious patients back into the community, and hindering the efforts to contain virus spread.[6] Therefore, a combination of RT-PCR and antibody testing methods would considerably improve diagnostic efficacy, even during early stages.[7]

What is the significance of serology testing for SARS-CoV-2?

Serological assays aid in understanding the immune responses to SARS-CoV-2 in a dynamic qualitative or quantitative manner and to identify individuals who were infected compared to those who were not. Data

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from serological tests are essential to determine the immune status of healthcare workers. Those who have developed the antibodies then can be deployed back to frontline work with less risk of illness while limiting virus spread.[11] Serological assays are also needed to conduct epidemiologic studies to assess the extent of virus spread in communities and to determine the infection fatality rate. Furthermore, serological assays can identify individuals with the required antibody response against SARS-CoV-2, who can be donors of hyperimmune plasma to treat sick individuals.[11,12] Lastly, serological assays can be used to select patients for clinical trials for vaccine or therapy development.[2]

What are the target antigens for the serology testing for SARS-CoV-2?

Coronavirus has four main structural proteins: nucleocapsid (N), spike (S), membrane (M) and envelope (E). The S protein consists of the S1 and S2 subunits. The globular head S1 subunit contains the receptor binding domain (RBD) and facilitates the attachment to host cells, mediated by its interaction with cell surface angiotensin-converting enzyme 2 (ACE2).[13] The S2 subunit comprises the stalk of the spike protein and facilitates fusion between viral envelope and host cell membrane after S1 binding induced conformational changes.[14] The S protein is highly immunogenic since it is located on the surface of the virus.[15]

The N protein plays an important role in the transcription and replication of viral RNA, packaging the encapsidated genome into virions[16] and inhibits the cell cycle process of the host cells.[17] The N protein is abundantly expressed during infections and also has high immunogenic activity.[8,9,18] Therefore, both N and S protein could be potential targets for the antibody-based detection of SARS-CoV-2.[9] However, the N protein homology between SARS-CoV-2 and SARSCoV-1 is 90 percent, compared with the S protein (77 percent), especially the S1 subunit including the RBD (66 percent).

Additionally, the percentage of amino acid identity among other endemic coronaviruses (OC43, HKU1, 229E, NL63) and MERS-CoV was higher for the N protein, compared with the S1 subunit,[2] suggesting that serodiagnostics using N protein could potentially demonstrate greater cross-reactivities among the endemic coronaviruses. Jiang et al. 2020 showed the N protein based antibody assays could exhibit a higher false-negative rate compared with the S1 subunit, and that S1 subunit purified from mammalian cells demonstrated the highest performance to distinguish COVID-19 patients from controls.[19]

On the contrary, the N protein antigen is generally produced in bacteria instead of mammalian cell lines. Therefore, critical human glycosylation would be missing and, in turn, decrease high-affinity binding to antibodies.[20] Therefore, the S1 subunit could be the specific target antigen for detecting SARS-CoV-2 antibodies.[2]

What antibodies are detected against SARS-CoV-2?

In infectious disease, IgM and IgG antibodies are commonly detected using serological tests due to their important role in tackling the viral infection. However, in many respiratory infections, in addition to IgM antibodies, IgA antibodies are produced in high titers during the early phase of the infection.[21,22] In general, IgM antibodies have a lower affinity compared to IgA or IgG antibodies, and pose an increased risk of cross-reactivity against antigenically similar epitopes, which are common in coronaviruses.[23]

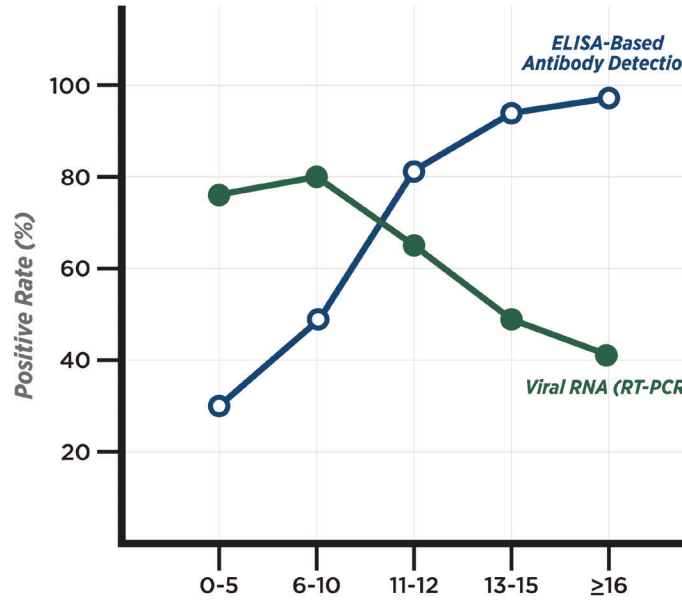

Figure 1. Positivity rate of patients based on detection of viral load by RT-PCR and antibodies by ELISA (Adapted from Liu et al. 2020.7).

During the SARS-CoV-1 infection of 2002, there was simultaneous seroconversion of all immunoglobulin classes (IgM, IgA and IgG),[24,25] and similar observations are being reported for the SARS-CoV-2 infection.[6,20,26] Guo et al. 2020 showed that the production of IgA, IgM, and IgG antibodies was positive as early as one-day PSO. IgM/IgA antibodies and IgG antibodies were detected at a median of five and 14 days' PSO, respectively.

Additionally, during the acute phase (days 1-7, PSO), a higher positivity rate was observed for IgA antibodies (92.7 percent) compared with IgM antibodies (85.4 percent),[6] indicating IgA antibodies could serve as a marker for acute infection. In another study, IgA antibodies were detected earlier than IgM antibodies, and the IgA values peaked at 20-22 days with persistently higher levels compared with IgM antibodies throughout the observation period (45 days PSO).

In contrast, IgM levels peaked at 10-12 days and declined after 18 days.[27] Jin et al. 2019 showed as the interval from RT-PCR confirmation to serological detection increased, the IgM positivity rate (75 percent) increased initially but subsequently declined (<50 percent). IgG positivity increased to 100 percent and remained stable thereafter. IgG levels were consistently higher than IgM levels,[8] suggesting IgG antibodies persist longer in the body, and could contribute to long-term immune memory against SARS-CoV-2.[28]

Does the presence of antibodies indicate if someone is immune against SARS-CoV-2 reinfection?

The presence of antibodies alone does not indicate that the person is immune against potential reinfection. It is important to determine whether the antibodies can confer protective immunity long-term, providing virus-neutralizing antibodies that block viral infection and help in clearing viral infection.[14,28] Therefore, neutralizing antibodies

play an important role in resolving viral infection. Plaque reduction neutralization tests (PRNT) *in vitro* are considered the gold standard for determining the ability of antibodies to neutralize virus and prevent viral replication.[28] Neutralizing antibodies primarily target S protein in coronaviruses, in particular the S1 subunit and the RBD contained within the S1 domain, preventing viral entry into the host cell.[29]

Studies from 2002 SARS-CoV-1 and 2012 MERS-CoV have reported neutralizing antibodies target the S protein, predominantly S1 or the RBD, and to a lesser extent S2,[30] and similar observations are emerging in SARS-CoV-2. Neutralizing antibody responses against SARS-CoV-1 begin to develop by week two, and most patients develop neutralizing antibodies by week three.[14] Okba et al. 2020 compared the performance of different ELISAs with PRNT assays to determine their ability to detect neutralizing antibodies to SARS-CoV-2. They found good correlation between antibody results in different ELISAs and the neutralizing test.

The strongest correlation was observed with the EUROIMMUN IgA ELISA for infection reduction rates of >90 percent ($PRNT_{90}$), suggesting the ability of EUROIMMUN ELISAs to detect SARS-CoV-2-specific antibodies.[2] However, it is currently unknown whether the presence of antibodies to SARS-CoV-2 is sufficient to confer protective-immunity *in vivo* in infected individuals. SARS-CoV-2 is a novel pathogen, and there is no information regarding the duration or long-term effectiveness of these antibodies in recovered patients. SARS-CoV-1 specific antibodies became undetectable in 91 percent of patients after six years of infection,[31] indicating longitudinal studies are required to further understand the potential role of SARS-CoV-2 specific antibodies in patients.

Conclusion

Detection of antibodies against SARS-CoV-2 virus complements viral testing. Antibody detection in combination with RT-PCR expands the detection window of SARS-CoV-2 infection and minimizes false-negative RT-PCR testing. It would provide valuable information to public health authorities making decisions to relax physical distancing measures and reopen businesses across the country. Antibody testing will help determine the extent of SARS-CoV-2 spread in communities. However, there are still questions that need to be answered such as how long would the antibodies last and if they are truly protective?[28]

It is important to understand the antibody kinetics in SARS-CoV-2 patients. Studies have reported both IgA and IgM antibodies can be detected earlier than IgG antibodies.[20,32] Additionally, IgA and IgM levels peak early post infection, but IgA is more robust and longer lasting. IgG levels peak at 21-25 days PSO.[20] However, further studies are required to understand the long-term kinetics of these antibodies in infected individuals. Additionally, whether these antibodies can provide protection from reinfection and what antibody titers are required for protective immunity are yet to

be determined. Furthermore, scientists are working on developing an effective vaccine, but the timeframe for its availability is unclear.

With many states in the U.S. looking to re-open businesses, it is important to be vigilant and continue practicing social distancing and good hygiene practices until the role of antibodies against the SARS-CoV-2 virus is better understood.

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Uncommon PCR cycling and reaction parameters – what and when

By John Brunstein, PhD

By now, the polymerase chain reaction (PCR) is no longer the new big thing in molecular biology and diagnostics; it's not only taken for granted as the underpinning method behind a vast number of clinical tests, it's the sort of thing taught in high school biology. Few, if any, readers of this column won't have at least a basic appreciation for the process (similar to that outlined in this space in the May 2013 edition, https://www.mlo-online.com/home/article/13005202/pcr-the-basics-of-the-polymerase-chain-reaction). Two primers, some buffer, and thermocycling including denaturation, annealing, and extension steps and you're good to go.

In a lot of assays, that's really about all there is to it, too. Aspects like making the PCR into a real-time assay involve complications with adding means for ongoing product detection, but those don't change the mechanics of how the product is formed. In this month's episode of The Primer, we're going to peek under the hood at some of the alterations that can be done to the underlying core process, how these impact product formation, and why or where you might find these applied.

Touchdown PCR

Specificity in a PCR reaction relates mechanistically to ensuring that the forward and reverse primers anneal productively at their intended priming sites, and not at a bunch of other unwanted loci. Part of the key to this is the "uniqueness" of each primer sequence, or how many near-match binding sites there exist in a template population as compared to intended annealing site(s). Ideally, one would design primers that have absolutely no significant similarity to template sequence elements other than the intended site.

In reality, unavoidable constraints on amplicon design based on minimum and maximum product sizes for efficient amplification, limited areas of sufficient genetic conservation pressure to serve as reliable primer sites, target genome size and GC content, and other factors can sometime leave an assay stuck with primers that have more than passing homology to unwanted secondary binding sites. While these will be thermodynamically unfavored compared to the perfect match, annealing occurs along a Boltzmann distribution curve: put more simply, at least some annealing will occur at less-than-perfect binding sites; and if your primers are less than ideal from a "uniqueness" standpoint, this can be a significant proportion. What's an assay to do in this situation?

One way to enhance specificity in this scenario is by what is called Touchdown PCR. In this, rather than use the predicted optimal primer-annealing temperature, one starts off the initial PCR cycles with annealing step temperatures well above that; often, as much as 10°C over predicted Tm. Over the next 10 to 20 cycles, the annealing temperature is decreased by ~0.5° - 1.0°C per cycle. What happens – thinking back to our Boltzmann curve – is that at temperatures well above ideal annealing, only a very small fraction of primers will have low enough thermal energy to bind, and such binding will be biased towards the perfect match sites. Thus, a single copy target gets preferentially (but very weakly) amplified in the first cycle, slightly less preferentially (but a bit more strongly) amplified in the second cycle, and so on until "optimal" Tm is reached. By this point, ideally, the number of perfect match priming sites from early cycle amplicons helps compete on a number of copies basis against the un- (or at least, less-) amplified spurious sites.

On this now selectively enriched template, a further 20-30 cycles or so of PCR at more usual optimal Tm, with no further decreases per cycle in annealing temperature, proceeds to drive the bulk of the amplification. At the cost of nothing more than some extra thermal cycles (minutes of time), this technique can help maximize specificity for non-ideal primers.

Asymmetric PCR

Traditional PCR makes the two strands of product amplicon in equal numbers. If you're going to detect product by gel or capillary electrophoresis, or by a double-stranded DNA (dsDNA) selective fluorescent dye, that's good. If, however, you want to use a hybridization-based product detection – including either product hybridization to fixed capture oligos (2D or liquid phase arrays), or fluorescently labeled probes using fluorescence resonant energy transfer (FRET) in its various guises – it's less ideal.

That's because the capture or hybridization probe oligo has to compete for product binding with the amplicon's complementary strand, and there are losses in detection efficiency arising from amplicon strand reannealing. If your detection method hinges on capturing just one strand of the PCR product, asymmetric PCR may be the solution you're looking for. In its simplest form, it consists of just limiting the amount of one primer (the one complementary to the desired detection strand) relative to the reverse (detected strand) primer.

Traditional cycling conditions are employed, and in early cycles where numbers of both primers available vastly outnumber template molecules, nominal two-fold amplification per cycle proceeds as you'd expect. As amplification proceeds and numbers of amplicons exponentially increase, the less-abundant primer becomes scarce by comparison to the detected strand primer and an increasing number of single-stranded products are formed where only the detected strand primer is available to successfully anneal and extend.

Denaturation allows the non-detected amplicon strand to then be available as template again. A key result of this – other than creating an excess of single-strand product – is that once the limited primer becomes scarce, amplification ceases to be exponential and becomes a much slower, linear process. Careful balancing of the ratio and total amounts of the two primers, and a relatively narrow optimal window for starting template concentration, are needed to ensure that sufficient levels of amplification can occur before this gradual shift to single detected target strand production takes over. Done properly, the result can be increased hybridization-based detection efficiency over classical "symmetric" methods.

Variations on this exist, most notably something referred to as LATE-PCR ("Linear After The Exponential PCR").

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Essentially, this alters the designed Tm of the limiting primer to take into account the thermodynamic reality that primer concentration impacts observed Tm – that is, the limiting primer behaves as if its Tm were lower than it would at concentrations equalling that of the opposing primer. This interferes with and reduces the real-life efficiency of the simple model described above. By intentionally raising the designed Tm of the limiting primer, this effect can be offset. For those interested in more on the topic, the original concept can be found in reference[1].

Nested PCR

Receiver operating characteristic (ROC) curves teach us that assay conditions are a trade-off between sensitivity and specificity. If increasing sensitivity in a PCR reaction is your goal, there is one method that can do so dramatically but simultaneously act to retain specificity – at a cost. Known as nested PCR, in essence, the process consists of a first "normal" PCR reaction (most commonly at a somewhat reduced number of cycles, 20 to 30), and then use of a small portion of this product as template for a second PCR in which the primer sets are designed to anneal to positions inside – "nested" – the first amplicon.

This allows for astronomical levels of amplification, but by forcing the second amplification to be specific to only correct products of the first amplification (that is, bearing the internal second priming sites), specificity is, in theory, retained. Such an approach can be useful when absolute limits of sensitivity are required, and/ or when samples are expected to contain inhibitors. In this second scenario, while only limited amplification may occur in the first stage, the effective dilution of template-borne inhibitors by taking a small sample onward gives the second stage a cleaner environment to work in, plus at least some amplicons to work from. Sounds great, so what's the cost?

Aside from small increased labor and reagents and consumables, the really significant cost is an operational one, which should be sounding alarms in every clinical laboratorian's mind – contamination! The process described above requires opening a reaction tube post-amplification and liquid handling amplicon containing products. This really should not be considered acceptable practice; the avoidance of this was the impetus behind development of real-time methods capable of detecting product without opening the reaction vessel, and a strong early driver of their adoption. There is, however, a way to do a form of nested PCR without opening the reaction vessel – that is to design the second (inner) primer set with a much lower annealing temperature (i.e. shorter primers and /or lower GC content than outer primers), and put both primer sets in the reaction at time of setup. A first set of thermocycling can be performed with an annealing Tm such that only the outer primer set significantly functions, with the inner set left in solution. After a number of such cycles, the annealing temperature is dropped to match the inner primers. While the outer primers will, of course, continue to anneal (and now, likely, mis-anneal at incomplete matches) under these conditions, two factors help to supress spurious products and favor the intended nested product.

First, some amplification has occurred during the first stage, so there is a relatively large number of first-stage amplicons compared to original bulk template, providing a stochastic advantage to the proper priming. Secondly, if the nested product is significantly shorter than the first-stage product, the extension time during the second stage can be shortened to give the nested product a kinetic advantage over the longer first amplicon. Challenges to this are in finding a suitable target sequence with inner and outer priming sites meeting all of these requirements. While this method avoids the calamity of having to open the product tube, it does not gain the dilution of inhibitor possible with the first method. For all of this, it can still give some boost to lower limit of detection with marginal or no loss of specificity.

Conclusion

There are far more variations on classical PCR conditions – both in reaction composition and thermocycling parameters – than can be covered in so short a space, but the three discussed here are among the ones the reader is most likely to encounter in common lab assays and may go some way to answering "why does this use a strange-looking thermal cycling profile?"

Not all PCRs are created equal, and often that's because someone is using clever tools to maximize reaction behavior against a particular task and detection method.

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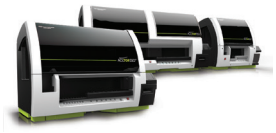

ACL TOP®Family 50 Series Hemostasis Testing systems offer the most advanced automation, quality management, and routine to specialty assays for mid- to high-volume clinical laboratories, including those with lab automation tracks.

Instrumentation Laboratory

SARS-CoV-2 Serology & Molecular Proficiency Testing

WSLH Proficiency Testing now offers proficiency testing for SARS-CoV-2 Serology and Molecular. As part of a national public health lab, WSLH PT provides reliable, affordable and easy-to use proficiency testing To enroll, visit: wslhpt.org.

WSLH Proficiency Testing

KL-6 Assay for Chemistry Analyzers

KAMIYA BIOMEDICAL is introducing a new assay for the quantitative measurement of Krebs von den Lungen-6 (KL-6) in serum or plasma samples on chemistry analyzers. The assay uses a specific monoclonal antibody to KL-6. Applications are available for most chemistry analyzers. For research use only in the U.S.

Kamiya Biomedical

SARS-CoV-2 Standard for COVID-19 research testing

The EDX SARS-CoV-2 Standard is intended for use as reference material for research testing of the novel coronavirus, allowing laboratories to validate assays. Additionally, the SARS-CoV-2 Standard can be validated for use by the laboratory as an external run control.

Bio-Rad Laboratories

Affordable Safe Solutions to Decap / Recap

Repetitive manual Decapping and/or Recapping of tubes exposes your staff to potential repetitive stress injuries. We offer a variety of models to fit any volume needs. Our Pluggo Decappers and KapSafe Recappers will eliminate potential injuries.

LGP Consulting

Powerful and Innovative

The LIAISON® MDX is an innovative and powerful thermocycler. Supported by an expanding menu of molecular assays, it is flexible allowing you to run real-time PCR for qualitative, quantitative and multi-analyte detection. The instrument can perform both IVD and Laboratory Developed Tests.

DiaSorin Molecular

KRONUS® 21-Hydroxylase Autoantibody (21-OHAb) ELISA

The KRONUS® 21-Hydroxylase Autoantibody ELISA Kit is for the qualitative determination of antibodies to steroid 21-OH in human serum, the measurement of which is useful as an aid in the diagnosis of autoimmune adrenal disease. *Manufactured Under Assigned Patents and Licenses*

KRONUS

Panther Fusion GBS assay

This assay detects the gram-positive bacterium Streptococcus agalactiae (GBS) in pregnant women and so providers can prevent its transmission to their babies. This assay features world-class performance and sensitivity, answering a crucial need for GBS detection.

Panther Fusion

Quantimetrix® Dipper POCT® Urinalysis Dipstick Control

Dipper POCT is a single-use liquid control delivering exceptional performance & efficiency.

- 3 months RT and 3 years refrigerated stability
- Patented design allows for visual verification of full dipstick immersion
- Minimized contamination risk, maximized convenience.

Quantimetrix

GEM®PremierTM 5000 System

GEM® Premier™ 5000 blood gas system with iQM®2 assures quality before, during and after every sample in lab and POC testing—for improved patient care. All-in-one, multi-use cartridge offers advanced simplicity.

Instrumentation Laboratory

ALCOR Scientific Proudly Presents the miniiSED®

The miniiSED® is a single position, fully automated ESR analyzer that works directly from the primary EDTA tube and produces an ESR result in 15 seconds. The miniiSED® is half the size of the iSED® making it the ideal ESR analyzer for small laboratories, POL's and emergency clinics.

ALCOR Scientific

CAPILLARYS 3 TERA

Sebia's CAPILLARYS 3 TERA provides high resolution capillary separation for HbA1c, Serum Proteins, Serum Immunofixation by capillary (Immunotyping), and Hemoglobins. The fully automated CAPILLARYS 3 TERA offers proven technology, enhanced throughput, increased walk-away capabilities and more flexibility for large volume laboratories.

Sebia, Inc.

Coagulation Analyzers

The ACL TOP Family 50 Series systems offer advanced automation and quality management for routine to specialty assays. The analyzers are designed for clinical labs and are standardized for superior performance across the testing process. Features include automated pre-analytical sample integrity checks, advanced quality and accreditation support, and enhanced security assures quality results and efficiency.

Instrumentation Laboratory

Coagulation Analyzers

The Chrono-log Model 490 4+4 Optical Aggregation Systems provide the ability to test platelet function in up to eight (8) samples simultaneously, with a throughput of approximately 80 tests per hour. HYPO and HYPER aggregability can be detected, especially important with COVID 19 patients, who may require individualized antiplatelet therapy based on risk of bleeding.

Chrono-log Corp.

Hemostasis System

The Sysmex CS-5100 hemostasis system is a fully automated coagulation system for high-volume labs. Preanalytical Sample Integrity (PSI) technology checks primary tube sample-volumes to identify and automatically manage problematic test samples prior to analysis, minimizing repeat testing.

Siemens Healthineers

Critical Care Blood Gas Analyzer

The Stat Profile Prime Plus is a comprehensive, whole blood critical care analyzer with 20 measured tests and 32 calculated results in a simple, compact, maintenance-free device. The test menu includes blood gases, electrolytes, metabolites, and co-oximetry.

Nova Biomedical

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LABORATORY INNOVATOR

CEO uses scientific data to drive better outcomes in the lab of the future

John Heiser is the CEO of **LabVantage Solutions** and oversees the informatics company's global strategy, innovative culture, and financial management. He joined LabVantage with a diverse business career in technology and life sciences, including a PhD in values-driven leadership.

Since joining LabVantage Solutions two years ago as CEO, how has your previous experience in technology and informatics benefited you in your current role?

I last led a company that developed and manufactured sophisticated process control solutions and saw how analytical insights could improve efficiency and processes to increase uptime. Turning data into knowledge ultimately leads to better outcomes, both for the firm and its end users. That is LabVantage's purpose.

We are applying the thinking from our collective experience to merge technology, informatics, and analytics to achieve the goals of greater productivity and efficiency, which leads to better outcomes, no matter the industry. Transforming data into workable knowledge ultimately leads to transformative results.

In April, a new "purpose-built biobanking lab information management systems (LIMS) accelerator for managing COVID-19 testing" was announced. Can you elaborate on its clinical benefits?

The benefits are twofold. Widespread laboratory testing is a critical component in the battle to control the coronavirus pandemic sweeping the globe. First, we mobilized our decades of expertise to rapidly deliver a new purpose-built biobanking LIMS to support COVID-19 testing and research. It incorporates our knowledge of biobanking along with numerous features designed to make it easy for laboratories to acquire, implement, and use our LIMS for their COVID-19 testing. The COVID-19 LIMS accelerator is also scalable, an important attribute as testing volumes escalate.

Second, our COVID-19 LIMS can be used to track the biospecimens and results that are helping people return to work safely, which is another critical, emerging task. We built a biobanking-specific LIMS, which is well-suited for the vast tracking required of all these samples. We are working with some major employers to ramp up testing capabilities, so they can bring their workforces safely back into manufacturing plants, warehouses, office buildings, and the like.

What has been the single, most important goal – LIMS or otherwise – for LabVantage Solutions related to the COVID-19 pandemic?

First and foremost, the health and safety of our employees worldwide, and those of our customers. True to our purpose, we are here to improve outcomes. Our solutions are contributing to collecting and analyzing data from COVID patients, which then yield insights that identify opportunities for treatment and spot important trends.

Simply stated, our focus on transforming data into workable knowledge helps our clients and their end users flourish.

How can current lab informatics options potentially impact future LIMS and Software-as-a-Service (SaaS) implementations and help streamline the workflows of clinical lab professionals?

When it comes to lab professionals, we are most interested in how they will use the system and what it needs to do for them. SaaS is important for the team choosing how to implement the LIMS in that it offers rapid deployment, ease of maintenance and upgrades, and reduces upfront capital expenses.

But to streamline the workflows, our approach has been to leverage four decades of knowledge, acquired through significant numbers of installations, and preconfigure the LIMS in an industry-specific way to meet the needs of users.

You can see this in our COVID-19 LIMS accelerator, which handles biobanking, testing, and reporting requirements of COVID-19 biospecimens. Similarly, we have a laboratory information system (LIS) that is built for medical testing laboratories in hospitals and other healthcare settings. It is designed with the industry-specific functionality that makes the workflows and processes in these lab settings easier, faster, better, more effective, and more efficient.

In addition, we are developing functionality specific to streamlining lab work. For example, we introduced a module for assigning work and resource planning that is essential for lab productivity; it has been a tremendous aid in assigning not only workers but maximizing instrument use, and we're seeing that is quite important as all this COVID-19 testing increases.

Where do you see the company's direction going over the next five to 10 years with an eye towards improving the accessibility of data and technology?

Our purpose, of turning scientific data into knowledge that drives better outcomes, is lasting. So, our direction will be to serve the lab of the future – however it looks. And we will do that by continuing to make sure our software supports labs in terms of driving efficiencies, productivity, and outcomes.

Analytics will play a big part of that, and we will continue to develop products that fit our purpose. This will continue to drive us, and it should be consistent with where the lab of the future is going because we know it's not just about collecting and processing data, but what you do with data that is transformative.

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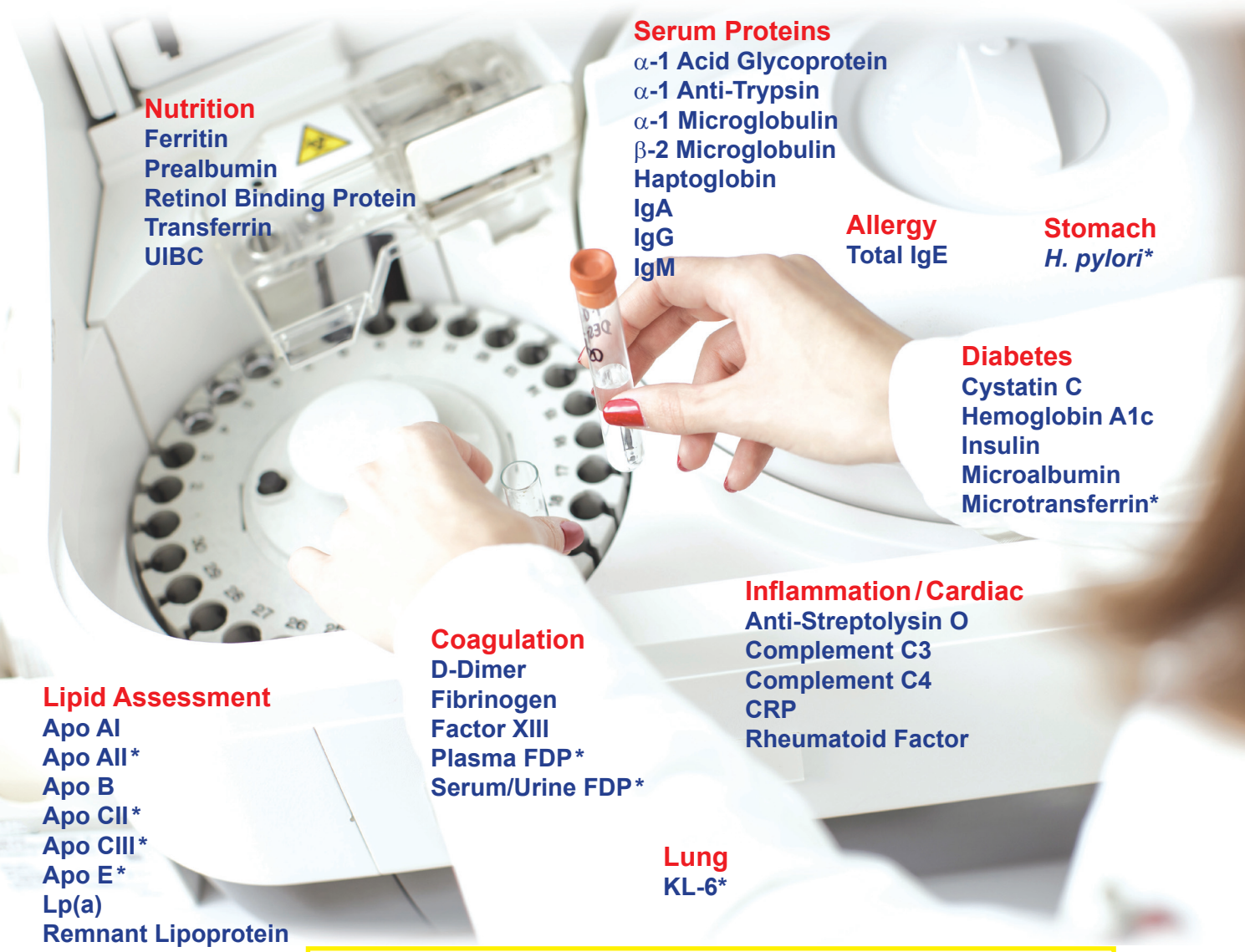

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α-1 Microglobulin
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New Products Now Available!!

- β-2 Microglobulin reagent for chemistry analyzers
- *H. pylori* Test Reagent* for chemistry analyzers
- KL-6 (Krebs von den Lungen-6)* reagent for chemistry analyzers
- Microtransferrin* reagent for chemistry analyzers
- Remnant Lipoprotein Cholesterol* reagent for chemistry analyzers
- Retinol Binding Protein reagent for chemistry analyzers
- UIBC (Unsaturated Iron Binding Capacity) for chemistry analyzers

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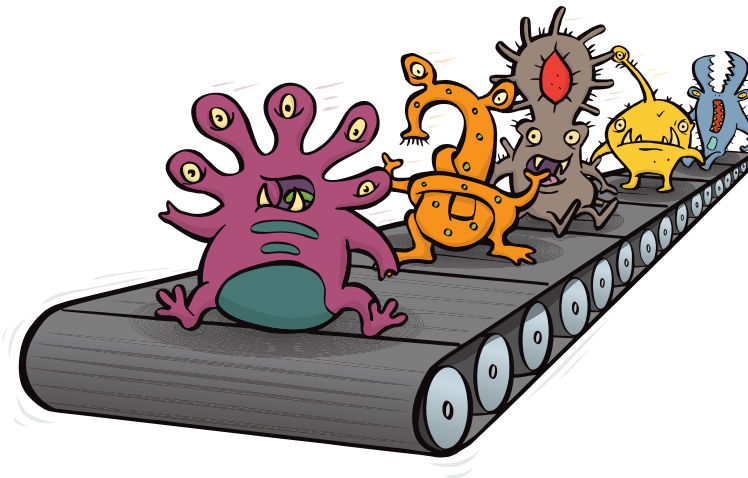

Better testing. Better workflows.

Improve laboratory workflows with infectious disease testing from the BioFire® FilmArray® Gastrointestinal (GI) Panel.

When using traditional methods at the stool bench, it can feel like the laboratory workflow comes to a screeching halt. Traditional testing is slow, labor intensive, and insensitive—often leaving clinicians waiting on results.[1] Fortunately, the BioFire GI Panel tests for 22 of the most common GI pathogens, all in about one hour. With fast, easy, accurate, and comprehensive testing, get things moving again with the BioFire GI Panel.

The BioFire FilmArray Gastrointestinal Panel

Fast, Easy, and Comprehensive. With only 2 minutes of hands-on time by any tech, on any shift, the BioFire GI Panel provides rapid results, all in about 1 hour.

Identify What Traditional Testing is Missing. Studies demonstrated the BioFire GI Panel detected 25%–36% more potential pathogens compared to traditional stool diagnostics.[2-5]

Better Patient Care. Patients tested with the BioFire GI Panel had an 84% reduction in time-to-result, were 11% less likely to be prescribed antibiotics, and received 17% more targeted therapy compared to patients tested with traditional methods.[2-4]

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Syndromic Testing: The Right Test, The First Time.

1. Riddle MS, et al. ACG clinical guideline: diagnosis, treatment, and prevention of acute diarrheal infections in adults. Am J Gastroenterol. 2016 April. 11(5):602. **2.** Beal S, et al. A gastrointestinal PCR panel improves clinical management and lowers healthcare costs. J Clin Microbiol. 2018 Jan. 56:1 e01457-17. **3.** Cybulski R, et al. Clinical impact of a multiplex gastrointestinal PCR panel in patients with acute gastroenteritis. Clin Infect Dis. 2018 Nov. 13; 67 (11):1688-1696. **4.** Axelrad J, et al. Impact of gastrointestinal panel implementation on health care utilization and outcomes. J Clin Microbiol. 2019 Feb. 57(3) e01775-18. **5.** Spina A, et al. Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicenter study of community-acquired gastroenteritis. Clin Microbiol Infect. 2015 Aug. 21(8):719-28.

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