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COVID-19 rapid testing

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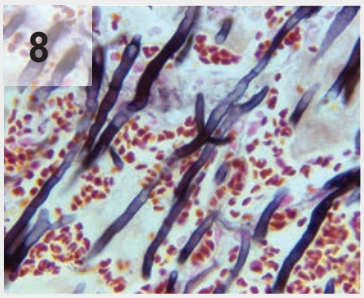

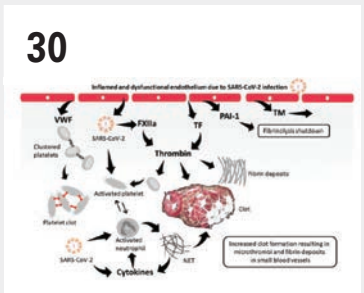

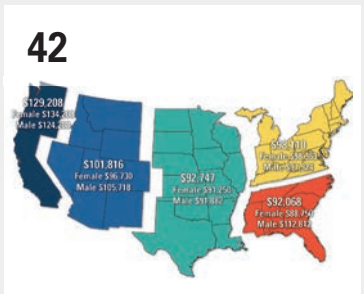

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Achieving the promise of IT interoperability

By Linda Wilson
Senior Editor

If ever there was a time when interoperable information systems were critical, the time is now during the COVID-19 pandemic.

Think about how various professionals in the healthcare system require timely access to critical pieces of information to both care for patients and monitor SARS-CoV-2's activity in local communities and the world.

For example, testing respiratory samples for the virus involves information sharing. Laboratories need to know the demographic information for the patient associated with a specimen. If they are not going to test the sample themselves, they need to share the patient's data with a reference lab. Once testing is complete, labs need to provide results to the patient and healthcare provider, report testing data to public health authorities, and bill an insurance company or government program.

Vaccinating patients is another example. Providers need to know the demographic and insurance information of the patients they vaccinate. They also need to know if patients have had a first dose of the vaccine, and if so, which one they have had. It also is important for providers to know if patients have had a reaction to a vaccine in the past or if their immune system is compromised.

Meanwhile, hospitals and emergency rooms need access to COVID-19 patients' medical histories, drug allergies, current medications, and demographic information, including socioeconomic variables.

But the flow of information does not always happen as smoothly as it could. Phone calls, faxes and paper forms are often part of the process.

This was not the vision policymakers had in mind when they appropriated taxpayer money to fund the installation of electronic health records (EHRs) at hospitals and doctor's offices throughout the country. This has happened through the Meaningful Use program, which is now called Promoting Interoperability. As of 2018, the Centers for Medicare & Medicaid Services (CMS) had paid more than $38 billion in incentive payments to reward hospitals and physician's offices that logged measurable goals related to using and sharing electronic information to provide high-quality, cost-effective patient care.

Signed into law in 2016, the 21st Century Cures Act also included provisions to improve data sharing, based on the lessons learned from implementing the earlier regulations. CMS and the Office of the National Coordinator for Health Information Technology (OIG) released final regulations in 2020 to implement these provisions; although both governmental bodies extended deadlines because of the pandemic.

One aim of the regulations is to prohibit information blocking, which involves actions that impede access to patient information. A common example of this is when an information technology vendor with proprietary software charges an excessive fee to build an interface, linking its system to another system.

Labs managers are all too familiar with this phenomenon. I am sure most of them have felt frustrated at some point trying to move patient data from their laboratory information system (LIS) to an EHR at a hospital or doctor's office.

As a result of the COVID-19 pandemic, we can see how important seamless information exchange is to the goals of providing high-quality patient care and managing public health crises. Hopefully, the regulations from the Cures Act will help propel the healthcare industry toward true interoperability.

On a separate note, Marisa Williams is *Medical Laboratory Observer*'s new Managing Editor. Earning her Master's in Writing at Johns Hopkins University and Bachelor of Science at the University of Toledo, emphasizing Forensics, she is the author of more than 100 independent books.

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

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Group Publisher/Executive Editor
Kristine Russell
krussell@mlo-online.com
Senior Editor
Linda Wilson
lwilson@mlo-online.com
Managing Editor
Marisa Williams
mwilliams@mlo-online.com
Guest Feature Editor
Kara Nadeau
Graphic Artist
Patti Connors
pconnors@endeavorb2b.com
Audience Development/List Rentals
Laura Moulton
lmoulton@endeavorb2b.com
Ad Traffic Manager
Tiffany Coffman
tcoffman@endeavorb2b.com
eProduct Coordinator
Mary Haberstroh
mhaberstroh@endeavorb2b.com

ADVERTISING
East Coast/Midwest Sales (except IL)
Classified/Recruitment Advertising
Carol Vovcsko
(941) 321-2873
cvovcsko@mlo-online.com
South/West Coast/Illinois Sales
Lora Harrell
(941) 328-3707
lharrell@mlo-online.com

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Fast Facts

Hospital COVID-19 Transfusions

The 2021 AABB COVID-19 Hospital Transfusion Services Survey gathered information on hospitals' use of COVID-19 convalescent plasma (CCP) to treat patients.

53%
of hospitals had transfusions of more than one CCP unit per patient.

28.7%
of hospitals have experienced delays in obtaining CCP units.

83%
of hospitals have enough CCP units to give transfusions to patients who meet the selection criteria.

14.6%
of hospitals frequently give CCP transfusions to ventilated patients.

38.6%
of hospitals infrequently give CCP transfusions to ventilated patients.

58%
of hospitals do not consider the timing of patients' diagnosis or admission in CCP exclusion criteria.

54%
of hospitals transfuse more than one CCP unit per patient.

88%
reported transfusing 0-24 CCP units in the last 7 days.

Source: https://www.aabb.org/docs/default-source/default-document-library/resources/2021-aabb-covid-19-hospital-transfusion-services-survey.pdf?sfvrsn=c2bd9f31_0

T cells mount attacks against many SARS-CoV-2 targets

La Jolla Institute for Immunology (LJI) suggested that T cells try to fight SARS-CoV-2 by targeting a broad range of sites on the virus – beyond the key sites on the virus's spike protein. By attacking the virus from many angles, the body has the tools to potentially recognize different SARS-CoV-2 variants, according to a news release from the institute.

The new research, published in *Cell Report Medicine*, is the most detailed analysis so far of which proteins on SARS-CoV-2 stimulate the strongest responses from the immune system's "helper" CD4+ T cells and "killer" CD8+ T cells, according to LJI.

The immune system is very flexible. By re-scrambling genetic material, it can make T cells that respond to a huge range of targets, or epitopes, on a pathogen. Some T cell responses will be stronger against some epitopes than others. Researchers call the targets that prompt a strong immune cells response "immunodominant."

For the new study, the researchers examined T cells from 100 people who had recovered from SARS-CoV-2 infection. They then took a close look at the genetic sequence of the virus to separate the potential epitopes from the epitopes that these T cells would actually recognize.

Their analysis revealed that not all parts of the virus induce the same strong immune response in everyone. In fact, T cells can recognize dozens of epitopes on SARS-CoV-2, and these immunodominant sites also change from person to person. On average, each study participant had the ability to recognize about 17 CD8+ T cells epitopes and 19 CD4+ T cell epitopes.

The new study shows that while the immune system often mounts a strong response against a particular site on the virus's "spike" protein called the receptor binding domain, this region is actually not as good at inducing a strong response from CD4+ helper T cells.

Without a strong CD4+ T cell response, however, people may be slow to mount the kind of neutralizing immune response that quickly wipes out the virus.

Among the many epitopes they uncovered, the researchers identified several additional epitopes on the SARS-CoV-2 spike protein.

Researchers from the University of California, San Diego, Australia's Murdoch University collaborated on the project.

Mount Sinai identifies three molecular subtypes of Alzheimer's disease

Researchers at the Icahn School of Medicine at Mount Sinai have identified three major molecular subtypes of Alzheimer's disease (AD) using data from RNA sequencing and published their findings in *Science Advances*, according to a press release from the medical school.

RNA is a genetic molecule similar to DNA that encodes the instructions for making proteins, while RNA sequencing is a technology that reveals the presence and quantity of RNA in a biological sample, such as a brain slice.

There is growing evidence that disease progression and responses to interventions differ significantly among Alzheimer's patients. Some patients have slow cognitive decline, while others decline rapidly; some have significant memory loss and an inability to remember new information, while others do not; and some patients experience psychosis and/or depression associated with AD, while others do not.

The research team analyzed RNA-sequencing data of more than 1,500 samples across five brain regions from hundreds of deceased patients with AD and normal controls, and identified three major molecular subtypes of AD, independent of age and disease stage.

These subtypes correspond to different combinations of multiple dysregulated biological pathways leading to brain degeneration. Tau neurofibrillary tangle and amyloid-beta plaque, two neuropathological hallmarks of AD, are significantly increased only in certain subtypes.

Many recent studies have shown that an elevated immune response may help cause Alzheimer's. However, more than half of AD brains do not show increased immune response compared to normal healthy brains. The analysis further revealed subtype-specific molecular drivers in AD progression. The research also identified the correspondence between these molecular subtypes and the existing AD animal models used for mechanistic studies and for testing candidate therapeutics.

Although the subtyping described by the researchers was performed postmortem using the patients' brain tissue, the researchers said that if the findings were validated by future studies, they could lead to the identification in living patients of biomarkers and clinical features associated with these molecular subtypes and earlier diagnosis and intervention.

Common HIV drugs may help prevent leading cause of vision loss

Scientists have identified a group of drugs that may help stop a leading cause of vision loss after making an unexpected discovery that overturns a fundamental belief about DNA, according to a University of Virginia news release.

The drugs, known as nucleoside reverse transcriptase inhibitors, or NRTIs, are commonly used to treat HIV. The new discovery suggests that they may be useful against dry macular degeneration as well, even though a virus does not cause that sight-stealing condition.

A review of four different health insurance databases suggests that people taking these drugs have a significantly reduced risk of developing dry macular degeneration.

The new discovery comes from researchers at UVA, the Salk Institute for Biological Studies, and collaborators around the world. The work rewrites scientists' understanding of DNA, revealing that it can be manufactured in the cytoplasm of our cells, outside the cell nucleus that is home to our genetic material.

The buildup of a certain type of DNA in the cytoplasm, Alu, contributes to macular degeneration, the researchers found. This buildup appears to kill off an important layer of cells that nourishes the retina's visual cells.

Based on this discovery, the researchers decided to look at drugs that block the production of this DNA, to see if they might help prevent vision loss. They found that people taking NRTIs were almost 40 percent less likely to develop dry macular degeneration.

Gestational diabetes can be tip-off to future increased risk for heart disease

Women with a history of gestational diabetes are at increased risk for heart artery calcification, a marker of increased risk for heart disease, throughout their childbearing years and into mid-life, even if they currently have normal blood sugar levels, a newly released study from Kaiser Permanente Division of Research shows.

The study, published in *Circulation*, is the first to look at heart disease risk in relation to changes in blood sugar levels in women who developed gestational diabetes and those who did not over a 25-year period, Kaiser Permanente Division of Research said.

The study found a 2-fold higher risk for heart artery calcification in women who had gestational diabetes and normal blood sugar, compared with women who did not have gestational diabetes and had normal blood sugar levels. The 2-fold higher risk was present in women with a history of gestational diabetes who many years later had blood sugar levels classified as normal, pre-diabetes, or type 2 diabetes.

Erica P. Gunderson, PhD, MS, MPH, Senior Research Scientist at the Kaiser Permanente Northern California Division of Research, led the study.

Yearly, in the United States, about 250,000 pregnancies develop diabetes.

Because gestational diabetes greatly increases a woman's lifelong risk for type 2 diabetes, the American Diabetes Association recommends that all women who develop gestational diabetes have a glucose tolerance test every 1 to 3 years. American Heart Association guidelines underscore that a history of gestational diabetes is important to consider in evaluating individual risk for atherosclerotic heart disease.

The new findings suggest physicians should monitor factors for heart disease in all women who develop gestational diabetes, before they develop prediabetes or diabetes. Women with a history of gestational diabetes may benefit from healthy lifestyle choices and weight loss.

Subset of COVID-19 patients have increased bleeding risk

Research has found that patients with COVID-19 are prone to serious blood clotting. However, a new study from Michigan Medicine found that aside from this heightened clotting risk, some COVID-19 patients have an unbalanced ability to break down clots as well, which is linked to a potential clinical biomarker seen in later stages of the disease, according to a news release.

The study was published in *Scientific Reports* and led by senior author Daniel Lawrence, PhD, Professor of Basic Research in Cardiovascular Medicine at Michigan Medicine.

The abnormal process of breaking down clots can contribute to a high bleeding risk, raising concerns about the current practice of giving COVID-19 patients high-dose anticoagulants throughout the duration of their disease course.

The study from Michigan Medicine included 118 COVID-19 patients and 30 healthy controls. In the COVID-19 patients, the team expected to see high levels of plasminogen activator-inhibitor-1, a molecule associated with stabilizing blood clots. However, they did not expect high levels of tissue-type plasminogen activator, the molecule responsible for removing the clots.

Almost half of the study's patients were supported by a ventilator and a quarter breathed just room air. Compared with the patients breathing room air, patients that required supplemental oxygen had significantly higher levels of plasminogen activator-inhibitor-1, but not of tissue-type plasminogen activator.

High levels of both tissue-type plasminogen activator (tPA) and plasminogen activator-inhibitor-1 (PAI-1) were associated with worse lung function, but high tPA was independently correlated with mortality. The levels of either molecule can increase independently of one another, but the research found a change in one can have consequences on the other.

The team asked whether COVID-19 plasma with the highest tPA levels might correlate with an enhanced, spontaneous breaking down of clots, as compared with low tPA COVID-19 plasma or control plasma.

After assessing 10 COVID-19 plasma samples with high tPA, 10 COVID-19 samples with low tPA, and 10 healthy control plasma samples, it was clear the high-tPA COVID-19 samples were found to significantly enhance spontaneous clot breakdown compared to the other two groups. The researchers said this means that high tPA may be a biomarker for high bleeding risk and poorer outcomes in COVID-19.

The researchers suspect the source of these high levels of tPA in COVID-19 patients, and the subsequent clotting issues, is because of damage to endothelial cells, which are cells that line blood vessels. If badly damaged, the blood vessels can actually break and cause bleeding.

The theory is that a hallmark symptom of COVID-19 ARDS, when fluid builds up in the lungs and causes trouble breathing and low oxygen levels in the blood, may trigger endothelial cell activation, which consequently promotes the release of tPA.

Review of laboratory diagnostic tests for invasive aspergillosis

By John Z. Deng, BS; Diego H. Caceres, BSc, MSc; Jeffrey D. Klausner, MD, MPH


Courtesy of the CDC

Aspergillus species, which are common environmental fungi, are known to cause aspergillosis, an invasive infection commonly observed in people with a weakened immune system. The most common *Aspergillus* species causing human disease are *A. fumigatus, A. flavus, A. terreus,* and *A. niger,* which can cause a wide range of clinical syndromes, such as allergic bronchopulmonary aspergillosis, aspergilloma, chronic pulmonary aspergillosis, and invasive aspergillosis. *Aspergillus* is widespread in the environment and can colonize the airway, making diagnoses of invasive pulmonary aspergillosis difficult to differentiate from colonization. Aspergillosis is one of the most commonly missed diagnoses in ICU patients based on a systematic review of autopsy studies.[1] Therefore, being suspicious of a possible aspergillosis infection and having the ability to diagnose it with the help of lab tests is critical.

Cases of invasive pulmonary aspergillosis that occur in critically ill patients with viral pneumonia have been reported recently.[2] Influenza-associated pulmonary aspergillosis (IAPA) has been reported globally with a variety of influenza strains with mortality rates as high as 57 percent.[2]

Because laboratory diagnostics are critical in the early detection and management of invasive aspergillosis per guidelines, it is important for laboratory scientists to be familiar with the range of diagnostic tests used in clinical practice. Two commonly used case definitions for invasive aspergillosis – by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG), as well as the *AspICU* collaboration – [3,4] require mycological evidence for the diagnosis of invasive aspergillosis. In the EORTC/MSG definition, *Aspergillus*-positive culture, galactomannan antigen detected, or *Aspergillus*-positive Polymerase Chain Reaction (PCR), are acceptable mycological evidence when aspergillosis host factors and clinical features are also present.[3] For the *AspICU* clinical algorithm, which was developed more for clinical trials than patient care, an *Aspergillus*-positive culture is an entry criteria for diagnosing aspergillosis in addition to clinical symptoms, abnormal radiographic features, and host factors.[4]

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LEARNING OBJECTIVES

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

1. Recall aspergillosis causative agents and describe its disease complications.
2. Discuss classification of diagnosis of *Aspergillus* disease based on EORT/MSG and the *AspICU*.
3. Describe all laboratory testing methods for the diagnosis of *Aspergillus* disease and their limitations.
4. Discuss the future recommendations for the proper diagnosis of *Aspergillus* disease.

We will review specimen collection procedures first and then commonly used tests to detect invasive aspergillosis, such as conventional diagnostics, immunodiagnostic, molecular tests and novel laboratory approaches. Lastly, *Aspergillus*, species antifungal susceptibility testing will be briefly discussed as well.

Literature review

We conducted a review of published literature including conferences abstracts and full papers to identify studies that investigated aspergillosis diagnostic test performance. We searched PubMed and Google Scholar using keywords "aspergillosis," "diagnostic tests," "beta-D-glucan," "galactomannan," "lateral flow assay," "culture," "histopathology," "microscopy," "anti-fungal susceptibility tests," "polymerase chain reaction," "next generation sequencing," and "volatile organic compounds." The range of reported sensitivity, specificity, and reproducibility from reported articles and commercial assay reports were extracted and the *Aspergillus* diagnostic tests were classified into three main categories: conventional diagnostics, immunodiagnostics, and molecular diagnostics. We summarized the strengths and weaknesses for different tests in each category and discussed isolate identification and antifungal susceptibility testing.

Specimens for invasive aspergillosis diagnosis

A key step to diagnosing aspergillosis is to select the right sample specimen for laboratory testing, since a poor-quality specimen can result in inaccurate laboratory results. While cerebrospinal fluid (CSF) and urine have been used, serum and lower respiratory tract specimens (bronchoalveolar lavage, sputum, or tracheal aspirate) are commonly used for aspergillosis diagnosis.[5] Since *Aspergillus* is commonly found in the environment, there is a possibility of contamination during the venipuncture. Thus, prior to collecting blood, it is important to use iodine tincture or chlorhexidine wipes to decontaminate the blood collection site.[5] Based on the laboratory diagnostic system used, the recommended amount of blood should be collected. Blood can easily be transported at room temperature to the laboratory without concerns that ambient temperature transport will affect lab results.[5] Since blood is a sterile sample, detection of *Aspergillus* would indicate an infection of invasive aspergillosis. Lower respiratory tract samples, however, are susceptible to contamination, since *Aspergillus* can colonize the airways or lungs, with reported frequencies of 36-91 percent, but not cause infection, especially in patients with chronic lung disease.[6] Thus, the collection procedure for lower respiratory tract samples is important. It is recommended that sputum samples be collected in the morning right after brushing teeth.[5] All bronchoalveolar lavage specimens should be collected via bronchoscopy, and all lower respiratory tract specimens should be collected using a sterile tube. Lower respiratory tract specimens can be transported to the laboratory within two hours at room temperature, but if one requires longer time for transportation, the specimen should be kept at 4°C.[5] Since a lower respiratory tract specimen is not sterile, it is difficult to associate a positive test result with infection and a blood/serum sample might be needed.[6]

Conventional Diagnostics

Conventional diagnostics rely on culture and microscopy for staining and visualization. Direct examination of the respiratory

specimen under the microscope for *Aspergillus* is one such example of conventional diagnostics. Direct examination allows rapid turnaround times of 2–4 hours. Technique could be improved by staining the specimen with calcofluor white and 10 percent potassium hydroxide to provide better sensitivity, since calcofluor white is a fluorescent dye that binds to the fungal cell wall, and potassium hydroxide helps visualize the hyphae of the fungus by dissolving the walls of human epithelial cells.[7] In addition to the cost for reagents and a fluorescence microscope, the main downside of direct examination is the difficulty to distinguish *Aspergillus* from other fungi with filamentous elements, which would require an experienced microscopist (**Table 1**).[7]

Culture

The most widely available conventional diagnostic for *Aspergillus* is fungal culture. Its wide availability stems mainly from a clinical aspect since a positive *Aspergillus* fungal culture is useful for diagnosing invasive aspergillosis. Fungal culture is a relatively simple technique, and most *Aspergillus* species grow rapidly in a fungal culture plate within 48 hours. A variety of solid and liquid fungal media can be used for growing *Aspergillus*, such as blood agar, chocolate agar, brain heart infusion broth, Czapeck agar, potato dextrose agar, cornmeal agar, and Sabouraud dextrose agar.[7] Additionally, antibiotics, such as chloramphenicol and gentamicin, are added to the fungal culture to prevent bacterial growth.[7] Besides antibiotics, protein synthesis inhibitors such as cycloheximide are occasionally added to inhibit environmental mold growth; although, this can also inhibit *Aspergillus*.[7] Fungal culture also allows for antifungal susceptibility testing. The drawback of fungal culture is that it is difficult to differentiate clinical colonization from infection and often requires an expert to be able to determine the species of the fungi.[7] Books and online articles that provide useful information on identification of common *Aspergillus* species are available (**Table 1**).

Histopathology

Histopathology, another type of conventional diagnostic, can be useful to determine whether the fungus is invasive within tissue and provides relatively quick results within 2-3 days. Most pathology and laboratory professionals use haemotoxylin and eosin (H&E) staining; however, studies have reported increased sensitivity when using Periodic acid-Schiff (PAS) stain and Grocott's (Gomori) methenamine silver stain (GMS).[8] For suspected aspergillosis, experts advise using H&E counterstaining and either GMS or PAS staining simultaneously to avoid delays in reporting results.[8] Despite histopathology's ability to determine invasion, microscopy cannot easily distinguish *Aspergillus* from other molds, such as *Fusarium, Scedosporium,* and Penicillium.[8] However, using immunohistochemistry with anti-*Aspergillus* antibody can reduce the difficulty of distinguishing *Aspergillus* from *Candida, Fusarium, Mucorales, Scedosporium, Paeciliomyces,* and dematiaceous fungi (**Table 1**).

Immunodiagnostics

Immunodiagnostic assays are used to detect pathogen-specific antigens or antibodies produced against foreign antigens. These tests are based on the antibody-antigen complex formation. Commonly used immunodiagnostics are galactomannan and beta-D-glucan tests, which are antigen tests (**Table 2**).

Galactomannan

Galactomannan is a cell wall component of *Aspergillus*. Immunodiagnostics assays that detect galactomannan antigen have

Conventional Diagnosis

Method	Strengths	Weaknesses
Direct examination of specimen using microscope, adding calcofluor white to immunofluorescence microscopy	~Rapid turnaround time around 2-4 hours. ~Potassium hydroxide: helps visualize hyphal elements.[7] ~Calcofluor white – fluorescent dyes that bind to fungal cell walls with high sensitivity, not specific for aspergillus[7]	~Inability to distinguish aspergillus from other filamentous fungi[7] ~Reagents cost and fluorescence microscope ~Expertise of laboratory technician ~Identification of fungal components: it is harder when calcofluor white is not use
Histopathology	~Useful to determine tissue invasion of fungus[8] ~Relatively quick results in a few hours[8] ~Periodic acid-Schiff stain and Gomari methenamine silver (GMS) stain can increase sensitivity[8, 9] ~Immunohistochemistry using anti-*Aspergillus* antibody can improve assay specificity.	~Difficult to distinguish infections by other molds (*Fusarium, Scedosporium, Penicillium*)[8] ~Normal tissue structures can be confused with yeast when using GMS stain alone. Co-localize GMS stain with H&E or PAS stain to make it easier to visualize.[8] ~Transversally cut hyphae can appear to be budding.[8]
Culture	~Allows for susceptibility testing and isolation of species[7] ~Simple technique ~*Aspergillus* grows rapidly - within 48 hours[9]	~Insensitive, requires expertise for species determination[7] ~Difficulty differentiating infection versus colonization.[9]

Isolate identification

Method	Strengths	Weaknesses
Microscopy	~Identifies specific fungal species[9] ~Books and articles can provide useful information on identification of cultures of *Aspergillus*[9]	~Difficult to identify fungal species accurately based on phenotype and morphology alone[21] ~Relies on clinical mycologist expertise, whose numbers are declining[21] ~Unable to distinguish cryptic species.
Matrix-assisted laser desorption/ ionization-Time of Flight (MALDI-TOF)	~Identifies specific fungal species rapidly[21] ~Protocol for identification is in the process of improving ~Identify cryptic species	~Requires extension of current databases[21] ~Need of consensus on protein extraction method
Molecular identification by sequencing	~Identifies different fungal species rapidly[21] ~Can identify cryptic species ~Can identify markers of resistance	~Limited commercial kits available[21] ~Limited comprehensive reference databases

Table 1. Strengths and Weaknesses of Laboratory Diagnostics for Invasive Aspergillosis.

been useful for the rapid, early detection of invasive aspergillosis. Serum and bronchoalveolar lavage fluid have been used as sample specimens.[9] The sensitivity of antigen detection in bronchoalveolar lavage fluid is higher when compared with serum in patients at high risk of developing aspergillosis, such as patients with hematological malignancies. Cerebrospinal fluid (CSF) specimens have been reported as useful specimens for the diagnosis of cerebral invasive aspergillosis, but the use of these types of samples lack validation studies.[9]

Previous studies of galactomannan enzyme immunoassay (EIA) have reported good sensitivity and specificity. In a meta-analysis that analyzed twenty-seven studies of patients with immunosuppression (hematological malignancy or bone marrow or solid organ transplant), Pfeiffer and colleagues reported the pooled sensitivity and specificity of the galactamannan EIA for proven invasive aspergillosis cases as 71 percent and 89 percent.[10] The pooled sensitivity was 61 percent and pooled specificity was 93 percent when probable invasive aspergillosis cases were included.[10]

A few commercial kits are available for galactomannan EIA, but only one is well validated and widely available. Despite its ease of use, galactomannan EIA might not be used in routine clinical care for several reasons. Clinically, galactomannan EIA has lower sensitivity in non-neutropenic patients and patients who have already received antifungal medications.[11] False positive results can also occur due to recent use of antibiotics – such as amoxicillin/clavulanic acid, piperacillin-tazobactam, and beta-lactam antibiotics – or other bacterial or fungal infections, such as with *Penicillium, Alternaria, Paecilomyces, and Histoplama.*

In addition to the varied clinical performance, another drawback is the reagent cost and the specialized equipment necessary to perform the test (**Table 2**).[12]

Lastly, *Aspergillus* lateral flow assays (LFAs) have been developed as a rapid, point-of-care test to detect an *Aspergillus* galactomannan antigen or an *Aspergillus* specific extracellular glycoprotein antigen. A primary benefit of LFAs is their low cost, because LFAs do not require expensive equipment or laboratory space. Thus, they can be utilized in low-resource settings to diagnose invasive aspergillosis. The specimen sample can be serum or bronchoalveolar lavage fluid. The test sensitivity and specificity are high, 90 percent and 84 percent respectively.[13] Higher sensitivity has been reported in patients with hematological malignancies. The assay that uses a monoclonal antibody, which binds to an *Aspergillus* specific antigen, does not cross-react with other antibiotic drugs.[13] Additionally, LFAs that detect antigen in serum specimens suggest that the infection is invasive, an important aspect for determining the clinical significance of infection.[13] However, a limited number of commercial LFA kits are available. Data on LFAs in clinical use is limited; thus, more research is needed (**Table 2**).

Beta-D-glucan

Another type of rapid immunodiagnostics is the beta-D-glucan assay, which uses serum as the specimen sample. Beta-D-glucan is another fungal cell wall component; however, the fungal antigen is not specific to *Aspergillus*. There is a beta-D-glucan antigen detection assay kit available for clinical laboratories that is commonly used and widely available. Using the assay involves creating a standard curve of beta-D-glucan levels, incubation of the sample and reading the assay plate. Most have a sensitivity of 93 percent and specificity of 77 percent.[14] In comparison with the galactomannan antigen EIA, the beta-D-glucan assay has a higher sensitivity and negative predictive value but a lower specificity.[14] False positives can also result from exposure to external glucan antigens found on surgical sponges and gauze, immunoglobulins, albumin, beta-lactam antibiotics, hemodialysis, tubes for sample collection, developing bacterial infections, and other causes. Besides the low specificity, another limitation of the beta-D-glucan assay is the cost of performing the test, given the concern for false-positives and false-negatives (**Table 2**).

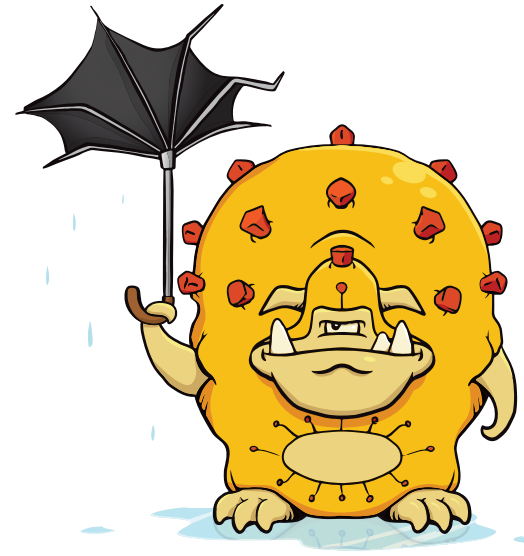
Immune precipitation

In immune precipitation, the antibody and antigen diffuse towards each other in the gel and create a band called a precipitin band. That process does require less specialized equipment to detect aspergillosis as compared to microscopy.[12] However, immune precipitation is time consuming and may take up to two to three days to obtain results.[12] As more immunodiagnostic assays became

Immunodiagnostic and Bio-markers

Method	Strengths	Weaknesses
Antibody detection (Precipitin antibodies)	~Requires less specialized equipment[12] ~Higher sensitivity for diagnosis of chronic pulmonary aspergillus[12]	~Low sensitivity in immunocompromised patients [12] ~Time consuming[12]
Bio-marker: Enzyme Immunoassay (EIA) (Galactomannan)	~Higher sensitivity for BAL compared to serum in patients with hematological malignancies, stem cell transplant, and solid organ transplantation[9] ~Useful for early diagnosis of invasive aspergillosis [15] ~CSF sample can be useful to diagnose cerebral invasive aspergillosis[9] ~Sensitivity of 68-74%[10] ~Specificity of 88-90%[10]	~Lower sensitivity in patients who received anti-fungal therapy.[11] ~Possible lower sensitivity in non-neutropenic patients[11] ~Cross reactivity with filamentous fungi, bacteria, and some antibiotics[7] ~False negative: 8-10%[15] ~False positive: 8%[15] ~Reagent cost and requires specialized equipment[12]
Bio-marker: Lateral Flow Assay (LFA)[13]	~Point-of-care testing[13] ~Rapid test (less than 60 minutes) and low cost.[13] No expensive equipment or laboratory space needed. ~Higher sensitivity in patients with hematological malignancies[13] ~Able to detect activity that indicates invasion.[13] ~Sensitivity – 90%[13] ~Specificity – 84%[13]	~Few commercial kits available[13] ~Limited data about validation on clinical forms different that invasive disease
Bio-marker: Beta-D-glucan assay	~Specimen sample (serum) easy to collect regardless of patient status[5] ~High negative predictive value[14] ~Beta-D-glucan detect aspergillosis earlier than other testing methods.[15] ~Sensitivity – 93%[14] ~Specificity – 77%[14] ~Negative predictive value – 98% [14]	~Panfungal assay can't distinguish etiological agent[15] ~False positive after exposure to surgical sponges and gauze for 3-4 days, or products that contains glucans. ~Hemodialysis, bacterial infections, administration of immunoglobulins, albumin, and certain antibiotics could result in elevated Beta-D-glucan levels.

Table 2 Strengths and Weaknesses of Laboratory Diagnostics for Invasive Aspergillosis.



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readily available, galactomannan and beta-D-glucan tests became more favored over the precipitin test. Immunodiagnostic assays that rely on antibody (Ab) testing are good for immunocompetent patients. However, in immunocompromised patients, those antibody assays are less sensitive due to inadequate antibody production in response to the infection. In these situations, an antigen detection assay has better analytical performance.

Molecular diagnostics

The most commonly used molecular diagnostic is polymerase chain reaction, which provides rapid detection of *Aspergillus* species using bronchoalveolar fluid and serum as specimen samples, with bronchoalveolar fluid having a higher risk of false positive results, because it is not sterile. The drawback of PCR tests for *Aspergillus* is the lack of standardized protocols across different laboratories, the difficulty with preparing samples, and the false positive frequency when using non-sterile samples.[15] Studies have reported higher sensitivity when multi-copy gene targets are used and when whole blood instead of serum is used.[15] However, whole blood is more difficult to process for PCR, and data is limited on its higher sensitivity.[15] Typically, PCR target genes include 18S rRNA, 28s rRNA, mtDNA, and internal transcribed spacer (ITS).[15, 16] Additionally, more advanced equipment, such as automated processors, can reduce performance variability compared to manual processing, but this advanced equipment costs more and requires more complex laboratory infrastructure than other types of diagnostics (**Table 3**). In a meta-analysis of 16 studies that included adult and children with hematological malignancy or hemopoietic stem-cell transplant, Mengoli and colleagues showed that the pooled sensitivity and specificity for one PCR-positive test was 88 percent and 75 percent, which was useful enough to exclude invasive aspergillosis.[16] However, they concluded that two PCR-positive tests were necessary to confirm an invasive aspergillosis diagnosis based on the pooled sensitivity and specificity for two PCR-positive tests of 75 percent and 87 percent respectively.[16] As of 2016, the Infectious Disease Society of America does not recommend *Aspergillus* PCR for clinical use, because experts do not agree on the utility of PCR in clinical diagnosis. Few commercial assays are validated, and there is a lack of standardization in *Aspergillus* PCR methods.[17]

A less commonly used specimen for molecular diagnostics is formalin fixed paraffin embedded (FFPE) tissue. Molecular testing of FFPE samples is possible using nine polymorphic short tandem repeat loci as well as detection of genes described previously in the PCR testing section above.[18] DNA is extracted from FFPE lung biopsies by lysing cells and purified through affinity chromatography.[18] After DNA extraction, genotyping was done using PCR. Azole susceptibility could be tested by identifying CYP51A mutations such as TR34, L98H, Y121F, and T289A.[19] Studies have reported that this method is capable of detecting *Aspergillus* in FFPE samples 41-94 percent of the time (**Table 3**).[18]

Next generation sequencing

An infrequently used molecular test due to its high costs is next generation sequencing, which can provide rapid and highly accurate results for diagnosing invasive aspergillosis.[20] Some studies have reported that next generation sequencing is able to detect *Aspergillus* when other assays cannot.[20] Differentiating invasive aspergillosis from colonization is clinically important, as missing the diagnoses can result in delayed treatment and consequently death.[20] In contrast, *Aspergillus* colonization in the airway is relatively common, and overtreatment may be deleterious to the patient.[20] The main weakness of next generation sequencing, however, is the lack of a comprehensive reference genome sequence database (**Table 3**).[20]

Recently, detection of *Aspergillus* metabolites has been developed using gas liquid chromatography/mass spectroscopy. The premise is that gas liquid chromatography/mass spectroscopy can detect volatile organic compounds, which are produced by *Aspergillus*, in the breath.[21] It is a fast and painless test with reported sensitivities of 94 percent and specificity of 93 percent.[21] However, additional research is needed to uncover new *Aspergillus* metabolite signatures and to determine its effectiveness for diagnosing invasive aspergillosis.[21]

Isolate identification

In addition to detecting *Aspergillus*, it is important to identify the species of the fungus. There are a few methods developed for isolate identification, such as microscopy, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF), and PCR with sequencing. Although microscopy can identify the genus and species of fungi, it is difficult to reliably characterize the species based on morphology alone, and it relies heavily on a mycologist's expertise.[21] Another downside of microscopy is that it is unable to distinguish cryptic species of *Aspergillus*, which are defined as species that are morphologically indistinguishable. MALDI-TOF and genetic sequencing can overcome that challenge and identify cryptic species. However, both lack a comprehensive reference database for identification.[21] Internal transcribed spacer sequencing has limited commercial kits available for isolate identification; whereas, MALDI-TOF has an established extraction protocol for commercial equipment (**Table 1**).

Antifungal susceptibility testing (AFST)

An important aspect of *Aspergillus* testing is AFST due to its clinical relevance, especially since resistance to commonly used antifungal medications in *Aspergillus fumigatus* has been increasing.[19] There are two types of resistance: intrinsic resistance, which is resistance that is natural and innate part of the fungus that emerges when the species emerges, and acquired resistance, which is resistance after exposure to antifungal medications.[19] Resistant strains of *Aspergillus* mean certain antifungals are not effective and use of these antifungals may result in unfavorable clinical outcomes. Azole-resistant *Aspergillus*, particularly *Aspergillus fumigatus*, have been reported with increasing frequencies ranging from 1-15 percent, depending on the country due to a mutation of the CYP51A, which is an azole target.[19] However, there are methods for detecting azole-resistant *Aspergillus* by looking for markers, such as TR_{34}/L98H and TR_{46}/Y121F/T289A.[19] Additionally, other mutations for the *CYP51A* gene, such as G54R/W/E, M220I/K/V, P216L, F332K, and G448S have been reported.[22]

AFST is a slow and technically demanding process that requires growing the fungal specimen in culture with discs of varying concentration of the antifungal substance. The most common antifungal medications tested for susceptibility are amphotericin B, anidulafungin, caspofungin, micafungin, isavuconazole, posaconazole, itraconazole and voriconazole. Broth dilution is another method used to measure the minimum inhibitory concentration of antifungal drugs and determine susceptibility. The broth dilution AFST method has been standardized by the Clinical and Laboratory Standards Institute (CLSI), which has a single breakpoint and epidemiologic cutoff values, and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), which has breakpoints and epidemiologic cutoff values. Commercial kits are also available and compare how well *Aspergillus* grows on the commercial growth agar with no antifungal agent, itraconazole, voriconazole, and posaconazole. However, susceptibility in vitro does not always predict how *Aspergillus* will respond to antifungal treatment clinically (**Table 3**).

Recently published studies have been utilizing MALDI-TOF MS to type and test for antifungal susceptibility for *Aspergilli*.[23] A few studies have looked at using MALDI-TOF MS for *A. fumigatus* and

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Molecular testing

Method	Strengths	Weaknesses
Polymerase chain reaction	~Rapid and early detection[15] ~High sensitivity with multi-copy gene targets[15] ~More advance equipment, such as automated processors, can reduce performance variability compared to manual processing.	~Difficulty with sample preparations[15] ~False positives on BAL samples is 10-25%[15] ~Low positive predictive value on BAL samples[15] ~Needs complex laboratory infrastructure ~Whole blood is more complex in processing than serum or plasma.
Next generation sequencing (microbiome)	~Rapid results and high accuracy[20] ~Able to detect *Aspergillus* when other clinical assays are negative[20]	~Current high costs, but could change in the future[20] ~Current lack of comprehensive reference genome sequence database[20]
Metabolites: Gas liquid Chromatography/ Mass spectroscopy	~Detects volatile organic compounds in breath[21] ~Sensiviity - 94%[21] ~Specificity - 95%[21] ~Fast and painless test[21]	~Additional research is needed to discover more metabolite signatures[21] ~More research is needed to determine usefulness for diagnosis and monitoring Invasive *Aspergillus*[21]

Antifungal susceptibility testing

Method	Strengths	Weaknesses
Antifungal susceptibility testing	~Can help guide clinicians on which antifungal medications to give	~Slow process ~Susceptibility in vitro testing does not always associate with response to antifungal therapy in vivo ~Lack of microbiological susceptibility breakpoint in most of the *Aspergillus* species ~Limited commercial kits for antifungal susceptibility testing ~*Aspergillus* resistance is an emerging problem

Table 3 Strengths and Weaknesses of Laboratory Diagnostics for Invasive Aspergillosis.

A. lentulus to provide quicker results for antifungal susceptibility.[23] The assay developed by Sanguinetti and Posteraro involves incubating the fungi with serial antifungal concentrations for 15 hours before analysis with MALDI-TOF, which allowed them to measure the minimal profile change correlation index, an alternative to the minimum inhibitory concentration.[23] Future directions in using MALDI-TOF MS for AFST involves reducing the time needed to obtain results and testing the accuracy of the method.

Diagnosis difficult despite range of testing options

The wide variety of laboratory diagnostics tests available to detect *Aspergillus* species provides information about the *Aspergillus* infection, such as genus and species, invasion into other tissues, and susceptibility to antifungals. Conventional diagnostics allow us to visualize and diagnose aspergillosis through microscopes, fungal culture, and staining techniques, but takes valuable time to provide results back to the clinician, which would delay treatment. On the other hand, immunodiagnostics – such as galactomannan and beta-D-glucan detection, and novel lateral flow assays – provide rapid results through the detection of biomarkers. Newer developments in molecular diagnostics – such as PCR, MALDI-TOF MS and volatile organic compound detection diagnostics – could provide rapid, accurate tests but will require further research and expansion of current reference databases for *Aspergillus*.

Studies have found that a combination of diagnostic assays – such as PCR with culture, PCR with beta-D-glucan, PCR with galactomannan, galactomannan with beta-D-glucan – have provided better diagnostic accuracy.[24,25] For example, when the lateral flow assay that detects an *Aspergillus*-specific antigen is combined with PCR, sensitivity of greater than 94 percent and specificity of greater than 86 percent are reported.[24] Galactomannan and PCR tests are reported to have high rates of agreement between the two assays (greater than 94 percent) in patients with hematologic malignancies, solid tumor malignancies, stem cell transplants, and primary or hereditary immunodeficiencies.[25] Therefore, experts suggest a combination of galactomannan and PCR for diagnosis of invasive aspergillosis in patients with hematologic malignancies, solid organ transplant, and stem cell transplants. However, a limitation is that the combination of immunodiagnostics and molecular testing do not confirm a proven invasive aspergillosis case, since that requires a tissue biopsy and histological techniques. Perhaps immunodiagnostic and molecular tests could be incorporated in future guidelines for confirming an aspergillosis diagnosis.

Despite the diagnostic tools at our disposal to detect aspergillosis, the clinical diagnosis of aspergillosis is still difficult and often relies on clinical guidelines, such as the EORTC/MSG and *AspICU* diagnostic criteria.[3,4] The EORTC/MSG guidelines were created for the classical immunocompromised host for aspergillosis with classifications as proven, probable, and possible with probable based on three categories: (1) host risk factors such as neutropenia, hematologic malignancy, or allogenic stem cell transplant, (2) clinical features such as halo, crescent, or cavitary sign on chest CT, and (3) mycological evidence from fungal culture, galactomannan test, or *Aspergillus* PCR.[3]

Classical cases of invasive aspergillosis are common in an immunocompromised host, since aspergillosis is an opportunistic fungus. However, more recent studies have reported aspergillosis in immunocompetent patients, some of whom might be critically ill with influenza.[2] These patients would typically not meet the classical EORTC/MSG criteria and do not typically display angioinvasion, which is a common level of invasion in an immunocompromised host.

The *Asp*ICU diagnostic criteria was developed for patients in the ICU without classic risk factors for aspergillosis. The criteria are (1) a positive *Aspergillus* culture, (2) clinical signs and symptoms such as dyspnea, hemoptysis, or pleuritic chest pain, (3) abnormal chest radiographic imaging, and (4) host risk factors that include high glucocorticoid treatment (>20mg prednisone equivalent).[4]

The need for increased awareness and education surrounding the diagnosis of invasive aspergillosis is critical. Finally, laboratory testing, especially for the detection of biomarkers and DNA, plays an important role in the control of *Aspergillus* outbreaks in hospitals, which have been seen due to contaminated water, ventilation systems, medicines and medical devices.

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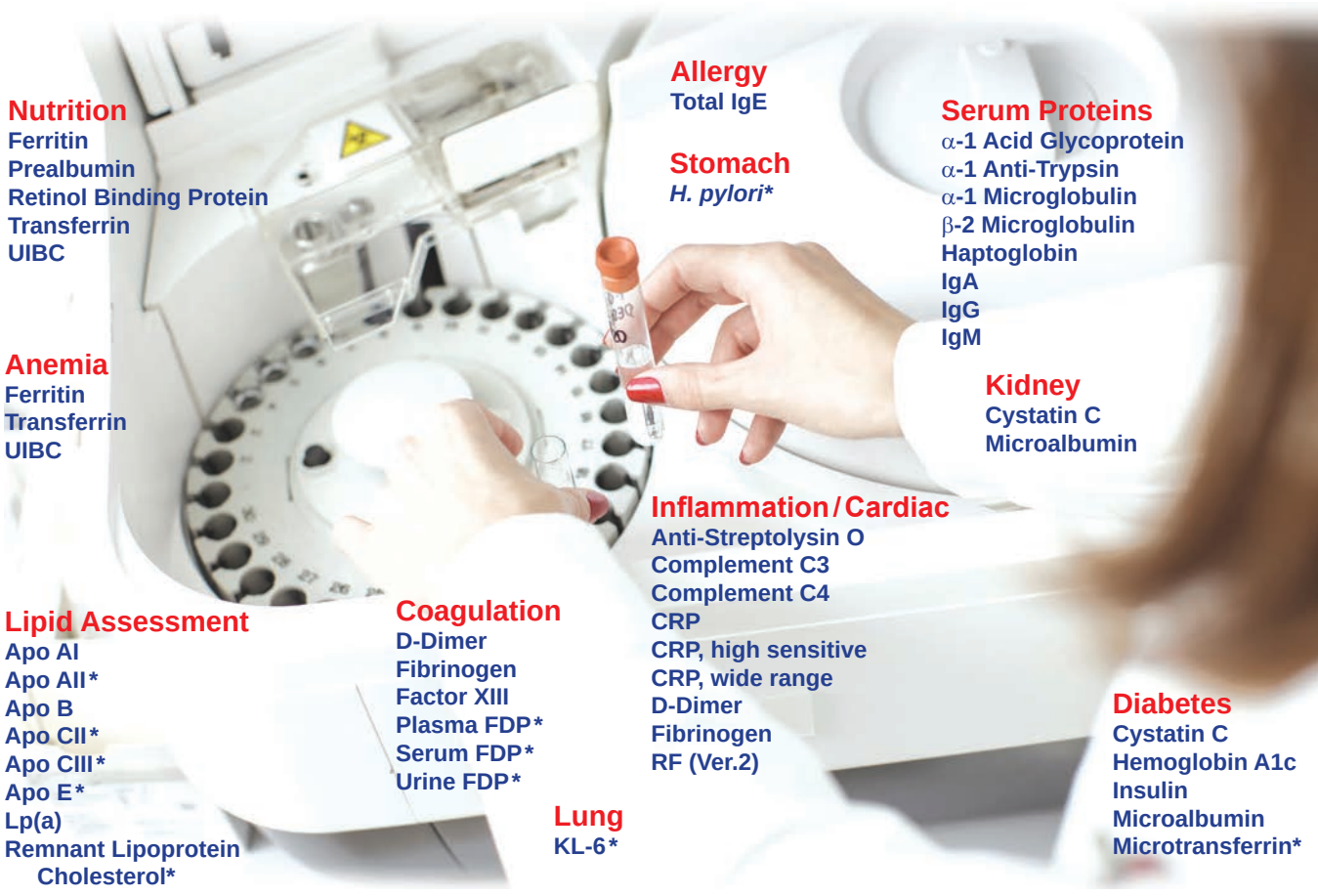

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Our review had several limitations. One is that this was not a systematic literature review, and we did not attempt to include every reported diagnostic, but only those used in common clinical microbiology practice. Second, we did not perform a pooled analysis of the sensitivity, specificity, and reproducibility for the different diagnostic tests, and instead reported a range of values that were available.

Future efforts on aspergillosis diagnostics are focused on developing rapid, highly sensitive, and highly specific tests, such as lateral flow assays and volatile organic compound detection tests. Additionally, research is moving towards finding the best clinical algorithm for the diagnosis of aspergillosis, given the complexity of the different disease states.

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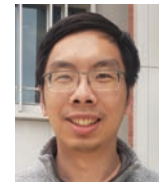

John Z. Deng, BS, is with the David Geffen School of Medicine at University of California Los Angeles (UCLA).

Jeffrey D. Klausner, MD, MPH, is a Professor of Preventive Medicine in the Division of Disease Prevention, Policy and Global Health, Department of Preventive Medicine at University of Southern California Keck School of Medicine.

Diego H. Caceres, BSc, MSc, is an Epidemiologist at the Mycotic Diseases Branch, Division of Foodborne, Waterborne and Environmental Diseases at the Centers for Disease Control and Prevention and a PhD candidate at Radboud University Medical.

TEST QUESTIONS

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

1. **All of the following *Aspergillus* species cause human disease but**
   - A. *A. fumigatus*
   - B. *A. clavatus*
   - C. *A. terreus*
   - D. *A. niger*

2. **Diagnosis of invasive aspergillosis is difficult to distinguish from colonization of *Aspergillus*, because it is widespread in the environment and can colonize the _____.**
   - A. skin
   - B. airway
   - C. hair
   - D. eyes

3. **The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Disease Mycoses Study Group (EORT/MSG) has the same invasive aspergillosis diagnostic recommendations that the AspICU.**
   - A. True
   - B. False

4. **The literature review that was conducted classified *Aspergillus* diagnostics into the following category(ies):**
   - A. immunodiagnostics
   - B. conventional diagnostics
   - C. molecular diagnostics
   - D. all of the above

5. **The two most common specimens that are used for diagnosing *Aspergillus* infections are_____.**
   - A. CSF and serum
   - B. serum and lower respiratory tract specimens
   - C. urine and CSF
   - D. lower respiratory tract specimens and urine

6. **Lower respiratory tract specimens should be collected after brushing teeth in the morning, because *Aspergillus* can colonize the airways but not cause infections in _____ to ____ percent of patients.**
   - A. 12; 52
   - B. 24; 89
   - C. 36; 91
   - D. 52; 91

7. **Direct microscopic examination allows for a ___ to __ hour turnaround time but is difficult to distinguish *Aspergillus* form other fungi with _____.**
   - A. 2; 4; filamentous
   - B. 5; 8; filamentous
   - C. 2; 4, hyphae
   - D. 5; 8; spores

8. **Culture for Aspergillus identification is most widely available and does not have any limiting factors.**
   - A. True
   - B. False

9. **Histopathology diagnostics cannot determine *Aspergillus* from other molds, such as_____.**
   - A. *Fusarium, Penicillium and Alternaria*
   - B. *Scedosporum, Penicillium and Cladosporium*
   - C. *Penicillium, Alternaria and Cladosproium*
   - D. *Fusarium, Scedoporium and Penicillium*

10. **Which enzyme immunoassay uses serum or bronchial lavage, shows varied clinical performance, is expensive and requires the use of specialized equipment?**
    - A. Beta-D-glucan
    - B. Galactomannan
    - C. PCR
    - D. none of the above

11. **Which immunoassay is a point-of-care assay to detect galactomannan, is low in cost and does not require expensive equipment?**
    - A. Beta-D glucan
    - B. Galactomannan EIA
    - C. Galactomannan LFA
    - D. PCR

12. **Beta-D-Glucan test methods and immune precipitation do not show quality test performance characteristics, showing either low sensitivity, low specificity or both.**
    - A. True
    - B. False

13. **The Infectious Disease Society of America does not recommend *Aspergillus* PCR for clinical use, because _____.**
    - A. there is lack of standardization
    - B. experts do not agree on the utility of the test for clinical diagnosis
    - C. few commercial assays are validated
    - D. all of the above

14. **Which molecular test is high in cost and has a limited database of reference genome sequences, but has a rapid turnaround time and is highly accurate for diagnosis of invasive aspergillosis?**
    - A. PCR
    - B. Next generation sequencing
    - C. Isolate identification
    - D. Microarray

15. **The main limitation of the MALDI-TOF for identifying *Apergillus* species is:**
    - A. relies heavily on a mycologist's expertise
    - B. its unable to distinguish cryptic species of *Aspergillus*
    - C. it lacks a comprehensive database for identification
    - D. it lacks a quick turnaround time

16. ***Aspergillus* susceptibility testing is important to include, because developing resistance from medication is increasing in infections with_____.**
    - A. *A. fumigatus*
    - B. *A. niger*
    - C. *A. terreus*
    - D. *A. flavus*

17. **The main limitation(s) for use of the MALDI-TOF in antifungal susceptibility testing includes**
    - A. long turnaround time
    - B. low testing accuracy
    - C. both A and B
    - D. none of the above

18. **Studies are showing that the combination of _____ have provided better diagnostic accuracy.**
    - A. immunodiagnostic and molecular tests
    - B molecular tests and conventional tests
    - C. immunodiagnostic and conventional tests
    - D. none of the above

19. ***Aspergillus* outbreaks that have occurred in hospital settings are due to_____.**
    - A. medicines and medical devices
    - B. ventilation systems
    - C. contaminated water
    - D. all of the above

20. **The future of quality aspergillosis diagnostics includes finding the best algorithms of combined tests, while using highly sensitive and highly specific test methods.**
    - A. True
    - B. False

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Quality control in the evolving landscape of molecular testing

By David Gauthier, MA, and Andy Quintenz

A laboratory in a cutting-edge field like molecular infectious-disease testing merits an exceptional quality control program to strengthen confidence in laboratory performance and support optimal patient care. However, molecular diagnostics is not as established as other diagnostics segments, and the corresponding QC standards and regulations are not as well-defined. The rapid innovation and evolving regulations around molecular testing can make it difficult to create and maintain an effective QC program, accounting for quality, efficiency, and regulatory requirements.

This article highlights relevant QC considerations for molecular diagnostics and presents effective QC solutions and best practices to support dynamic and evolving molecular laboratories.

Improving laboratory performance with effective QC practices

Systematic quality control is a laboratory's first line of defense against potentially disastrous consequences. This is especially true for clinical laboratories because results directly impact patient treatment. Additionally, any change in the test system or process (e.g., new reagent lots, new instruments, etc.) could potentially affect the quality and integrity of the results. Procedural errors, instrument malfunctions, environmental changes and any other variables can also alter test results. While some of the resulting effects are obvious, other changes are subtle and only detectable with a methodical quality control analysis.

By adhering to thorough QC procedures, molecular laboratories can closely monitor testing for both random and systematic errors, as well as immediate data shifts and abnormal long-term trends. The early detection of anomalies allows laboratories to address issues promptly, before they adversely impact patient data.

A standard QC program might only rely on in-kit controls and proficiency surveys. As discussed below, supplementing this with independent third-party controls, as well as a QC data management software, considerably strengthens QC measures, while mitigating any bias that may result from in-kit materials. A rigorous QC program not only helps fulfill regulatory requirements, but it also increases result confidence, enhances efficiency, minimizes budgetary waste, and simplifies laboratory procedures.

Photo by National Cancer Institute on Unsplash

A QA/QC program for molecular diagnostics should include independent third-party controls and quality management software.

Streamlining QC with data management software

Integrating data management software into molecular laboratories is a transformative step in modernizing, simplifying, and strengthening laboratory procedures. Following a rigorous quality control program is critical, especially when dealing with patient specimens. It can be challenging to prioritize quality while focusing on saving time, complying with regulations, and minimizing expenses. Automating data management is the solution to greatly simplify this QC process.

Challenges with molecular QC data management

Many molecular laboratories spend hours manually entering and analyzing QC data in spreadsheets or basic software programs. In addition to taking up valuable time better spent elsewhere, the process of manually recording data is tedious and substantially error-prone.

Effective QC analysis should take into account QC results over time and compare them to other laboratories (peer groups). Performing this level of analysis by manually gathering and entering outside data from detailed spreadsheets after sourcing it is challenging and causes delays in noticing errors and variations in the data. Time is everything in a clinical laboratory: the longer it takes to identify errors, the more patient results could be affected.

As molecular diagnostics expands within and across laboratories, the amount of QC data to be collected rises, making the analysis of higher volumes of data more complex.

Supporting molecular laboratories with QC data management software

Effective QC data management software programs automate all the steps of a QC data system, from collection to analysis. Data can flow directly from instruments and laboratory information systems (LIS) to QC data management software, eliminating the undesirable manual data recording step. This generates

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a reliable, accurate and standardized database of results. Laboratories can monitor data in real-time as it is transmitted to the software. Consequently, meaningful errors that might otherwise go unnoticed can be promptly identified and resolved. In molecular infectious disease testing, this can be accomplished by monitoring cycle threshold values, for example. Statistical capabilities of the software can conduct a comprehensive QC analysis, including bias and imprecision calculations, alerting the user of shifts that may need immediate attention, while monitoring values over time to identify subtle trends that could affect the overall laboratory performance. When changes in lots (reagents or standards), operators, the environment, and instrument maintenance occur, the software will identify any resulting shifts or concerning trends.

A powerful QC data management software platform should also provide access to data from peer groups, an invaluable resource to compare testing performance (snapshot and long-term trends) across molecular laboratories. This tool is not only a good preparation for proficiency testing, but also helps identify problem areas while assisting with troubleshooting efforts.

The automation of QC documentation and analysis is another source for time-saving that also offers the advantage of presenting the information needed to monitor quality efficiently. It makes regulation compliance straightforward by easily printing detailed QC reports for auditors, rather than spending time compiling data manually from spreadsheets.

Every feature of powerful QC data management software helps molecular laboratories take control of their QC and develop comprehensive awareness of their performance strengths and weaknesses. A QC program monitored by powerful data management and analysis tools helps save time and cost while increasing confidence in results.

Evaluating assay performance with independent control materials

Independent quality control materials bring molecular infectious disease testing to the level of standardization and uniformity needed in clinical testing.

A QC program is only as effective as its controls. If QC materials do not perform as expected, potentially harmful errors could go unnoticed. Even careful data analysis cannot identify anomalies if not accurately represented by the data.

It is critical that controls closely mimic patient specimens by consisting of whole inactivated pathogens diluted in the relevant clinical matrix. This allows those controls to capture inconsistencies and changes in a similar manner to patient specimens.

Many controls are made of synthetic materials diluted in matrices unrelated to patient specimens (although whole organisms may be available). Moreover, in-kit controls are optimized to function with specific assay platforms and reagents. Indeed, assay reagents are developed and tested by assay manufacturers with the same materials making up the controls provided in their kits, hence introducing a potential prejudicial bias. Additionally, when assays also include standards, the in-kit control(s) typically derive from those standards. Therefore, if an issue impacts the standards, the in-kit controls are affected in the very same manner, making everything appear as normal, while the underlying issue may continue to impact patient results. Hence, it is critical to use well designed, independent, and unbiased third-party QC controls in addition to (or instead of) the in-kit controls. Such independent molecular controls are designed to mimic clinical specimens and consist of whole inactivated pathogens in relevant clinical matrices, so they are affected by the entire analytical process in the exact same manner as the same pathogens found in the patient specimens. Hence, unlike with synthetic controls, each of the extraction, amplification and detection steps is fully controlled in an unbiased and thorough manner.

If the independent controls and standards are not rigorously manufactured, they could also introduce a drift in the QC data. Hence, their manufacturing should rely on ddPCR (droplet digital PCR) to ensure their absolute quantification whether they are assayed or unassayed. This helps to maintain every lot of those independent controls and standards within a tight range of acceptance criteria. This is particularly important for standards and controls of molecular quantitative assays.

Supplementing a QC program with independently manufactured controls allows an unbiased, comprehensive, and rigorous analysis of instrument and assay performance, while minimizing inventory waste, since their usage is not restricted to a single lot of assay reagents.

Investing in laboratory excellence

A comprehensive QC program is a form of risk mitigation. Like an insurance policy, it provides an extra layer of security, protecting from possible complications and mistakes. Effective QC programs limit the risks of potential harm to patients, liability of the laboratory, economic consequences, and damage to a laboratory's reputation.

Clinical laboratories are constantly under pressure to increase efficiency and lower spending. While independent controls and QC software may seem to be an added expense on the surface, a limited QC program may ultimately cost a laboratory much more than that investment. Money not spent up front on problem detection and prevention can result in much higher failure costs in the long run. Quality should not be sacrificed for price. For example, controls made of synthetic materials and/or in-kit controls optimized for specific instruments or reagents may not be attuned to nuanced variations in patient specimens.

In summary, investing in a comprehensive quality control program lowers the risk of inconsistent and inaccurate results that if allowed to leave the laboratory could result in expensive retesting or lead to misdiagnosis. An effective QC program avoids potentially severe consequences by capturing mistakes before any incorrect results are reported. Because a testing laboratory's first priority is its patients, adopting rigorous quality control measures is an investment in a higher standard of patient care. Supplementing a laboratory's QC program with independent third-party QC controls and QC data management software demonstrates the laboratory's dedication to exceptional patient care, economic efficiency and forward-thinking.

The authors acknowledge the contributions of Sarah Lehman for this article.

David Gauthier, MA, is Senior Product Manager at **Bio-Rad Laboratories**. He is responsible for the Exact Diagnostics portfolio of molecular controls, standards and verification panels at Bio-Rad.

Andy Quintenz serves as Scientific and Professional Affairs Manager for **Bio-Rad Laboratories** Quality Systems Division.

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COVID-19 highlights need for laboratory data sharing and interoperability

By Kim Futrell, MT(ASCP), MSHI

Secure sharing of patient data across healthcare systems and organizations – better known as interoperability – is widely recognized as part of the solution to improve patient outcomes. The laboratory is an important part of the move to interoperable healthcare systems because of the tremendous amount of data it manages and the value of that data in making patient care decisions. The SARS-CoV-2 pandemic has made the need for an interoperable healthcare system blatantly evident.

COVID-19 reiterates the value of interoperability

Transmitting data on COVID-19 results to government officials often remains a manual process involving paper and faxing. Currently, each state has its own guidelines for COVID-19 response, so statistics are inconsistent and vary based on the information source and location.

An interoperable healthcare system would greatly improve the nation's response to COVID-19. For instance, a patient with symptoms could visit his or her provider, and the provider would have immediate access to their full medical record to address any underlying conditions. This scenario would alleviate time wasted tracking down records and reduce or eliminate duplicate testing. When a patient with COVID-19 moves from primary care to emergency care, quick decisions and tracking of laboratory results is necessary. Shared data improves the providers' ability to provide prompt and effective patient care.

Interoperability requires removal of data silos

The ability to deliver targeted clinical interventions efficiently and effectively is a key aspect of healthcare value. To facilitate this value, providers need access to comprehensive, timely patient data, including information about existing chronic conditions that may affect treatment decisions. A significant factor in the lack of shared patient data is the healthcare industry's data silos that can make patient care cumbersome and, in some cases, dangerous.

Interoperability allows the sharing of data from electronic health records (EHRs) and other information systems across organizations and geographic locations. If patients travel to another state for vacation, then go to the emergency department (ED), their records from their home healthcare provider (from another state) are readily available in the ED, as those systems are electronically connected and able to share data. With this need evident, interoperability has been long-awaited in healthcare, and there is finally some effort in this direction.

Eliminating Interoperability barriers is slow

Yet, progress toward a connected healthcare system is slow. The U.S. healthcare system has spent billions of dollars to incorporate the use of EHRs through the Meaningful Use program, now renamed Promoting Interoperability, making their use commonplace in hospitals and within the majority of ambulatory provider offices.

Laboratory information systems predate EHRs and have been a strong component of laboratory workflow for decades. Often within healthcare organizations, these two information systems are interfaced and share data, creating a foundation for data sharing. However, the industry has made less progress in connecting systems and data among providers and across geographic locations. The technology is available, but data is still is not easily shared.

The Promoting Interoperability program relies on provider grants and incentives and does not encourage data sharing. In addition, the U.S. healthcare reimbursement system remains fragmented and does not promote interoperability. Only where revenue comes into play does integration occur. For example, most medical claims are transmitted electronically, because this accelerates payments. Laboratories are generally well-connected to the internal providers who order tests, because this link supports reimbursement.

ELR: Key to pandemic response and core to healthcare interoperability

Electronic Laboratory Reporting (ELR), the electronic transmission of laboratory data from laboratories to public health entities for reportable conditions, was included as part of the Meaningful Use program and has been in place for quite some time in many healthcare organizations.

The Laboratory Response Network (LRN), created by the Centers for Disease Control and Prevention (CDC) more than two decades ago, uses a variety of networks to handle emerging threats, such as a pandemic. The primary function of the LRN is to provide rapid detection of bio-threat and emerging agents of infectious diseases. The strength of the LRN lies in its standardized approach and its tiered capability construct – with sentinel clinical laboratories serving at the foundation to quickly recognize, rule-out, or refer potential bio-threat agents to the LRN reference laboratories. The LRN structure for biological threats is national laboratories, reference laboratories, and sentinel laboratories, including more than 150 state and local public health, military, international, veterinary, agriculture, food, and water testing laboratories. LRN labs must report their results to the CDC through an electronic portal called the Public Health Information Network Messaging System (PHIN-MS).

The LRN receives about 20 million reports annually, of which approximately 80 percent are electronic, via an HL7 interface. Nevertheless, participation in electronic reporting remains uneven, with larger labs reporting electronically, but hospital labs less likely to do so.

21st Century Cures Act pushes interoperability but faces delays

The 21st Century Cures Act, signed into law in December 2016, was also a push toward nationwide clinical data sharing. The Cures Act intends to provide $6.3 billion over a decade to the National Institutes of Health (NIH) for the Precision Medicine Initiative, Cancer Moonshot, BRAIN Initiative, and regenerative medicine using adult stem cells.[1]

Part of the act's overarching goals are to achieve widespread interoperability among health IT systems and improve patient accessibility to their medical information. The Cures Act includes

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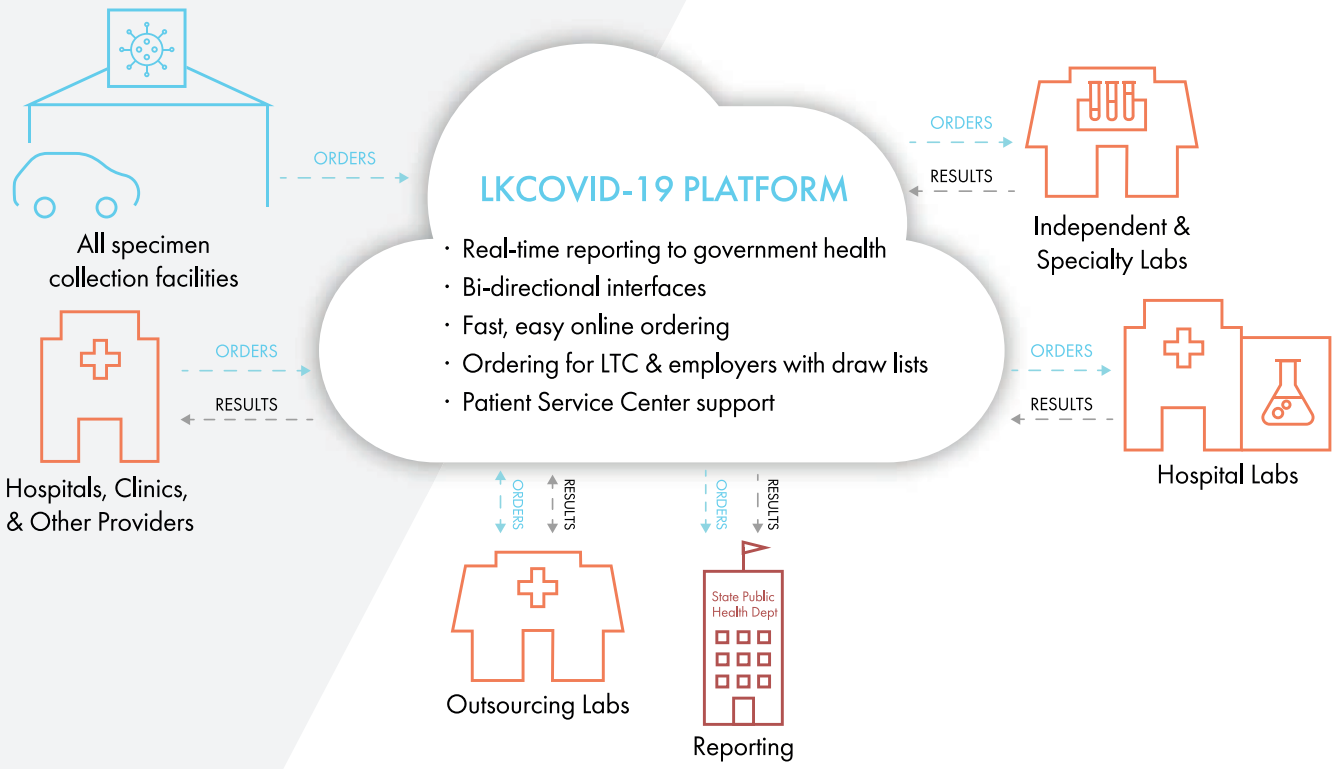

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specific measures that promote these initiatives, and they've been implemented through separate rules issued by the Office of the National Coordinator for Health Information Technology (ONC) and the Centers for Medicare & Medicaid Services (CMS).

Due to the COVID-19 pandemic, in October 2020, ONC announced a second extension of the timeline for implementation of its regulations related to the 21st Century Cures Act until 2021.[2] Enforcement for specific regulations implemented by CMS ranges from 2021 to 2022.[3]

CDC COVID-19 Reporting Guidelines	
Laboratories should make every reasonable effort to provide the following data elements to state and jurisdictional health departments:	
1	Test ordered – use harmonized LOINC codes provided by CDC
2	Device identifier
3	Test result – use appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by CDC
4	Test result date (date format)
5	Accession # / Specimen ID
6	Patient age
7	Patient race
8	Patient ethnicity
9	Patient sex
10	Patient residence ZIP code
11	Patient residence county
12	Ordering provider name and nonpharmaceutical interventions (as applicable)
13	Ordering provider ZIP code
14	Performing facility name and CLIA number
15	Performing facility ZIP code
16	Specimen source – use appropriate LOINC, SNOMED-CT, or SPM4 codes, or equivalently detailed alternative codes
17	Date test ordered (date format)
18	Date specimen collected (date format)
The following additional demographic data elements should also be collected and reported to state or local public health departments:	
1	Patient name (last name, first name, middle initial)
2	Patient street address
3	Patient phone number with area code
4	Patient date of birth
5	Ordering provider address
6	Ordering provider phone number

Figure 1

Laboratory interoperability requires standards

A key element of laboratory interoperability is the use of terminology standards that reduce the time spent exchanging, tracking, and reporting tests. Observational data findings from a provider encounter, laboratory data, and other diagnostic information are translated via standard terminologies that can be understood by all information systems. These terminology standards include LOINC and SNOMED-CT codes.

Additionally, there is a collaborative group called the Systemic Harmonization and Interoperability Enhancement for Lab Data Shield (SHIELD) that is working to "improve the quality, utility and portability of electronic laboratory data through the harmonized implementation of semantic data standards that have been appropriately qualified by a sole authoritative source."[4] SHIELD, which is an initiative of the Medical Device Innovation Consortium, supports the provision of standardized codes from manufacturers to laboratories to consistently identify tests in the LIS and EHR. This standardization allows laboratory data to be consistently interpreted by information systems, thereby promoting interoperability. Improving the semantic interoperability of laboratory data between organizations allows diagnostic information to be more efficiently used to support clinical decisions.

There is also a COVID-19 Interoperability Alliance, which is a collaboration between healthcare industry stakeholders to provide a collection of value sets for clinical, demographic, and administrative terms relating to COVID-19. The Alliance's goals are to provide these resources to the healthcare industry to support data aggregation and interoperability that will allow the nation to gain a greater understanding of the pandemic.[5]

The CARES Act addresses lab data reporting

Laboratory testing is one of the most significant parameters necessary to track and mitigate a pandemic. Public health management of the COVID-19 response needs to be highly organized, including accurate laboratory testing, standardized test results, data sharing, and the capture of demographic information to allow for contact tracing, mitigation, and control of the virus.

In response to the SARS-CoV-2 pandemic, the Coronavirus Aid, Relief, and Economic Security Act (CARES Act), a $2.2 trillion economic stimulus bill, was signed into law in March 2020. The CARES Act intends to provide timely economic assistance to American families and small businesses, addressing a number of areas of need (e.g., direct payments, unemployment, payroll taxes, retirement funds, support for hospitals, PPE, testing supplies, vaccination costs, etc.).

A portion of the CARES Act, Section 18115, requires laboratories to report results of COVID-19 testing daily to the Secretary of the Department of Health and Human Services and

appropriate state or local public health departments. See **Figure 1** for the required data elements.[6]

Data sharing needed to advance healthcare system and address pandemic

Lack of data interoperability in healthcare, with clinical and financial data residing across different systems, has caused extensive challenges for many years. Disparate data has prevented both payers and providers from achieving a comprehensive view of the patient record and slowed the shift to value-based care. Lack of shared data has also diminished the healthcare industry's ability to proactively respond to the COVID-19 pandemic.[7]

Quickly and effectively communicating lab orders and results across care locations and between treating providers is of extreme value in patient care. Sharing diagnostic information through integration of the LIS to other information systems, including the EHR, is a crucial cornerstone of interoperability. Sharing patient data is also a foundational need for the management of public health crises, such as COVID-19. The importance of interoperability needs to be understood, communicated, and embraced within the healthcare system, backed with regulatory measures and an incentive structure that encourages interoperability between vendors and healthcare organizations.

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Kim Futrell, MT(ASCP), MSHI, is the Senior Strategic Marketing Manager at **Orchard Software**.

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Rapid molecular and antigen tests to detect SARS-CoV-2 deliver results quickly and efficiently

Role of rapid testing during the COVID-19 pandemic

By Linda Wilson

As the COVID-19 pandemic has surged into its second year, rapid tests to detect SARS-CoV-2 and deliver results quickly have become more prevalent.

The gold standard to diagnosis COVID-19 is polymerase chain reaction (PCR), but turnaround times for these tests have lagged at times during the pandemic for a variety of reasons, such as the sheer volume of specimens or shortages of supplies, testing platforms, and personnel.

As a result of inconsistent turnaround times for PCR tests, rapid tests have gained prominence during the pandemic. The number of rapid tests – which the Infectious Disease Society of America (IDSA) defines a rapid test as one that returns results in less than one hour – [1] have been increasing, too.

There are two major categories of rapid tests: molecular and antigen. While rapid antigen tests usually are antibody-based and capture SARS-CoV-2 antigens (typically N protein), the rapid molecular tests detect viral RNA.

The tests often can be performed at the point-of-care, such as a doctor's office or pharmacy, reducing the number of steps between specimen collection and results. The tests also are moving into the home. For example, the U.S. Food and Drug Administration granted emergency use authorization (EUA) in November 2020 to the first COVID-19 test that consumers can perform completely at home.[2]

The Department of Health and Human Services (HHS) has said that rapid tests should supplement laboratory testing in certain situations, such as emerging outbreaks, or locations where laboratory-based tests are not readily available, such as rural areas. Using rapid testing also makes sense in situations, such as congregate living facilities, where people are tested often. This would include nursing homes, prisons, and college dormitories.

Indeed, the federal government has purchased and distributed rapid tests to screen people living in nursing homes, and it has developed rules about how often staff and residents should be tested.

In the case of the antigen tests specifically, their speed, simplicity and low cost could foster better containment of the virus by allowing millions of people to be tested daily, increasing the number of infected people who quarantine and reducing of spread of the virus.

However, antigen tests tend to be less sensitive than molecular tests. And some studies have suggested that the antigen tests may not detect the virus in patients who have had symptoms for more than five days, leading to concerns about how well the tests perform, particularly among asymptomatic people.[1]

According to the Centers for Disease Control and Prevention (CDC), the specificity of antigen tests is generally as high as most molecular tests, which means that false positive test results are not likely if the manufacturer's instructions are followed correctly. The CDC also notes, however, that false positive results may occur in communities where the rate of infection is low, which is true for all in vitro tests.[3] The agency defines low prevalence as when the positivity rate for NAAT tests is less than 5 percent, or where there are fewer than 20 new cases per 100,000 people within the last 14 days.[3] (See chart on page 28).

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COVID-19 Rapid Molecular Test Updates					
Company Name	**Website**	**Name of Molecular Test**	**Type of Test**	**Platform/Application**	**Time to Results**
Abbott Diagnostics Scarborough	https://www.abbott.com/IDNOW.html	ID NOW COVID-19	RT, Isothermal Amplification	ID NOW System	~13 minutes
BioFire Diagnostics	https://www.biofiredx.com/products/the-filmarray-panels/filmarray-respiratory-panel-ez/	BioFire Respiratory Panel 2.1-EZ (RP2.1-EZ)	RT, Nested multiplex PCR, Multi-Analyte	CLIA-Waived BioFire FilmArray 2.0 EZ Configuration System	about 45 minutes
Cepheid	https://www.cepheid.com/coronavirus	Xpert Xpress SARS-CoV-2/Flu/RSV	Real-time RT-PCR, Multi-Analyte	GeneXpert II, IV, XVI, Infinity 48, Infinity 80, and Xpress Systems	25-36 minutes
Cue Health	https://www.cuehealth.com/what-is-cue/how-cue-detects-covid-19/	Cue COVID-19 Test	RT, Isothermal Amplification	Cue Health Monitoring System with Cue Cartridge Reader and Cue Health App	20 minutes
Lucira Health	https://www.lucirahealth.com/	Lucira COVID-19 All-In-One Test Kit	Prescription Home Testing	"Test Unit: a single-use, battery operated, disposable unit with lyophilized reagents for multiplexed amplification and electronic readout for detection of SARS-CoV-2 RNA"	30 minutes
Mesa Biotech	https://www.mesabiotech.com/	Accula SARS-Cov-2 Test	RT and Amplification	Accula System Dock	~30 minutes
Roche Molecular Systems	https://diagnostics.roche.com/us/en/products/params/cobas-sars-cov-2-influenza-a-b-nucleic-acid-test.html	cobas SARS-CoV-2 & Influenza A/B Nucleic Acid Test for use on the cobas Liat System	Real-Time RT-PCR, Multi-Analyte	cobas Liat System	20 minutes

COVID-19 Rapid Antigen Test Updates					
Company Name	**Website**	**Name of Molecular Test**	**Type of Test**	**Platform/Application**	**Time to Results**
Abbott Diagnostics Scarborough	https://www.globalpointofcare.abbott/en/product-details/binaxnow-covid-19-home-test-us.html?gclid=Cj0KCQiA3smABhCjARIsAKtrg6lTuP971b7afazobpLNeHbrWD9iILyldBfiE_0fY-4YVHUTSDRkHFQaAsDYEALw_wcB	BinaxNOW COVID-19 Ag Card Home Test	Lateral Flow, Visual Read, Prescription Home Testing	NAVICA mobile app in collaboration with eMed digital health solution	15 minutes
Access Bio	https://accessbiodiagnostics.net/carestart-covid-19-antigen/	CareStart COVID-19 Antigen test	Lateral Flow, Visual Read	N/A	10 minutes
Becton, Dickinson and Company (BD)	https://www.bd.com/en-us/offerings/capabilities/microbiology-solutions/point-of-care-testing/bd-veritor-plus-system-for-rapid-covid-19-sars-cov-2-testing	BD Veritor System for Rapid Detection of SARS-CoV-2	Chromatographic Digital Immunoassay, Instrument Read	N/A	15 minutes
Ellume Limited	https://www.ellumehealth.com/products/consumer-products/covid-home-test	Ellume COVID-19 Home Test	Lateral Flow, Fluorescence, Instrument Read, Over the Counter (OTC) Home Testing, Screening	Bluetooth connected analyzer for use with an app	15 minutes
Luminostics	https://luminostics.com/	Clip COVID Rapid Antigen Test	Lateral Flow Immunoluminescent Assay, Instrument Read	Clip Analyzer	30 minutes
LumiraDx UK Ltd.	https://www.lumiradx.com/us-en/what-we-do/diagnostics/test-technology/antigen-test	LumiraDx SARS-CoV-2 Ag Test	Microfluidic Immunofluorescence Assay, Instrument Read	LumiraDx Instrument	~12 minutes
Quidel Corporation	https://www.quidel.com/immunoassays/quickvue-sars-antigen-test	QuickVue SARS Antigen Test	Lateral Flow, Visual Read	N/A	10 minutes
Quidel Corporation	https://www.quidel.com/immunoassays/sofia-2-flu-sars-antigen-fia	Sofia 2 Flu + SARS Antigen FIA	Lateral Flow, Fluorescence, Instrument Read, Multi-Analyte	Sofia 2 Analyzer	15 minutes
Quidel Corporation	https://www.quidel.com/immunoassays/rapid-sars-tests/sofia-sars-antigen-fia	Sofia SARS Antigen FIA	Lateral Flow, Fluorescence, Instrument Read	Sofia Analyzer/ Sofia 2 Analyzer	15 minutes

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Pathophysiology of COVID-19-associated coagulopathy and its impact on laboratory measures of coagulation

By Andreas R. Rechner, PhD; John V. Mitsios, PhD; Christine Dahler, GCE

Severe Acute Respiratory Syndrome Coronavirus – 2 (SARS-CoV-2) is the virus that causes COVID-19, which has ravaged the world since January 2020, infecting and killing millions of people worldwide. Based on the global numbers of the total number of individuals infected, approximately 2 percent of confirmed SARS-CoV-2 infections succumb to the disease. The infection may be asymptomatic or may cause a broad range of symptoms including, but not limited to, mild symptoms of the upper respiratory tract, cytokine storm, multi-organ failure, life-threatening sepsis, and COVID-19-associated coagulopathy, which causes serious thrombotic complications. The exploration and understanding of the mechanisms behind the severe course of COVID-19 is crucial to improve treatment, recovery, and ultimately the survival rate of hospitalized patients. Laboratory parameters are not only essential to better understand the physiological mechanisms of the disease, but also to monitor disease progression and evaluate the effectiveness and success of treatment.

The cytokine storm

An early sign of severe COVID-19, and a predictor for worse outcomes, is the occurrence of the cytokine storm. A cytokine storm is an unregulated and excessive release of pro-inflammatory cytokines, first locally in the infected lung and later, systemic throughout the body.[1] High plasma levels of inflammatory markers [C-reactive protein (CRP), serum amyloid A (SAA), ferritin, procalcitonin] and cytokines [Interleukin-6 (IL-6), Interleukin-10 (IL-10) and Tumor Necrosis Factor-α (TNF-α)] are indicators of a hyperinflammatory response and an underlying cytokine storm.[2,3] The increase in pro-inflammatory cytokines (in particular, IL-6, TNF- α, and IL-10), which are elevated in patients with COVID-19, are indicators of a cytokine storm and are associated with disease progression and outcomes in severe COVID-19 patients.[4,5] Currently, it is unclear if immune hyperactivity, dysregulation of the inflammatory response to the viral infection, or immune dysregulation causes the progression to severe COVID-19.[1]

COVID-19-associated coagulopathy

The cytokine storm, with its excessive release of pro-inflammatory cytokines, may also play a key role in the pathophysiology of COVID-19-associated coagulopathy by activating endothelial cells and leukocytes – in particular, neutrophils – which in response, produce neutrophil extracellular traps (NETs), a process called NETosis. NETs promote thrombus formation, amplify cytokine production, and play a significant role in the pathophysiology of COVID-19.[6] In addition to the activation of endothelial cells and neutrophils, the direct interaction of the virus with the contact system, part of the innate immune system – specifically with that of factor XII (FXII) and plasma prekallikrein – may also contribute to the highly prothrombotic environment at the site of infection.[7] The formation of activated

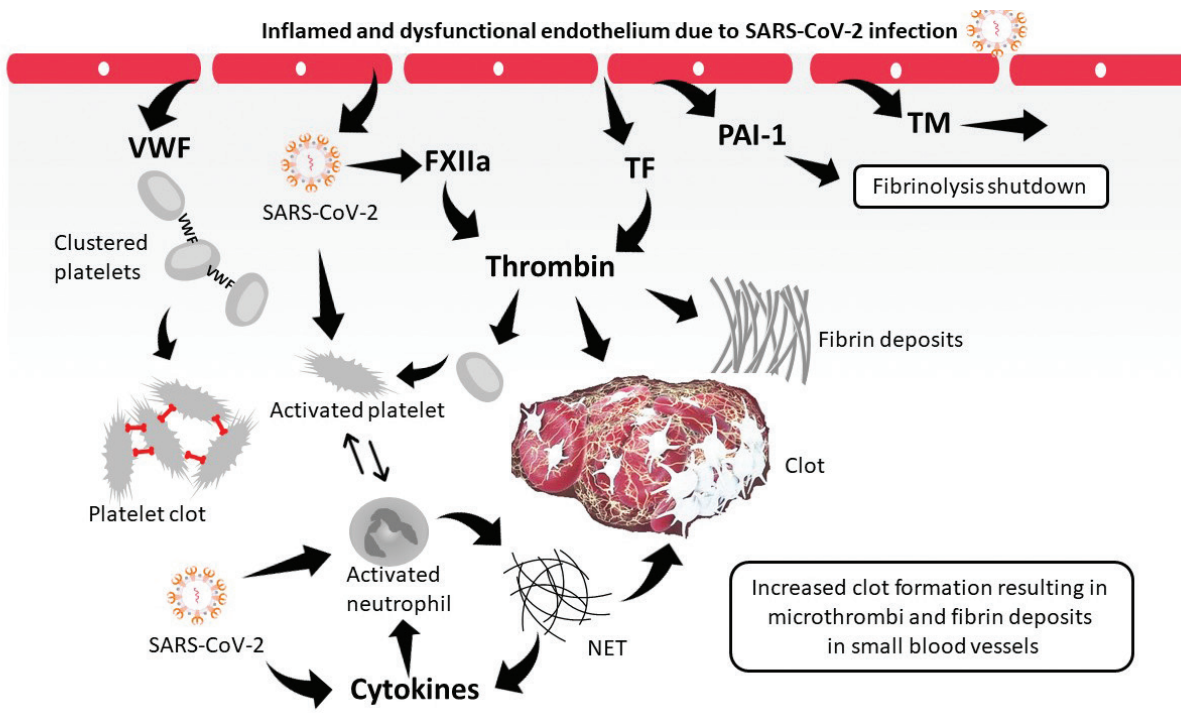

Figure1. Scheme of the pathophysiological mechanisms of COVID-19-associated coagulopathy.

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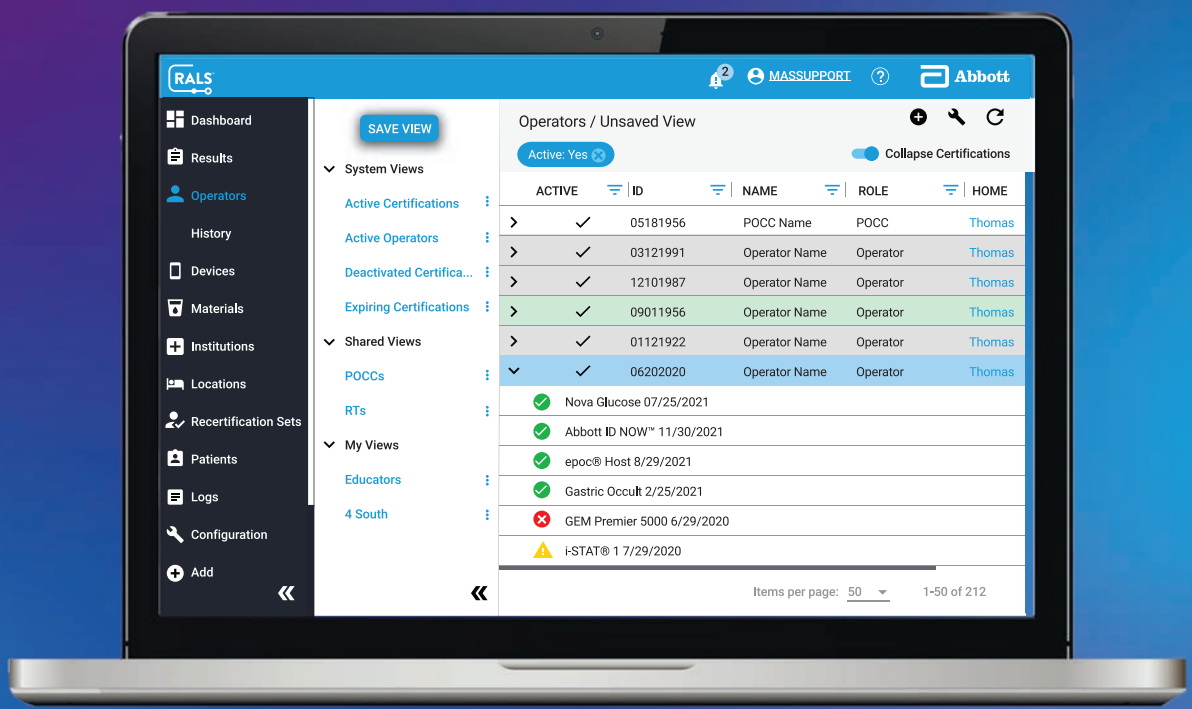

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FXII via SARS-CoV-2 contact may not only initiate thrombosis via the intrinsic pathway of coagulation [e.g., increase thrombin generation, fibrin formation (microthrombosis), fibrinolysis, and increased D-dimer levels], but also the production of bradykinin, which increases vascular dilation and permeability. Thus, SARS-CoV-2 directly and indirectly influences the coagulation system, creating a highly prothrombotic state in patients with COVID-19. **Figure 1** schematically summarizes the pathophysiological mechanisms of COVID-19-associated coagulopathy.

The more than a century-old concept of Virchow`s triad shows how three factors contribute to thromboembolic risk: endothelial dysfunction, blood flow/stasis, and hypercoagulability. The three factors highlighted by Virchow can explain the venous and arterial hypercoagulable state seen in COVID-19 patients (**Figure 2**).[8]

In summary, thrombosis in COVID-19 patients is a complex and multifactorial process, and COVID-19-associated coagulopathy must be considered to successfully treat and prevent potentially lethal thrombotic complications.

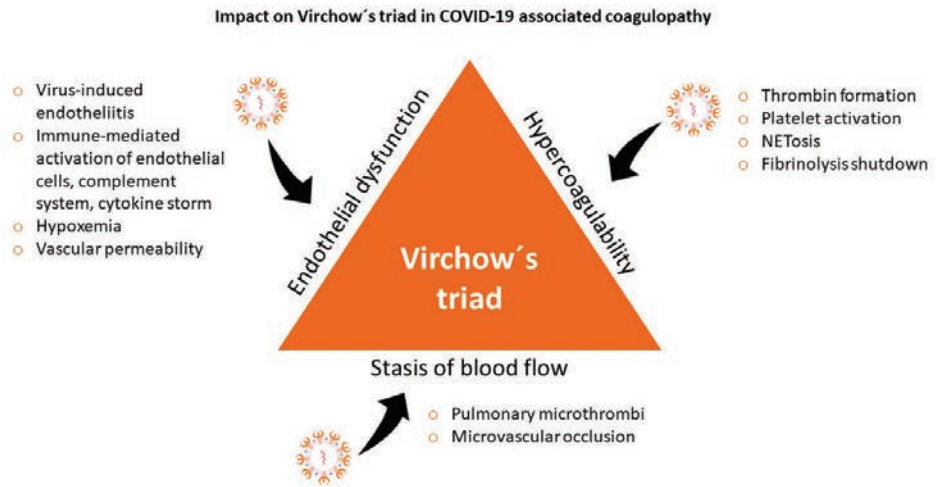

Figure 2. The impact of SARS-CoV-2 infection on Virchow´s triad of thrombosis

Thrombosis in COVID-19

While it is not unusual for infections to raise the risk of thrombosis, the highly prothrombotic state in COVID-19 patients causes an unprecedented range of thrombosis-related disorders in affected patients. From benign skin lesions on the feet (e.g., COVID toe), to life-threatening thrombotic events, the SARS-CoV-2 virus has demonstrated a strikingly high prevalence of deadly blood clots. Early studies have shown that approximately 25-45 percent[9-11] — or even up to 70 percent[12] — of critically ill patients have a confirmed venous thromboembolism (VTE), such as deep vein thrombosis (DVT) or pulmonary embolism (PE). Approximately 70 percent of COVID-19 patients who died had met

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Laboratory Coagulation Parameter	Change in COVID-19	Indication
D-dimer	↑↑	Increased clot formation
Prothrombin time	↑	Unbalanced extrinsic coagulation
Fibrinogen	↑ (acute phase) ↓ (DIC phase)	Inflammation DIC
Platelet count	↓/↑	Increased platelet consumption
Von Willebrand Factor (VWF)	↑↑	Endothelial dysfunction and platelet activation
Coagulation Factor VIII	↑↑	Thrombotic risk
Plasminogen Activator Inhibitor-1 (PAI-1)	↑↑	Endothelial dysfunction/fibrinolysis shutdown
Prothrombin fragment 1+2	↑↑	Increased clot formation
Soluble thrombomodulin	↑↑	Endothelial dysfunction/decreased anticoagulant activity of endothelium

Table 1. Significantly altered laboratory parameters of coagulation and their indication in COVID-19

the International Society of Thrombosis and Hemostasis (ISTH) criteria for disseminated intravascular coagulation (DIC).[13] Analyzing available study data, the weighted mean prevalence of VTE was found to be as high as 31.3 percent in COVID-19 patients,[11] while the pooled incidence of VTE in COVID-19 patients admitted to the ICU was 28 percent.[14] The incidence of VTE can steadily increase in hospitalized patients during severe COVID-19, from 16 percent after 1 week to 42 percent after 3 weeks.[15] When comparing those findings with the incidence of VTE in patients with pneumonia following respiratory tract infections, the rate of VTE in COVID-19 patients is 7- to 8-fold higher.[11]

An autopsy on 12 deceased COVID-19 patients revealed DVT in 7 of 12 patients (58 percent), while PE was the direct cause of death in 4 patients (33 percent).[16] A histological analysis of pulmonary vessels in COVID-19 patients showed thrombosis and endotheliitis throughout the pulmonary vasculature, with alveolar capillary microthrombi being ninefold more prevalent in COVID-19 patients than in patients with H1N1 influenza.[17] The high incidence of thromboembolic events in COVID-19 patients, which are also frequently the cause of death, highlight the importance of diagnosing and treating COVID-19-associated coagulopathy. One of the most relevant laboratory parameters for diagnosing and monitoring COVID-19-associated coagulopathy is the determination of plasma D-dimer levels. Other laboratory parameters of coagulation are also altered in COVID-19 patients, as described in **Table 1.**

Following a COVID-19 diagnosis, hemostasis testing, monitoring, and therapy have been shown to play a decisive role in COVID-19 patient management.

D-dimer – a primary marker for COVID-19-associated coagulopathy

Elevated D-dimer levels were found to be a crucial laboratory marker to indicate a thrombotic risk in COVID-19 patients.[18,19] However, when interpreting D-dimer results, several aspects must be considered. For example, D-dimer levels can increase with age and are elevated during pregnancy.[20] In addition, high plasma D-dimer levels are also observed in a variety of clinical conditions including but not limited to DIC, sepsis, inflammation, DVT/PE, immobility, liver disease, malignancy, recent surgery, preeclampsia, and trauma. D-dimer is primarily used to rule out DVT/PE in low risk-patients (non-hospitalized), due to its high negative predictive value, and to diagnose and monitor DIC in conjunction with other laboratory parameters. It is also important to note that D-dimer is not a clearly defined antigen. It consists of multiple D/E-fragments of different molecular weights. Thus, the D-dimer antigen is heterogenous, as are the antibodies used to measure it. As a result, the D-dimer assays cannot yet be standardized to an international standard. Each assay uses its own calibration material, which means comparing or transferring D-dimer results has to be done with great caution. Some assays use fibrinogen equivalent units (FEU), while other assays report in D-dimer units (DDU). D-dimer results are more commonly reported in FEUs (FEU = 2xDDU).

One of the first reports highlighting the importance of D-Dimer was a study of 191 hospitalized COVID-19 patients in Wuhan (137 survivors and 54 non-survivors), which identified elevated D-dimers levels greater than 1 µg/mL FEU (odds ratio: 18.42, 2.64–128.55; p=0·0033) on admission as a strong predictor of in-hospital death.[21] Another retrospective study from Wuhan, China, looked at 343 COVID-19 patients, of whom 330 survived and 13 died.[22] A D-dimer cut-off of 2 µg/mL was derived by receiver operator characteristics (ROC) curve analysis which yielded 92.3 percent sensitivity and 83.3 percent specificity in predicting in-hospital mortality. In addition, using Kaplan-Meier curves, patients with COVID-19 with D-dimer levels >2 µg/mL FEU on admission were shown to be 50 times more likely to die than patients with D-dimer levels <2 µg/mL FEU.

Comparable results regarding the D-dimer cutoff and its predictive power were found in a study on 248 hospitalized COVID-19 patients with 17 non-survivors.[23] ROC curve analysis revealed a cutoff of >2.14 µg/mL FEU of D-dimer on admission to predict death, with an odds ratio (OR) of 10.17 (95 percent CI 1.10-94.38, P=0.041).

Another multicenter retrospective study that included 400 hospitalized patients showed that an elevated D-dimer >2.5 µg/mL at initial presentation was predictive of coagulation-associated complications during hospitalization, including thrombosis, bleeding, critical illness, and death.[19]

As increased D-dimer levels are common in COVID-19, the exclusion or diagnosis of VTE using plasma D-dimer may need to be adapted. The widely used cut-off for the exclusion of VTE in non-COVID-19 patients of <0.5 µg/mL FEU is not suitable. A study analyzing VTE in 100 COVID-19 patients admitted to the intensive care unit (ICU) calculated an optimal cutoff for the exclusion of VTE of <2.0 µg/mL applying ROC curve analysis to their data set.[24] However, a cutoff value of >8.0 µg/mL provided an optimal sensitivity and specificity for the diagnosis of VTE in COVID-19 patients. The authors conclude that those two D-dimer cutoffs may be useful to identify patients with a low or high probability for the presence of VTE.

The above studies indicate that D-dimer can be used as an early prognostic marker for COVID-19 progression, including mortality and thrombotic complications, as well as a helpful marker to improve management and outcomes in COVID-19 patients.

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Primary Presenter

John Toffaletti, PhD, Professor of Pathology
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Learning Objectives

- Describe the physiology of urea and creatinine production and handling of these metabolites by the kidney
- Understand physiology of the kidneys and how kidney handling of urea and creatinine change with disease severity
- Apply and interpret the BUN/Creatinine ratio in critical illness

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Other markers of COVID-19-associated coagulopathy

Although D-dimer has been used extensively as a laboratory marker in COVID-19, other laboratory markers of coagulation are also altered.[25] For example, prothrombin fragment 1+2, an early indicator of thrombin formation, was found to be markedly increased in COVID-19 patients.[26] In addition, fibrinogen, von Willebrand Factor (VWF) and factor VIII (FVIII), hemostasis-associated acute-phase reactant proteins, are also elevated in COVID-19 (**Table 1**).[25,27] Fibrinogen levels are increased in hospitalized patients due to hyperinflammatory response.[19] However, early studies have shown a markedly reduced fibrinogen mimicking levels associated with DIC in patients with severe COVID-19.[13] The significant increase in FVIII and VWF not only confer an increase in thrombotic risk in COVID-19 patients, but are also markers of endothelial activation.[25]

High plasma levels of VWF promote tethering of platelets to the inflamed endothelium, which may lead to platelet activation as shown by increased P-selectin expression of platelets isolated from COVID-19 patients (**Fig. 1**).[28] Platelets from severe COVID-19 patients were assessed under high shear conditions using the PFA-200 system, which showed decreased closure times compared to those of patients with intermediate COVID-19, indicating that platelets of patients with severe COVID-19 are hyperactive. [28]

Increased plasma levels of soluble thrombomodulin and plasminogen activator inhibitor-1 (PAI-1) have also been reported.[25] Only thrombomodulin bound to the extracellular membrane of endothelial cells can convert activated thrombin (FIIa) from being prothrombotic to being antithrombotic. An increase in soluble thrombomodulin means a decrease in membrane-bound thrombomodulin and its ability to attenuate clot formation. PAI-1 inhibits fibrinolysis, the endogenous process of resolving blood clots. However, under high concentrations, as seen in inflammatory responses, PAI-1 can tip the balance of coagulation in the direction of thrombosis.

Furthermore, Nicolai and colleagues[28] evaluated the formation of neutrophil extracellular traps, or NETosis, in patients with COVID-19. Conducting an experiment in which platelet-rich plasma isolated from either healthy donors or COVID-19 patients was incubated with control neutrophils, and analyzing the formation of NETs by confocal microscopy, an enhanced NETosis was noticed in severe COVID-19 patients.

The alteration of all those coagulation parameters in plasma are pointing in the same direction: COVID-19-associated coagulopathy is a hypercoagulable and highly prothrombotic state requiring thorough treatment and monitoring.

Therapy and monitoring of COVID-19-associated coagulopathy

Various societies and expert groups have issued treatment and monitoring recommendations for COVID-19-associated coagulopathy.[29-33] Daily measurements of D-dimer and fibrinogen levels, prothrombin time (PT), and platelet count are indicated to detect and monitor COVID-19-associated coagulopathy, as well as the success of anticoagulant therapy. Anticoagulation with prophylactic to therapeutic doses of preferably low-molecular weight heparins (LMWHs), or unfractionated heparins (UFH), is strongly recommended. Dosing depends on disease status and risk profile of the patient.[29-32] Therapeutic monitoring of the heparins using anti-FXa-assays – but not the APTT (activated partial thromboplastin time) – is preferred. Anticoagulant therapy success may be monitored using plasma D-dimer levels.

Several clinical trials are currently underway to help optimize treatment protocols from drug dosing, application timing, and the choice of the right anticoagulant drug(s) and other potentially helpful drugs, such as the antiplatelet drug clopidogrel, tissue plasminogen activator (tPA), thrombomodulin, and antithrombin. Anticoagulation predominantly with LMWH is now standard of care for hospitalized COVID-19 patients. But the high incidence of thrombotic complications in COVID-19, despite anticoagulation with LMWH, clearly shows that there is still a lot room for improvement in treating and managing COVID-19-associated coagulopathy.

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Andreas R. Rechner, PHD, works in Global Scientific Marketing for Hemostasis & Plasma Proteins at **Siemens Healthcare Diagnostics Products** in Marburg, Germany.

John V. Mitsios, PhD, is a senior clinical consultant for hemostasis at **Siemens Healthineers**.

Christine Dahler, GCE, works in hemostasis global marketing at **Siemens Healthineers**.

Neutralization of red cell antibodies using soluble proteins: application to anti-CD38 interferences

By Ghislain Noumsi, MD, MBA, SBB, and Lauro Guerra, MLS, SBB

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Soluble substances with structures similar to blood group antigens have been used for decades in immunohematology testing to inhibit red cell antibodies with corresponding reactivity. This is a particularly useful technique to separate specificities in samples with multiple antibodies, to identify antibodies against a high prevalence antigen, or to eliminate reactivity attributed to a panreactive antibody, allowing for the identification of potential underlying non-neutralizable antibodies.

In this test, the neutralizing substance is first incubated with the serum, allowing the substance to bind to the variable regions of the target antibody. After incubation, the treated serum is then used to perform antibody identification. Several substances with blood-group antigen specificity are naturally present in body fluids, such as saliva, urine and plasma (**Table 1**)[1]. In addition, recombinant blood group proteins have been recently produced for other blood group antigens specificities, extending the potential application of the neutralization technique to a higher number of red blood cell antibodies.[2]

The resolution of drug-interferences during antibody identification is another area where the neutralization technique has been proven to be useful. Monoclonal antibodies represent a new and rapidly evolving class of immunotherapeutics used in the treatment of various conditions, such as solid tumors, leukemia and infections, as well as cardiovascular and inflammatory diseases.[3]

On November 16, 2015, the U.S. Food and Drug Administration (FDA) approved the first monoclonal antibody, daratumumab or DARA (anti-CD38), for the treatment of patients with multiple myeloma.[5] Since then, several trials have been performed to extend the application of this new drug, or to develop other monoclonal antibodies with similar indications.[6] During the phase I and II trial with DARA, the investigators observed some unexpected interferences in routine immunohematology testing. More specifically, all patients receiving DARA showed a positive indirect antiglobulin test (IAT), rendering difficult the identification of true red cell antibodies among these patients, and complicating the selection of suitable products for transfusion.[7] These observations have been confirmed by numerous reports and are well-documented.[8,9,10]

Mitigating DARA interferences in immunohematology

Experts have proposed several methods to overcome the anti-CD38 interferences in immunohematology testing: chemical or enzymatic treatment of red cells; use of umbilical cord red cells for testing; extended phenotyping before or genotyping after initialization of DARA treatment; blocking of CD38 epitopes on the red cells using DARA-Fab fragments; and neutralization of DARA in patient's serum using human soluble CD38 or mouse anti-DARA idiotype antibody.

Chemicals and enzymes

Among chemicals and enzymes used in routine immunohematology, dithiothreitol (DTT), papain and trypsin have been shown with different degrees of reproducibility to mitigate the interaction of DARA. DTT is a thiol-reducing agent that denatures red cell surface CD38 by disrupting the disulfide bonds in the molecule's extracellular domain, therefore, preventing anti- CD38 from binding to the red cell.[11] Papain and Trypsin are proteolytic enzymes that remove sialic acid residues on the red cell surface, modifying the structure of the corresponding proteins. By altering or cleaving the CD38 protein on the red cell, papain and trypsin have been shown to reduce interferences due to DARA treatment.[12,13] The downside to using these chemicals and enzymes is the fact that, in addition to modifying CD38 expression on the red cell, they also denature other blood group antigens, impairing the identification of the corresponding antibodies in the patient's serum (**Table 2**). Patients who have developed those specific antibodies are at high risk of a hemolytic transfusion, unless antigen negative blood is systematically provided to compensate for the effects of the enzyme treatment. This is particularly true for some DARA-patients who might develop anti-Kell antibodies after exposure to Kell antigens, or for who an existing anti-Kell may not be easily identified using DTT treated cells.

Umbilical cord red cells

Cord red cells have shown to be nonreactive with DARA. The interferences observed when testing adult red cells were not seen when using cord cells. Some laboratories have successfully established protocols using cord blood to mitigate the interferences observed in DARA patients.[14] However, these techniques are not readily available in all laboratories because of the limited supply of cord cells. In addition, some red cells antigens are not expressed or only weakly present on cord cells,[15] limiting their effectiveness to positively identify numerous clinically significant antibodies in DARA-patients.

Antibody	Source of neutralizing substance
Anti-P1	Hydatid cyst fluid, pigeon droppings, pigeon egg whites
Anti-Lewis	Plasma or serum, saliva
Anti-Chido, Anti-Rodgers	Serum (contains complement)
Anti-Sda	Urine
Anti-I	Human breast milk

Table 1. Source of substances used for neutralization of certain antibodies. Source: See reference 1 and 4.

DTT		Papain	Trypsin	
Kell Indian JMH LW Dombrock	Cromer Cartwright Lutheran MER2 Knops	Chido-Rodgers Indian JMH MNSs Ge2, Ge4 Duffy	Indian JMH Lutheran MER2 Chido-Rodgers MN	Knops Dombrock Cartwright Ge2, Ge3, Ge4 Scianna

Table 2. Examples of red cells antigens denatured or weakened by treatment with DTT, papain or Trypsin. Source: See reference 15.

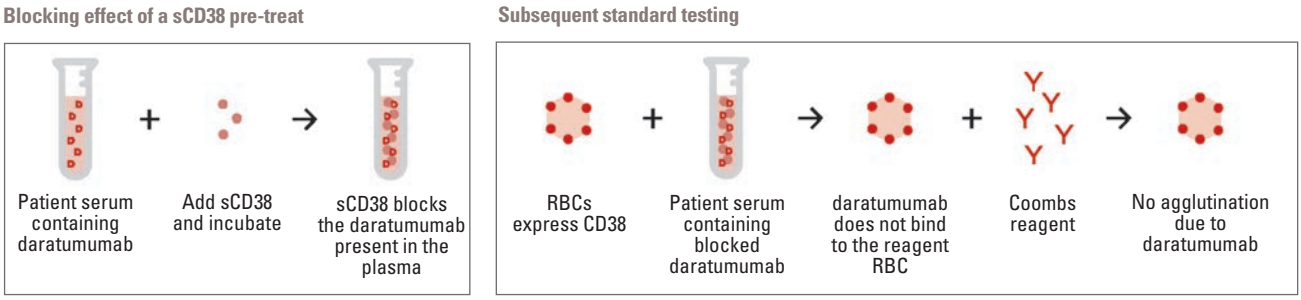

Figure 1. Neutralization of DARA using sCD38

Extended phenotyping or genotyping

Performing an extended blood-group phenotyping prior to initiating DARA treatment, or blood group genotyping after initiation of DARA, is an alternative strategy for dealing with the interferences seen during immunohematology testing.[10] The rationale supporting this strategy is that extended phenotypically matched blood can be provided to the DARA-patient if needed, without the need for extensive additional testing. In order for this strategy to be efficiently implemented, there is a need for coordination between the oncologist in charge of the patient, the pharmacy, and the immunohematology laboratory. In addition, blood group genotyping needs to be accessible in a timely and cost-effective manner to guarantee optimal benefits for the patient.[16] Also, according to current practices across laboratories, providing extended phenotypically matched blood does not eliminate the need for performing antibody screening or identification for transfused DARA-patients. Identifying suitable blood donors that are extendedly matched to the patient is also an issue, especially in DARA-patients needing multiple blood transfusions.

Blocking and neutralization

Blocking the DARA binding site by using an anti-CD38 idiotype antibody demonstrated some success in laboratory experimentation.[7,17] However, this method has not been widely used because of the high cost for producing such highly DARA-specific antibodies and the difficulty optimizing a complex assay in routine immunohematology testing. The use of soluble substances – i.e. proteins – for neutralizing the reactivity of DARA is another method that was demonstrated in laboratory experiments. However, reagents available during these initial studies were not very concentrated and required larger volumes for neutralization, which posed a risk of diluting weakly reactive antibodies. Subsequently, a recombinant soluble CD38 (sCD38) was developed, allowing the use of smaller volumes of reagent for use for neutralization. With the development of a concentrated sCD38 reagent, and the fact that immunohematology laboratory technologists are familiar with neutralization techniques, it seemed logical to implement a CD38-neutralization method as an optimal approach to dealing with these interferences. CD38 neutralization uses a sCD38 reagent that, when incubated with the DARA-patient serum, will neutralize the reactivity of anti-CD38 present in the serum (**Figure 1**), allowing for the detection of any underlying antibodies.[18]

By testing the patient's plasma with sCD38-treated plasma, the absence of reactivity observed in the IAT can be considered as a negative antibody screen. To develop the sCD38 neutralization assay, multiple examples of different red cell antibody specificities were spiked with DARA, to a final concentration of ~0.5mg/mL DARA. Recombinant soluble CD38 (sCD38) was then added to the sample and incubated for 30 minutes at room temperature. After inhibition, spiked samples were tested using IAT-LISS, IAT-PEG and Gel methods (**Table 3**).

Conclusion

Different strategies are available for mitigating the interference of daratumumab (anti-CD38) with immunohematology testing or providing alternative approaches for hemotherapy, each with its pros and cons. One of these strategies, neutralization, which is based on the principle of inhibition, and has been in use in blood bank laboratories for decades. This test effectively overcomes the CD38 interferences and allows for accurate identification of potential underlying alloantibodies against red cell antigens, which may be present in the patient's plasma but masked by daratumumab interference. Additionally, the ruling out of underlying alloantibodies with a negative antibody screen with sCD38-treated plasma eliminates the need for an AHG crossmatch.

Antibody specificity	Tested red cells	IAT-LISS		IAT-PEG		Gel	
		Sample + DARA	Sample + (DARA+sCD38)	Sample + DARA	Sample + (DARA+sCD38)	Sample + DARA	Sample + (DARA+sCD38)
Anti-D high (titer = 1:32)	D+	4+	4+	4+	4+	3+	3+
	D-	1+	0	1+	0	2+	0
Anti-D low (titer = 1:2)	D+	2+	1+	2+	1+	2+	2+
	D-	1+	0	1+	0	2+	0
Anti-C	C+	1+	W+	2+	1+	3+	2+
	C-	1+	0	1+	0	2+	0
Anti-E	E+	2+	1+	2+	2+	2+	2+
	E-	1+	0	1+	0	2+	0
Anti-K	K+	2+	1+	2+	2+	2+	2+
	K-	1+	0	1+	0	2+	0
Anti-Jka	Jka+	1+	W+	1+	1+	2+	2+
	Jka-	1+	0	1+	0	2+	0
Anti-Fya	Fya+	2+	2+	3+	3+	3+	3+
	Fya-	1+	0	1+	0	2+	0
Anti-S	S+	2+	1+	2+	2+	2+	2+
	S-	1+	0	1+	0	2+	0

Table 3. Neutralization of anti-CD38 using sCD38 in samples containing various red cell antibody specificities

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Ghislain Noumsi MD, MBA, SBB, is the Director, Medical Affairs Asia-Pacific for **Grifols Diagnostic Solutions**. Noumsi is an immunohematologist specialist with clinical and laboratory expertise in the resolution of complex cases in transfusion medicine, and proven track record in the laboratory pharmaceutical industry.

Lauro Guerra, MLS, SBB, is a Laboratory Manager in **Grifols** Immunohematology Center, San Marcos, TX . Guerra is a medical laboratory technologist, with experience in immunohematology laboratory testing, using advanced pre-transfusion serological techniques and methods.

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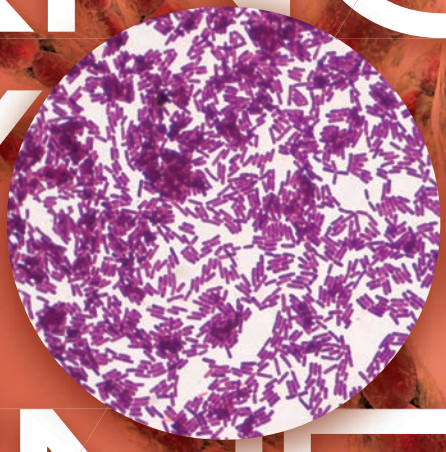

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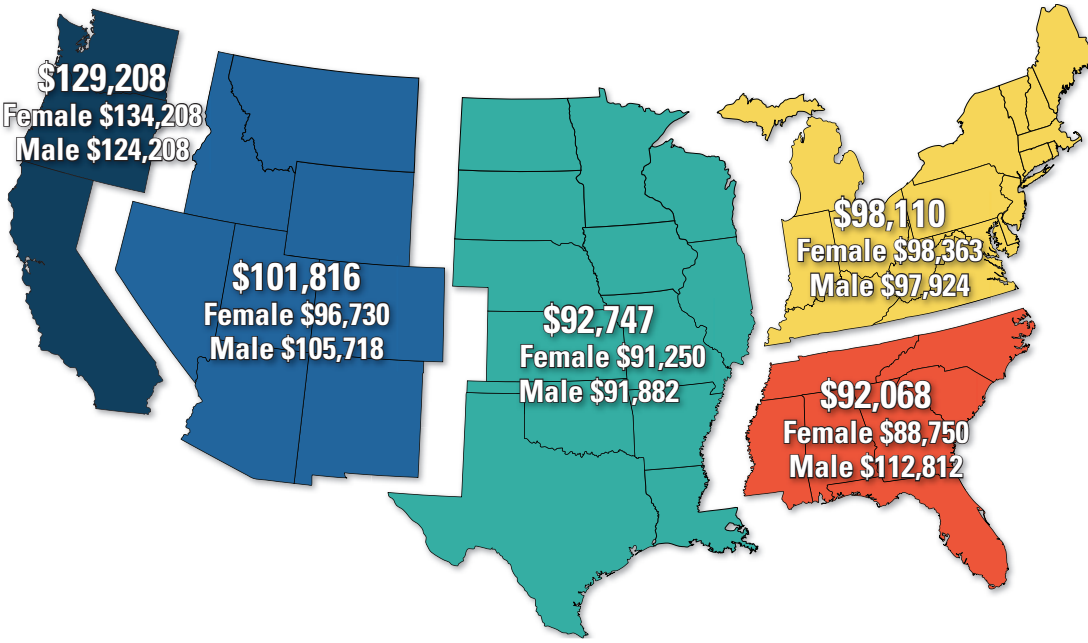


MLO's 2021 Annual Salary Survey of laboratory professionals

By Kara Nadeau

According to the results of Medical Laboratory Observer's 2021 annual salary survey of laboratory professionals, average salary has increased and job security remains high, but the COVID-19 pandemic has exacerbated the profession's staff shortages, with 80 percent of respondents noting shortages having a "moderate to large impact" on operational efficiency.

We present key findings from the annual survey, as well as insights from U.S. lab professionals on compensation, job security and satisfaction, education and training, changing roles and responsibilities, technology adoption, and how COVID-19 has impacted their teams and operations over the past year.

Average salary on the rise

Average compensation of laboratory professionals across the board rose $4,044, from $93,844 in 2020 to $97,888 in 2021. While men still report the highest average salary (at $103,233), the average salary for female lab professionals grew slightly more than that of their male colleagues from 2020 to 2021, with females earning $4,509 more on average, and males earning $3,454 more. The average expected base salary for all lab professionals in 2021 was far higher than last year, at $92,500 compared with $72,500.


Terri Brown

"While compensation is getting better over the past couple of years, for our region specifically, there is much work to be done," said Terri M. Brown, MHA, MT(ASCP), LSSGB, Director of Laboratory and Pathology Services at Mary Lanning Healthcare in Hastings, NE. "In order to remain competitive in a market where there are multiple positions open for any one technologist available, hospitals and clinics have to be creative in their pay practices. I would like not only to see higher hourly wages, but also student loan forgiveness, hiring bonuses, differentials, and loyalty bonuses (staying at one institution for greater than three years) paid to technologists and technicians."

When asked if they received a pay increase in 2020, 63 percent of respondents said "yes," which was down from 2019 (at 71 percent). Although 10 percent more lab professionals reported bonuses in 2020 compared with 2019, or 38 percent versus 28 percent, with regards to raises, about half (48 percent) said they anticipate a 2-4 percent pay increase, and 21 percent expect their raise to be less than 2 percent.

2021's snapshot

The information gathered from the survey is based on 312 respondents. This year's composite clinical lab professional is female, between 56-65 years old and holds a salaried, management position in a hospital lab. She has been in the lab profession an average of 26 years and has worked for her current employer for 17 years.

Some positions see large pay leaps, others drop since last year

Salaries increased in 2021, compared with 2020, for most lab positions, with pay for both medical lab scientists and medical lab technicians up 24 percent ($80,500 and $65,000 respectively), lab directors up 10 percent ($126,938) and pathologists up 7 percent ($160,000).

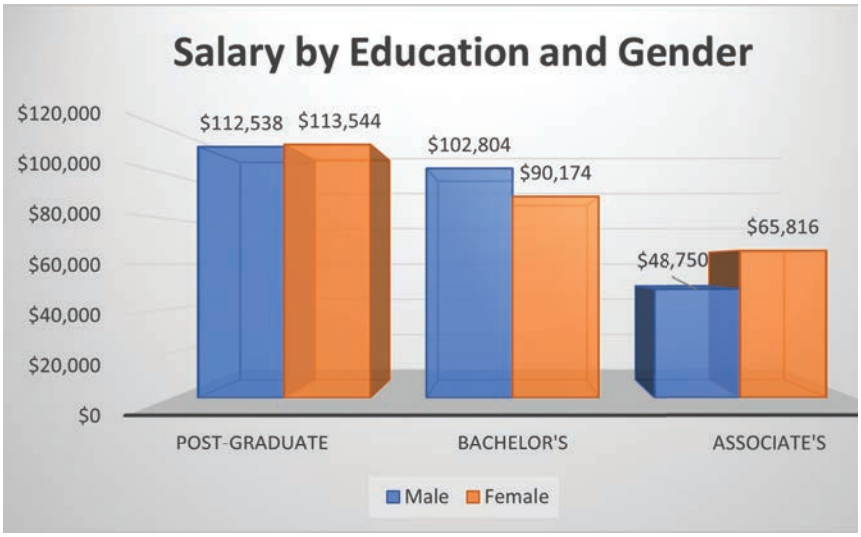

Nicole Radford

Other positions reported more moderate average salary gains. For example, salaries for lab managers, administrators, and supervisors were up 4 percent ($93,879), while chief and assistant chief medical technologists saw salaries increase 4 percent ($86,833).

The average pay for section managers and department heads was down 6 percent compared with last year ($77,703), while the salary for LIS/EHR/EMR managers was down only slightly, by less than 1 percent ($84,500).

Nicole Radford, FACHE, MS, MT(ASCP), Director of the Laboratory at Swedish American in Rockford, IL (part of UW Health), feels clinical laboratory

SALARY SURVEY HISTORY	
2021	$97,888
2020	$93,844
2019	$83,538
2018	$79,006
2017	$84,654
2016	$71,491
2015	$80,985
2014	$71,086



compensation is definitely a contributing factor to the national shortage of lab professionals. She explains that other positions in the medical profession pay better, attracting young professionals who might otherwise choose a career in the lab.

"While salaries have improved for medical laboratory scientists, the salaries of other professions have as well – and some have improved at a faster pace. This makes it difficult to entice college students to choose this profession. In fact, my own daughter has told me that she can get just as much education and make much more money as a nurse," Radford said.

Lab size and location make a difference

Professionals working in larger labs continue to earn higher salaries. Among the largest labs, those with more than 100 employees, the average salary was $115,873 in 2021. The average salary for those working in labs with 51-100 employees was $101,535, labs with 21-50 employees $91,336, 11-20 employees $86,560, and 1-10 employees $83,128.

As for testing volume, the highest average salaries were reported at either end of the spectrum, with those in labs running more than 2 million tests earning $115,516 on average in 2021, and those in labs running fewer than 25,000 tests earning an average annual salary of $107,900. In between this range, average salary increased by volume of tests performed:

- 25,001 to 50,000 tests: $81,917
- 50,001 to 100,000 tests: $87,891
- 100,101 to 500,000 tests: $91,053
- 500,001 to 1 million tests $93,596
- 1-2 million tests: $102,695

Lab professionals working in the Pacific region of the United States once again report the highest pay at $129,208 on average annually, up from $121,825 in 2020. Those working in the Mountain States reported the largest pay gains over last year, up 14 percent (from $89,625 to 101,816), followed by the Southeastern States, with a 10 percent increase in average annual salary (from $83,649 to $92,068), and the Central Region, with a 6 percent increase (from $87,365 to $92,747). Lab professionals in the Northeast saw only a slight increase (from $97,778 to $98,110).

Job security is high because staffing is low

Even though 32 percent of those surveyed said their labs furloughed employees during the pandemic, the vast majority of respondents reported their job security as "very secure" (54 percent) or "somewhat secure" (40 percent). Among those surveyed, 22 percent have been with their current employer for more than 30 years, with an additional 20 percent reporting a tenure of between 20 and 30 years with the same employer.

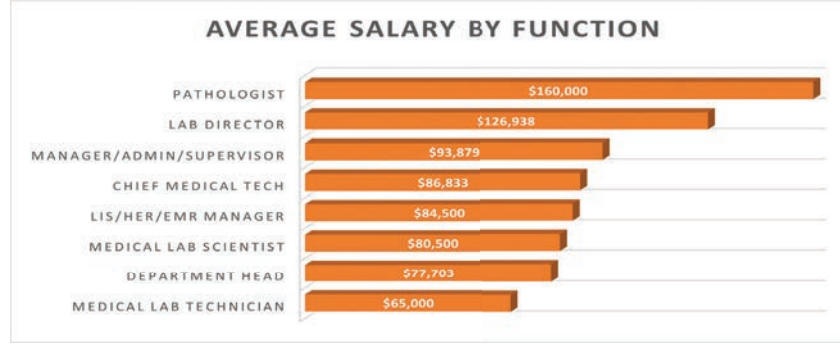
"Currently, jobs are very secure in the clinical laboratory world," said Misha M. Tate, MBA, BS, MT (ASCP) SM^{CM}, Director of Laboratory Services, Mon Health Medical Center, Morgantown, WV." Due to the COVID-19 pandemic, there is a high demand for qualified personnel to perform point-of-care testing for COVID, as well as more complex molecular tests for COVID. Qualified lab personnel will always be needed to perform testing, interpret results and troubleshoot analyzers. Hopefully, the awareness of the need for lab personnel will catch the attention of the younger generation and encourage them to pursue a lab career."

When asked what impact the current shortages of medical personnel have on their lab operational efficiency, 80 percent of those surveyed said it has a "moderate or large impact," compared with 73 percent last year. Yet, only 23 percent said the shortage caused their lab to outsource more tests during 2020.


Thomas Marallo

"The impact for many labs is additional workload spread over existing staffing levels," said Thomas G. Marallo, MT (AMT), Director, Laboratory Services, Infection Control Officer, Upper Connecticut Valley Hospital in Colebrook, NH. "There may be opportunities for overtime or additional shifts, but burnout is an ever-present omen."

Competition from the nursing field, an aging population of individuals in the lab profession, and the changing nature of lab



work are three major factors driving staff shortages, according to Marallo.

"The lack of new graduates sufficient to replace the aging body of med techs is a trend that has been developing for at least 10 years - maybe longer. Young students interested in a medical professional role are gravitating towards nursing as a career choice due to the better pay and less rigorous academic requirements. I have also spoken to some students interested in the lab as scientists, but they are turned off by the increases in automation that effectively 'takes the science out of the job,' to quote one student. I'm not sure if I agree with that perspective, but it is true for some labs that keeping an automated platform in operation is more of a biomedical/computer chore than it is a medical laboratory science function," Marallo said.

Benefits remain stable across the board

With regards to benefits for lab professionals, the 2021 survey results were similar to past years. Nearly all respondents said their employers offer health insurance, dental insurance, vision insurance, and a 401(k) plan or pension. There were some slight increases and decreases in other benefits categories:

- Paid time off increased from 85 percent in 2020 to 87 percent in 2021
- Paid holidays increased from 58 percent in 2020 to 63 percent in 2021
- Childcare increased from 5 percent in 2020 to 6 percent in 2021
- Life insurance decreased from 88 percent in 2020 to 86 percent in 2021
- Disability insurance remained stable at 78 percent in 2020 and in 2021
- Flex time decreased from 13 percent in 2020 to 12 percent in 2021
- Overtime pay decreased from 33 percent in 2020 to 31 percent in 2021

More than one-third of respondents (35 percent) say their employers provide paid COVID-19 specific leave.

Education remains a priority for lab professionals

As in past years, the majority of those lab professionals surveyed hold bachelor's or post-graduate degrees, at 61 percent and 30 percent respectively. An additional 7 percent hold associate degrees, and only 1 percent said their highest level of education attained is a high school diploma. Again, lab professionals with post-graduate degrees earned the most, with a reported $113,758 average annual salary in 2021.

While females with post graduate degrees earn on average more than males ($113,544 versus $112,538), males with bachelor's degrees report higher earnings than their female counterparts ($102,804 versus $90,174). Among those with associate degrees, female lab professionals again outpace males with regards to salaries ($65,816 versus $48,750).

As Brown explains, there is an urgent need to attract students into lab degree programs to sustain the future of the industry. "With so many preparing for retirement, we have to do everything we can to fill classes for both four-and two-year degrees," said Brown. "Without staff, we will be forced to resource out valuable patient tests and exist with stat-labs that are limited in capacity and menu. Having the ability to perform most of the requested testing that comes through our hospital laboratory is vital to exceptional, safe, high-quality healthcare," Brown said.

The majority of survey respondents (76 percent) hold certifications from the American Society for Clinical Pathology, 13 percent from state governments and 10 percent from the National Credentialing Agency for Laboratory Personnel (NCA).

As for continuing education, 25 percent of respondents say they earned between 11-20 hours of continuing education credits in 2020, and 18 percent earned more than 20 hours.

Greater recognition, collaboration and expanding roles

Those interviewed for this article agree that the pandemic has highlighted the importance of the lab profession, instilling pride in those individuals who are playing a critical role in the diagnosis of millions of patients who have contracted the SARS-CoV-2 virus in the U.S.

"I try to remind my staff regularly of the enormous impact that they're making on the health and well-being of our community," said Radford. "While this has always been the case, it has never been so 'front and center' as it has now during the COVID-19 pandemic. Not just from the perspective of testing, but also the care that we're helping provide for the sickest of sick in our community. The expediency of our results has helped expedite the care to the patients when our hospital has been at heightened census – particularly ICU beds. I have seen such pride from my team, as well as the teams of other clinical laboratory leaders that I know."

During the pandemic, lab professionals have found themselves collaborating with other healthcare stakeholders to overcome challenges. All areas of healthcare have experienced supply shortages, and lab is no exception. When asked how their lab department was affected by the COVID-19 pandemic, over half of survey respondents (55 percent) said they have reorganized and evaluated supply usage and storage.

Brown describes how her team worked with the sterile processing department for their health system on a strategy to address supply shortages during the pandemic.

"When testing became widely available, we realized quickly that supplies for testing were strained. We were unable, like so many, to obtain viral media and nasopharyngeal swabs for testing. Our brilliant team of physicians, pathologists and technologists put their minds together and were able to produce our very own viral transport media and 3D print collection swabs. Our sterile processing department came up with a process for the safe sterilization and packaging of the swabs. This carried us through the peak of testing demands from late April through the summer months in Nebraska. We still have these supplies on hand, ready to use at any time when commercial materials again become strained," Brown said.

Tate explains that some staff in the lab may have been reassigned to new tasks as testing needs changed. "During the height of the pandemic, many hospitals decreased or ceased performing elective operating room (OR) procedures," said Tate. "This led to staff being re-assigned to perform tasks that they were not accustomed to, such as transporting supplies and samples back and forth to COVID testing stations."

Brown and her team have also found themselves shifting staff resources to meet changing needs during the pandemic. Early on, before COVID-19 testing was available in their lab and normal volumes of cultures and other testing decreased dramatically, they redeployed several staff members in microbiology to the collection of samples. In the late spring 2020, when testing became available, they not only continued to use multiple staff members for collections, but also had one or two technologists dedicated to running tests during their entire shifts.

Brown is not alone. Among those surveyed, 33 percent said they have had to reassign lab employees to other areas to address COVID-19 testing demands. Brown describes how her team has addressed knowledge gaps among staff members assigned to new roles.

"Prior to the pandemic, there was always a focus on having enough generalist technologists on staff, so we could shuffle where we needed to when workflow changed," said Brown. "Now, we realize more than ever that we need not

only generalists, but the specialties, such as microbiologists, are just as in demand. We had to get creative and cross train support laboratory staff members to function in roles they never had been asked to serve before. Those staff rose to the challenge, and we are very proud of what we accomplished and continue to provide for our patients."

There was a noticeable decrease in number of labs performing outreach to other organizations in an effort to build test volumes, compared with last year, as 39 percent said their labs have performed minimal or no outreach efforts at their organizations, compared with 29 percent in 2020. Those performing outreach varied by type of organization:

- Physician's practices: 51 percent
- Nursing homes: 37 percent
- Community members: 32 percent
- Home care: 18 percent
- Other laboratories: 17 percent

A heightened focus on process efficiency drives adoption of new technologies

When asked how their lab department was affected by the COVID-19 pandemic, more than one-third (33 percent) of survey respondents said they updated their processing policies, and 29 percent reviewed test and utilization costs, as well as reimbursement levels.

An increasing number of lab professionals report that their organizations have automated or further automated new procedures – 51 percent, up from 42 percent last year.

"The capabilities of the various lab information systems that exist today have come a long way in assisting clinical laboratory management in keeping a finger on the pulse of operations," said Radford. "With the improvements that have been seen, we have more information that can help make better decisions; this information is at our fingertips."

"However, this is not something that will just come because you have it," she added. "It takes time and experience to comfortably utilize the myriad of data at our access and not be overwhelmed by 'analysis paralysis.' The details are what drives our day-to-day work in the clinical laboratory, so it's easy to get lost in the minutia of the information. It is important to keep the overarching purpose of the data review top of mind to remain focused, and let the information work for your operation's benefit."

Tate sees clinical labs increasing their use of data analytics to improve upon operational and financial challenges, as well as using the information to support value-based care.

"This is especially evident in antibiotic stewardship," said Tate. "Through electronic reporting and data sharing, labs are readily able to gather information about their own entity, or even other entities, throughout the nation. By collaborating with pharmacy, infectious disease physicians and other key stakeholders, they can help identify any trends that may cause an increase in the incidence of multi-drug resistant organisms. Keeping our patients safe is of the utmost importance. By decreasing the incidence of these multi-drug resistant organisms, we also decrease pharmacy costs and decrease patient length of stay."

On the topic of molecular diagnostics adoption, more than 80 percent said they have embraced it in microbiology, up from 73 percent in 2020. Other top areas for molecular diagnostics include chemistry (14 percent), hematology (6 percent) and blood bank (3 percent).

"One major trend that is actually making our profession more 'fun' is the introduction of more molecular testing in a sample-to-answer format," said Radford. "We're seeing more and more small and mid-sized analyzers that give the capability to perform molecular testing without the need for extraction material, clean rooms, and all of the other aspects of a molecular lab. This has increased the ability for smaller clinical labs to provide the great technology to the patients of their community without necessarily increasing the cost – and, in some instances, decreasing the cost. One of the best ways to demonstrate the impact that this technology has had is the fact that many microbiology labs are more automated now than they ever were in the not-so-distant past."

Looking toward the future

The lab professionals we interviewed for this article agree that there is a bright future for the clinical lab profession, with strong job security and an increased recognition for the role labs play in the supply chain of patient care.

"Since the average age of Med Tech is increasing, and many are retiring, there are jobs available, and I think a good tech in the right lab can count on a secure future," said Marallo.

The COVID-19 pandemic, with its pressing need for rapid and accurate testing results, has shined a spotlight on the work of clinical lab professionals.

"There has been a drastic change in how clinical labs are portrayed," said Tate. "Lab testing has historically been more of a behind-the-scenes role that is now being brought into the spotlight. People are more aware of some of the different types of testing that take place in a clinical lab. They have also become more aware of the constraints that cause delays in testing."

"The pandemic has brought to light that there are more professionals in healthcare than doctors and nurses," said Brown. "For the first time in my 18-year career, I feel the world actually knows that the medical laboratory science profession exists. We have always been here, behind the scenes, working to serve our patients. I am hopeful that this recognition of our work will increase the interest in our profession and help us for years to come as we face severe staffing shortages."

But more work is needed to address industry challenges, including competition from other areas of healthcare, most notably nursing, that can divert young professionals away from lab careers with promises of greater compensation and growth opportunities.

One way for clinical lab to raise its profile in the healthcare field, better promote its contributions to patient care and advance both individual lab professionals and the profession as a whole is through greater collaboration throughout the care continuum.

As Radford explains, this requires lab leaders to be positive and proactive champions for their teams. "I know of many more laboratory professionals who are making a difference outside of the four walls of the lab," said Radford. "However, in order for this to happen and for the value of the contributions from the lab to be realized, the department leader has a responsibility to 'manage up' what his/her team has to offer. Participation on multi-disciplinary committees is one key way for the lab to make a name for itself. Often, the department leader(s) represent the laboratory on these committees. A recommendation that I would have is for the leaders to tap into the resources within the ranks of the department, giving others an opportunity to participate as well. This is actually a win-win for everyone involved. The leader has an opportunity to decrease the many meetings on their schedule, and the staff members have an opportunity to grow."

Kara Nadeau has 20+ years of experience as a healthcare/medical/technology writer, having served medical device and pharmaceutical manufacturers, healthcare facilities, software and service providers, non-profit organizations and industry associations.

At the center of most lab operations is clinical chemistry, which performs tests on body fluids to assess human health and monitor chronic diseases. As is the case in other parts of the laboratory, clinical chemistry is becoming increasingly automated, with sophisticated instrumentation capable of performing hundreds to thousands of tests per hour. As six in 10 Americans have been diagnosed with at least one chronic medical condition, according to the Centers for Disease Control and Prevention (CDC), the field of clinical chemistry is likely to continue its prominent role in patient care.

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LABORATORY INNOVATOR

Machine learning's role in molecular diagnostics

By Linda Wilson

Helen Cha Roberts, PhD, the President of Seegene Technologies since 2016, heads the U.S. subsidiary of **Seegene**, which develops syndromic molecular tests using machine learning. Roberts received an undergraduate degree in molecular and cell biology and genetics from University of California Berkeley, and a PhD in Vertebrate Molecular Genetics from Stanford University School of Medicine.

Why did you become interested in molecular genetics?

I had very inspirational mentors along the way. Both my undergraduate and graduate thesis advisors were incredibly dedicated to teaching. I remember a late night in the lab during graduate school where I was in my PI's (principal investigator) office until about 2 a.m., which was not unusual at all, as he often came back to the lab after dinner with his family. We were both so excited about an *in situ* hybridization experiment that I was working on to identify the localization of the transcript (mRNA) of a novel gene. After a lively discussion, he left for home, and I finished up the experiment. I got such exciting results that I just curled up on the top of my desk and dozed off, until he came back to the office early the next morning. I was too excited to go home and wanted to tell him the results right away. I had many, many late nights and early mornings in the lab; however, when I told him the news that morning, it completely solidified for me why I love this field so much.

Why is molecular diagnostics important?

Molecular tests are highly accurate diagnostics tools that examine genetic markers to identify what is causing a patient's symptoms, helping clinicians identify a treatment plan. Real Time-PCR (RT-PCR) can be used to evaluate a patient sample based on the presence or absence of a genetic marker of a pathogen. If a pathogen is present, the test for that pathogen will amplify the genetic marker sequence, indicating that the patient is positive for that pathogen. Standard diagnostic tests that employ the TaqMan strategy typically only look at one genetic marker at a time.

What are the benefits of syndromic assays?

Because many different pathogens produce similar symptoms, there are significant treatment advantages to utilizing highly accurate diagnostic tests that can evaluate more than one genetic marker or pathogen at a time and ensure timely diagnosis. Syndromic panels are designed, so that a panel of tests looking at multiple pathogens at the same time can be selected based on a patient's symptom(s). When the test is run, it simultaneously evaluates whether a patient is positive for up to 25+ target sequences at once, representing a panel of pathogens known to cause certain symptoms, plus internal controls. Importantly, unlike traditional diagnostic testing, syndromic results can also indicate if a patient is infected with several pathogens at once.

How does digital technology and machine learning allow a diagnostics company to develop tests faster, and what impact does this have on patient care?

When the process is automated, the development of tests takes only a few days from start to finish. In contrast, such a test developed by experienced professionals manually would take more than a year. Since the advent of digitalized platforms, machine learning technology has accelerated the development of syndromic assays.

This includes COVID-19 tests, which can be developed in just a few days. At the start of the pandemic, China made a massive amount of genomic data publicly available, making it possible to use a big-data, auto-surveillance, in-silico platform to sift through the genetic data.

How do you think the field of molecular diagnostics will evolve over the next 3-to-5 years?

The future is already here: COVID-19 diagnostic variant tests are now available and capable of screening COVID-19 and identifying multiple mutant variations in a single reaction. Some of the mutations appear to increase the ability of the virus to bind to the cell receptor and improve the ability of the virus to infect a patient. Being able to know which variant is affecting a specific patient may allow providers to adjust treatment or vaccination strategies.

I see machine learning playing an important role in advancing developments in molecular diagnostics. The new era in diagnostics will both simplify and automate the R&D process to enable scientists to create syndromic tests that meet unmet medical needs, where a scientist or company only needs to have the idea for a test for it to become a reality. In the future, I envision even more sophisticated technologies where a worker could press a key, and the computer would handle all in-silico processes. The only manual step needed would be to validate the test using an actual sample to ensure that it works.

I also predict opposing trends of centralization in large local reference labs, providing quick turnaround times, and decentralization of molecular testing with point-of-care testing. Mobile, point-of-care and at-home diagnostics, combined with telemedicine, will likely see some of the biggegst growth. With at-home diagnostics, it will be easier to test more people within a short period of time. In fact, people would be able to test themselves, before they even start exhibiting symptoms, with high-sensitivity molecular tests.

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① U.S. Food and Drug Administration. StatStrip Glucose 510K Notification K181043. Accessed online at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm

② US Food and Drug Administration Product Classification. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPCD/classification.cfm?ID=NBW

③ U.S. Food and Drug Administration. Self-monitoring blood glucose test systems for over-the-counter use. Draft guidance for industry and Food and Drug Administration staff. https://www.fda.gov/media/119828/download

④ US Food and Drug Administration Product Classification. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPCD/classification.cfm?ID=NBW

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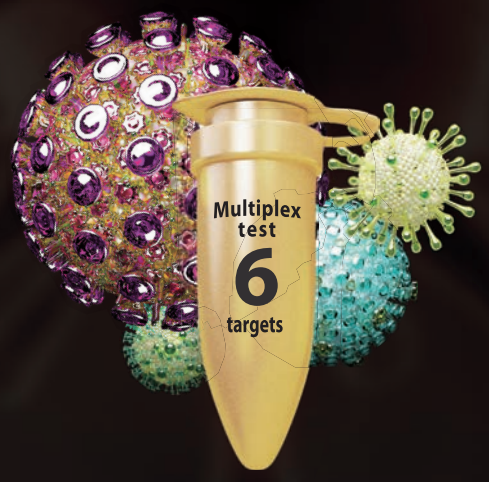

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