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STATE OF THE INDUSTRY: Disease Management

Labs move into a new phase of the pandemic

CE Allergenicity and diagnostics

Eliminating race-based reporting of eGFR

Pandemic impacts diabetes testing

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- *Trichomonas vaginalis*
- Bacterial vaginosis
- Candida vaginitis/*Trichomonas vaginalis*
- HSV 1 & 2
- HPV
- HPV 16 18/45
- Group B Strep
- Zika Virus†

INFECTIOUS DISEASE

- HIV-1 Quant Dx
- HCV Quant Dx
- HBV Quant
- CMV*
- Flu A/B/RSV
- Paraflu
- AdV/hMPV/RV
- SARS-CoV-2†
- SARS-CoV-2/Flu/A/B†
- SARS-CoV-2/Flu/A/B/RSV*
- GI Panels*

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Back to the basics in diagnostic testing

By Linda Wilson
Senior Editor

As vaccination rates climb — albeit more slowly now than earlier in the year — labs in many parts of the country are handling fewer SARS-CoV-2 diagnostic tests. At the same time, newly vaccinated people are emerging from their homes and re-engaging in their pre-pandemic routines. They may be determined to catch up with healthcare services they put off earlier in the COVID-19 pandemic.

At least, that is the scenario labs should be prepared to address.

I expect labs will see testing levels for wellness exams, chronic disease management, and surgeries return to (and possibly exceed) pre-pandemic levels.

There is evidence of pent-up testing demand. For example, a February 2021 report from the Urban Institute found that 36% of adults 18-64 years old either put off or went without necessary medical care during the pandemic. This happened for two reasons: (1.) They were concerned about exposure to COVID-19; (2.) Their healthcare providers reduced services, making it difficult to obtain appointments.

But now, both the volume of SARS-CoV-2 testing and the positivity rates on those tests have dropped nationally.

Data from the Coronavirus Resource Center at Johns Hopkins University chronicles the course of the pandemic in the United States, including the post-vaccination decline in infections and testing. On April 12, 2020 — back when testing supplies were limited and only symptomatic people were tested — the United States conducted 129,358 tests, or 39 per 100,000, with a positivity rate (based on a seven-day rolling average) of 21.2%.

Then came the 2020 holiday season. Despite pleas from public health officials to stay home, lots of Americans traveled, and the result was predictable. On November 28, 2020, the United States conducted 1,659,731 tests, or 506 per 100,000, with a positivity rate of 9.3%. On January 10, 2021, the United States conducted 2,044,361 tests, or 623 per 100,000, with a positivity rate of 13.1%.

The situation was quite different by June 10, 2021, when the United States conducted 778,470 tests, or 237 per 100,000, with a positivity rate of 1.9%.

Labcorp executives have been watching testing trends and commented on them in the company's first quarter 2021 earnings report and 2021 guidance, which it released in April 2021. The reference lab said it expects its 2021 revenue from COVID-19 testing to plummet by 35%-50%, and its revenue from its base testing business to grow by 13.5%-16%.

There are many basic diagnostic assays involved in the routine of care of patients, and labs could see demand increase for all of these during the remainder of 2021.

For example, wellness exams and pre-surgery consultations could include tests to assess cholesterol, potassium, and glucose levels as well as kidney and liver function. Complete blood count (CBC) and coagulation studies (PT/PTT) also are common tests.

Labs also are likely to see more demand for tests to manage chronic diseases. Unlike annual wellness exams and pre-surgery assessments, chronic-disease testing involves repetition of the same tests over time, such as to monitor HbA1c levels in patients with diabetes or blood-clotting rates in patients on anticoagulation therapy.

That means it is back to the basics for many labs in the United States.

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

MEDICAL LABORATORY OBSERVER

MEDICAL LABORATORY OBSERVER Vol.53, No.7

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VERIFIED

MLO - MEDICAL LABORATORY OBSERVER

(ISSN: 0580-7247). Published monthly, with an additional issue in August, by Endeavor Business Media, LLC., 2477 Stickney Point Rd, Suite 221B, Sarasota, FL 34231 (941) 388-7050. Subscription rates: $127.60/year in the U.S.; $154.88 Canada/Mexico; Intl. subscriptions are $221.43/year. All issues of *MLO* are available on microfilm from University Microfilms International, Box 78, 300 N. Zeeb Rd., Ann Arbor, MI 48106. Current single copies (if available) $15.00 each (U.S); and $20.00 each (Intl.). Back issues (if available) $17.60 each (U.S.); $22.00 each (Intl.). Payment must be made in U.S. funds on a U.S. bank/branch within the continental U.S. and accompany request. Subscription inquiries: subscriptions@endeavorb2b.com. *MLO* is indexed in the *Cumulative Index for Nursing and Allied Health Literature* and *Lexis-Nexis*. *MLO* Cover/CE, Clinical Issues, and Lab Management features are peer reviewed. Title® registered U.S. Patent Office. Office of publication: Periodicals Postage Paid at Nashville, TN 37209 and at additional mailing offices. **Postmaster:** Send address changes to Omeda (MLO Medical Laboratoy Observer), PO Box 3257, Northbrook, IL 60065-3257.
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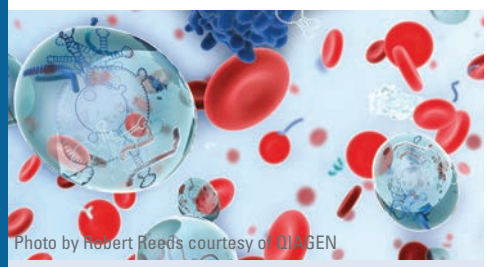

Photo by Robert Reeds courtesy of QIAGEN

Fast Facts

Liquid Biopsies

A survey of oncologists by InCrowd sought to understand how oncologists learn about new biomarker tests, including the use of liquid biopsy testing.

61%

of oncologists preferred liquid biopsies, because they said samples were easier to obtain and less invasive than other biopsy types.

19%

of respondents said that the increased use of liquid biopsies would provide better detection rates.

16%

of respondents thought that liquid biomarker testing provided a broader reach of testing and treatments.

66%

of oncologists believe liquid biopsy testing will continue to increase over time.

25%

of respondents reported that liquid biopsies helped personalize and target their decision-making.

26%

of oncologists anticipate that liquid biopsy use helps avoid additional testing and associated complications.

33%

of cancer biomarker tests currently ordered in the solid tumor space are liquid tests.

Source: Onc_Biomarkers2021-May-Webinar-Deck.pdf (incrowdnow.com)

Link between blood sugar and liver disease progression

There are no approved drugs to treat nonalcoholic fatty liver disease, but controlling blood sugar over time may help decrease the risk of liver scarring and disease progression, according to a study by Duke Health as reported in a news release.

In the study, the average three-month blood glucose levels of patients with non-alcoholic fatty liver disease influenced their chance of having more severe scarring in the liver, which can lead to liver failure.

Appearing online in the journal *Hepatology*, the study examined the documented glucose levels of 713 adult patients with fatty liver disease prior to a liver biopsy.

Researchers found that higher average blood glucose levels in the year leading up to a liver biopsy were associated with more severe swelling of liver cells. For every 1 percentage point increase in hemoglobin HbA1c (a measure of average glucose levels) in the year preceding biopsy, the chances for severe fibrosis rose by 15%.

Similarly, researchers also found that those with moderate glucose control over a period of five years, rather than good control, had more severe swelling of liver cells and a higher likelihood of having advanced liver scarring.

Lead author of the study, Duke Health endocrinologist Anastasia-Stefania Alexopoulos, MBBS, said severe liver disease related to fatty liver is on the rise. Alexopoulos says these findings are particularly significant for diabetes patients because a significant portion of the patient population also has nonalcoholic fatty liver disease.

Alexopoulos also said the findings may lead clinicians to reconsider their diabetes treatment approach to prescribe diabetes medications that are known to both improve glucose control and promote weight loss.

"A lot of the times in diabetes, we're thinking about heart disease, high blood pressure, cholesterol — we're thinking about all these complications," Alexopoulos said. "I want fatty liver to be added in there."

New bacterial weaknesses potentially targets AMR

In the perpetual arms races between bacteria and human-made antibiotics, there is a new tool to give human medicine the edge, in part, by revealing bacterial weaknesses and potentially by leading to more targeted or new treatments for bacterial infections.

A research team led by scientists at The University of Texas at Austin has developed chemical probes to help identify an enzyme, produced by some types of E. coli and pneumococcal bacteria, known to break down several common types of antibiotics, making these bacteria dangerously resistant to treatment.

"In response to antibiotic treatment, bacteria have evolved various mechanisms to resist that treatment, and one of those is to make enzymes that basically chew up the antibiotics, before they can do their job," said Emily Que, Assistant Professor of Chemistry. "The type of tool we developed gives us critical information that could keep us one step ahead of deadly bacteria."

In a paper published online in the *Journal of the American Chemical Society*, the researchers zeroed in on the threat posed by the bacterial enzyme called New Delhi metallo-beta-lactamase (NDM). They set out to create a molecule that glows when it comes into contact with the NDM enzyme. When these chemical probes are added to a test tube, they bind to the enzyme and glow. Such a tool could be used to alert doctors to what kind of bacterial threat is affecting their patients and tell them which antibiotics to use.

NDM breaks down antibiotics in the penicillin, cephalosporin and carbapenem classes, which are some of the safest and most effective treatments for bacterial infections. Other classes of antibiotics exist, but they may carry more side effects, have more drug interactions, and may be less available in some parts of the world.

In addition to indicating the presence of the NDM enzyme, the florescent chemical probe developed by Que and Walt Fast, Professor of Chemical Biology and Medicinal Chemistry, may help find a different way to combat these resistant bacteria. One treatment option that doctors use with resistant bacteria is to combine common antibiotics and an inhibitor. Although there is no known clinically effective inhibitor for NDM-producing bacteria, Que's probe could help find one.

Once the probe has bound to the enzyme and begun to glow, if an effective inhibitor is introduced, it will knock the probe loose and the glow would stop. This allows scientists to test a high volume of potential drugs.

The study also examined a process called nutritional immunity, which comes from the human body's production of proteins in response to an infection. The proteins snatch up all the

available metals in the body, such as the zinc required to make NDM, rendering the bacteria more susceptible to attack.

Que's probe can also be used to study nutritional immunity and NDM, because it will glow only in the presence of the zinc needed to form the enzyme.

Chip under skin may identify stroke

For patients who have experienced certain common types of stroke, a small chip inserted under the skin may help physicians predict their likelihood of experiencing a second stroke and, therefore, their likelihood of benefiting from preventive therapy, according to a news release from Massachusetts General Hospital.

The findings come from a recent clinical trial, which was supported by Medtronic and published in the *Journal of the American Medical Association*. It was led by investigators at Massachusetts General Hospital (MGH) and Northwestern University Feinberg School of Medicine.

Each year, approximately 800,000 strokes occur in the United States, and as many as one-fourth occur in people who experienced a previous stroke. Investigators have been searching for ways to identify patients who are likely to experience a recurrent stroke, as these individuals could be candidates for taking certain medications such as blood thinners. One group of patients who face an elevated risk of recurrent strokes are those with atrial fibrillation — an irregular and often rapid heart rate — that often goes undetected and untreated. (Irregular heartbeats can allow blood to pool in the heart, which can cause clots to form and travel to the brain.)

Recent research has shown that a small chip inserted under the skin can monitor the heart rate and rhythm, and help physicians detect atrial fibrillation in patients who previously experienced what's called a cryptogenic stroke, one with no identified cause despite thorough patient testing. Now investigators have tested the chip — less than 1¾" long and 1/6" thick and called an insertable cardiac monitor — in patients who experienced a stroke caused by narrowing of a large artery like the carotid artery, or blockage of a small artery deep in the brain where atrial fibrillation would be unexpected.

In the Stroke of Known Cause and Underlying Atrial Fibrillation (STROKE AF) trial, 492 patients were randomized and completed 12 months of follow-up after receiving either an insertable cardiac monitor within 10 days of an initial stroke or usual care consisting of external cardiac monitoring through electrocardiograms or other tracking methods.

The chip detected atrial fibrillation in 12.1% of patients, compared with 1.8% detected through usual care. The team noted that the episodes of atrial fibrillation were not brief, with most lasting at least one hour. Most stroke experts would recommend that patients with this degree of atrial fibrillation start taking blood thinners to prevent a future stroke.

The FDA approves first Alzheimer's drug since 2003

The U.S. Food and Drug Administration (FDA) approved Aduhelm (aducanumab) for the treatment of Alzheimer's, a debilitating disease affecting 6.2 million Americans. This first new treatment approved for Alzheimer's since 2003.

The agency granted approval of the drug even though its advisory committee and other experts voiced concerns about the drug's efficacy and safety, based on the evidence produced during clinical trials.

The FDA said Aduhelm was approved using the accelerated approval pathway, which can be used for a drug that provides a meaningful therapeutic advantage over existing treatments for a serious disease. Under that pathway, the FDA said it is requiring the drug's developer, Biogen, to conduct a new randomized, controlled clinical trial to verify the drug's clinical benefit. If the trial fails to verify clinical benefit, the FDA said it may withdraw approval of the drug.

Editors Clarification

Two Abbott hematology analyzers (CELL-DYN RUBY and Sapphire) were in the June issue feature on coagulation analyzers; however, neither perform coagulation tests. *MLO* regrets the error.

Lung protein elevation may predict COPD

Airway mucus consists of various proteins, such as long mucins MUC5AC and MUC5B, both of which contribute greatly to the proper gel-like consistency of this most essential bodily fluid.

UNC School of Medicine researchers led by mucin expert Mehmet Kesimer, PhD, had previously discovered that the total mucin concentrations in the lungs are associated with COPD disease progression and could be used as diagnostic markers of chronic bronchitis, a hallmark condition for patients with COPD. Kesimer and colleagues now report that one of these mucins, MUC5AC, is more closely and reliably associated with the development of COPD than is its brother, MUC5B.

The research, published in *The Lancet Respiratory Medicine*, shows that MUC5AC is found at elevated levels in smokers who had not yet developed COPD but whose lung function wound up decreasing over the course of the three-year study. Former smokers at-risk for COPD, on the other hand, had normal levels of MUC5AC at the start of the study and maintained proper lung function over three years. MUC5AC hyper concentration in the lungs may be a key factor in predicting the risks and rates of progression to more severe disease, according to the study.

Recent nationwide efforts have focused on early- or pre-COPD to predict the risks of progression to COPD amongst smokers.

"Currently, we cannot forecast which individuals in the at-risk smokers group will progress to COPD, because we don't have an objective biological marker to underpin the disease-causing pathways. Our research shows that MUC5AC could be a predictor of who will develop COPD from the large group of aging 'at-risk' smokers," said Kesimer, Professor in the UNC Department of Pathology and Laboratory Medicine, and member of the UNC Marsico Lung Institute. "We think MUC5AC could be a new biomarker for COPD prognosis, and it could be a biomarker for testing the effectiveness of therapeutic strategies."

MUC5AC could also become a target for pharmaceutical developers whose goal it is to halt COPD disease progression and help patients live more normal, active lives.

Smoking cigarettes has long been known to be a major risk factor for COPD, but Kesimer's work suggests that quiting smoking decreases the odds of developing COPD as we age.

Photo by Alicia Jones on Unsplash

What do cats, dogs, horses, and ticks have in common? Allergenicity and component resolved diagnostics

Tara Bruner, MHS, PA-C, DFAAPA

A young girl asks her parents for a pet, perhaps a dog or cat. The parental answer is,"No, your brother is allergic."This is a common scenario, as up to 60% of Americans own a domesticated pet.[1]Through diagnostic testing, this answer could be different. With the use of blood allergy testing and component resolved diagnostics (CRD), answers could be,"Sure, we can get a female dog instead of a male dog."Another answer could be,"Yes, we can get a dog, but not a cat, because we know your brother is only allergic to cats, not dogs."

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LEARNING OBJECTIVES

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

1. Describe the clinical history of allergy testing.
2. Describe what component resolved diagnostics is and how it differs from whole allergen testing.
3. Describe the protein families for pet allergens and their impact on clinical strategies to manage allergic sensitization.
4. Recall what Alpha-Gal syndrome is and how it is diagnosed through in vitro testing.

In another scenario, a patient presents to the emergency room late in the evening with a symptomatic allergic reaction to something, but the patient's medical history is unclear about exposure to specific allergens. In this case, too, allergy diagnostics help complement a patient's history and aid in the diagnosis and management of allergic diseases.

How allergy diagnostics help

An in vitro (blood) whole allergen test can identify sensitization, but just as with many other laboratory tests, there is more to the story. A whole allergen is made up of a variety of proteins, and these proteins may or may not have an association with clinical symptoms. Within the whole allergen, there may be a variety of proteins, some of which may be cross-reactive, which means they have a similar protein structure to other clinically non-relevant proteins. Other proteins may be unstable to heat or digestion, or they may be species-specific proteins that can drive clinical symptoms and are often associated with significant risk for systemic reactions.

The use of specific IgE (sIgE) tests aid in the diagnosis of allergen sensitization alongside a thorough patient history. If the whole allergen is positive via in vitro blood testing or skin prick testing, additional in vitro blood testing is often available to tell"the rest of the story."This is known as allergen component resolved diagnostics (CRD).

History of allergy testing

Tracing the history and evolution of allergy testing shows the trajectory of allergen diagnostics. Skin testing dates back

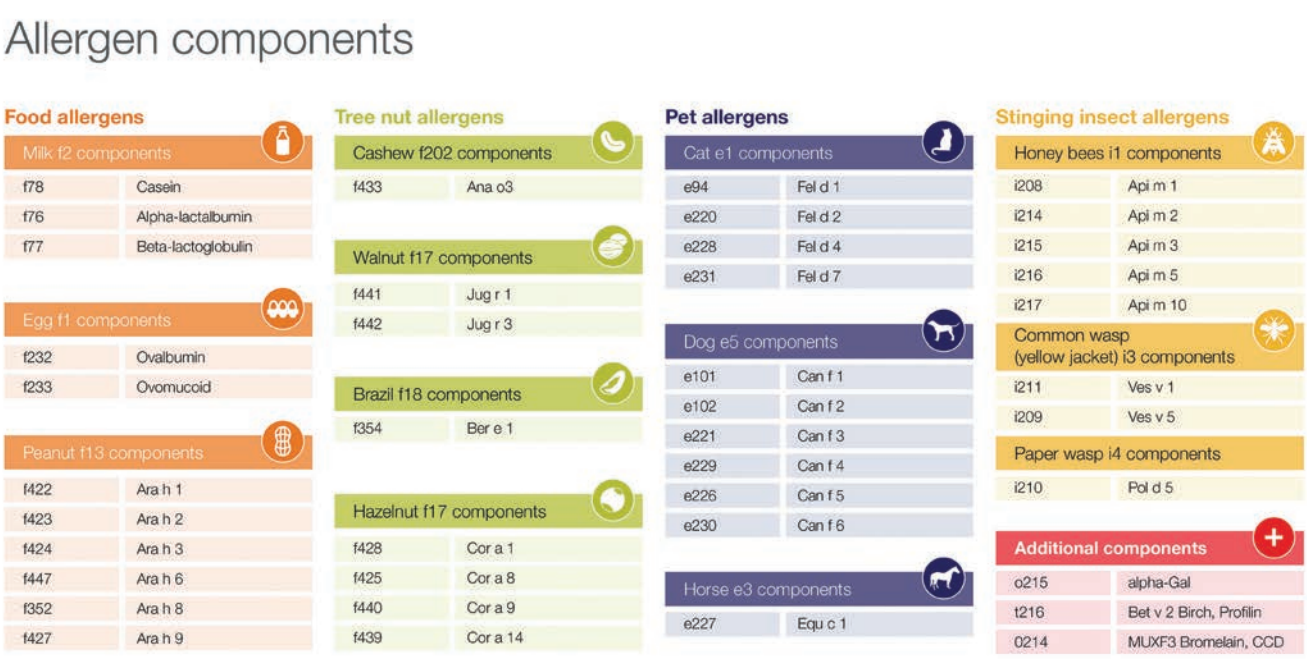

Figure 1. Examples of allergen components available in the U.S.

to 1865, when Charles H. Blackley, a physician who suffered from allergic rhinitis, abraded his skin with a lance, then applied grass pollen to the wound, resulting in intense itching and a large cutaneous response.[2] This scratch testing procedure led to the first applied skin prick tests (SPT)[3] by Lewis and Grant in 1924.

In 1967, Immunoglobulin E was the last of the five immunoglobulins (IgG, IgM, IgD, IgA) to be discovered and was attributed to type I hypersensitivity allergic reactions.[4,5] Blood allergy testing became commercially available through radioallergosorbent (RAST) testing in 1974. Improvements to this method of testing came in 1981, with the introduction on an enzyme linked assay. Currently, there is a third generation of immunofluorescence[5] testing, which binds allergens to a 3-dimensional polymer sponge, allowing for superior surface binding.[6]

Once components for milk and egg became commercially available in the United States, this immunofluorescence method and instrumentation expanded to allergen component resolved diagnostics in 2005.[7] Allergen components have been extensively researched and have been available globally for over two decades. CRD will continue to advance well into the future, as molecular allergology continues to enhance the diagnosis of allergic disease.

Both whole allergen and component allergen testing are sandwich IgE assays, allowing for solid phase binding of the relevant and specific antibodies. Therefore, it is imperative to have reliable source material bound to the sponge or well; this is done utilizing either native purified proteins or recombinant DNA technology.[6] However, skin prick testing for allergen components is not readily available. The ease of in vitro blood testing only requires a venipuncture and provides fully quantitative measurement and reproducibility of results.[6]

Allergen components have been extensively researched and have been available globally for over two decades. CRD will continue to advance well into the future, as molecular allergology continues to enhance the diagnosis of allergic disease.

Previously, in the July 2020 issue of *Medical Laboratory Observer*, Lakiea Wright, MD, noted that component testing is emerging as critical indicator for anaphylactic risk through the use of available food allergen components and stinging insect components.[8] Listed in **Figure 1**, there are examples of many clinically relevant allergen components currently accessible to laboratories and clinicians that have been approved by the U.S. Food and Drug Administration (FDA). Several allergen components are available in the categories of peanut and tree nuts, pets, stinging insects, and even a carbohydrate component called alpha-Gal.

Automation and CRD within the lab

Using automated instruments with high walk away times maximizes efficiency of CRD, as the calibration, quality control, and curve control are all in-house, except for carriers, allowing for lab efficiency.

Fully automated reflex testing with whole allergen positivity puts orders back on track, allowing a faster turnaround time for component allergen results. Automation also provides flexibility by being able to batch and run testing daily, as well as offering different ways to run the instrument. Workflow can be enhanced through optional automated track connectivity and integrative-operation software systems. This type of laboratory automation integrates sample handling to improve efficiency, quality, and safety across various analyzers in the laboratory.[9]

In addition, component resolved diagnostic testing is reimbursed at three times the whole allergen rate.[10] Keeping this testing within the laboratory, as opposed to a more costly and time consuming send out, is beneficial to patients, gives providers clarity on treatment options, and makes the laboratory more

Pet allergen components – Proteins[1]

Uteroglobin/ Secretoglobin	Kallikrein	Lipocalins	Serum Albumins
• Sensitization during childhood can be a predictive marker of cat allergy in adolescence. • A cat-specific marker of sensitization. • Fel d 1, a uteroglobin and the major cat allergen. • A uteroglobin expressed in skin and salivary glands, its synthesis is related to sexual hormones.	• Can f 5, a prostatic kallikrein, was isolated from urine of male dogs and is considered a major allergen. • Therefore, patients sensitized only to Can f 5 may tolerate female dogs or castrated male dogs. • Patients sensitized to Can f 5 may show allergic reactions to seminal fluid.	• Lipocalins are the most important allergen protein family. • Most are major allergens. • Synthesized in salivary glands and dispersed into the environment by saliva and dander.	• Highly cross-reactive molecules generally considered minor allergens. • Abundant in saliva and dander. • Respiratory allergens present in animal dander and fluids such as milk, serum, urine, and saliva.

Provide a more precise diagnosis[1]

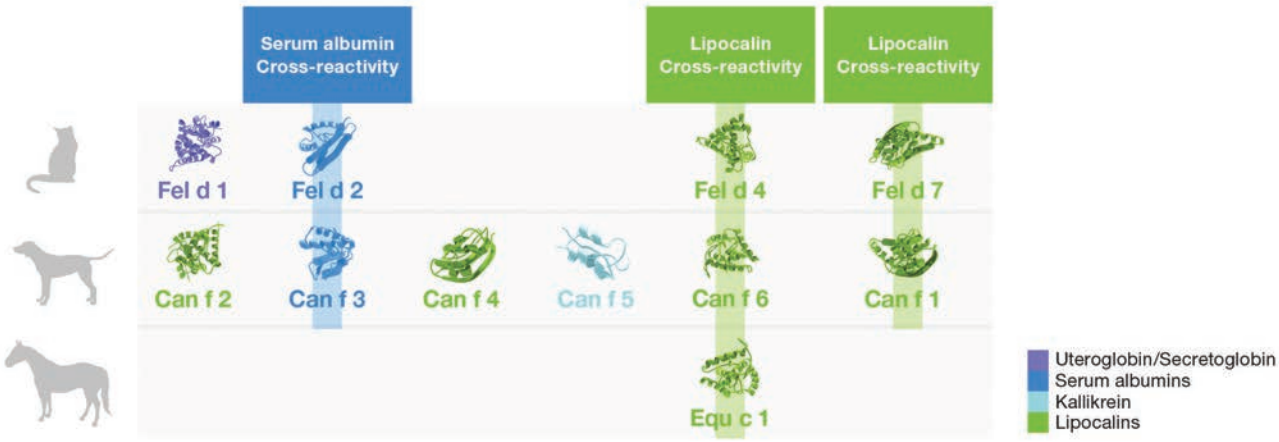

Figure 2. Pet component families and demonstration of primary sensitization versus cross reactivity with bar through similar homology proteins.

efficient and valuable. A collaborative initiative with all relevant stakeholders allows for the appropriate use of allergy CRD and increased opportunities for value-based testing.

Value to patients

Now that allergen components have been defined, what is the value to the patient? In the scenario described about a family wanting to obtain a pet, but being limited because of a child's allergic symptoms, CRD can aid in that patient's dilemma. Pet allergen components can help define a primary allergen sensitization that is likely responsible for clinical symptoms, versus an irrelevant cross reactivity with other pets. In the case of dogs, CRD can help determine the sex of the pet that a patient may tolerate.

Domestication of animals has turned what were once exclusively outdoor allergens into indoor allergens, increasing the cumulative exposure to mammalian aeroallergens, specifically to dogs and cats. For those who are sensitized and have clinical symptoms, this is problematic. Symptoms for respiratory diseases, such as asthma and rhinitis, can complicate the cohabitation and range from mild to life threating asthma exacerbations.

Dog, cat and horse component resolved diagnostics have been widely studied and demonstrate the positive impact they can have on the management of patients, up to and including the ability to predict severity and development of asthma and rhinitis.[11] Polysensitization to pet allergen components has been correlated with increased bronchial inflammation in those with severe asthma.[1,11,12] Availability of CRD offers more personalized diagnostic work-ups, which facilitates identification of primary sensitization, versus cross reactive sensitizations between animal species.

Proteins of pet allergens

Protein families for relevant pet allergens can be classified into secretoglobins, kallikreins, lipocalins and serum albumins.[12] These allergens are present in pet dander, saliva and urine, and are distributed into the environment through

Test Menu:

pH

PCO_2

PO_2

$SO_2\%$

Hct

Hb

Na

K

Cl

TCO_2

iCa

iMg

GLU

Lac

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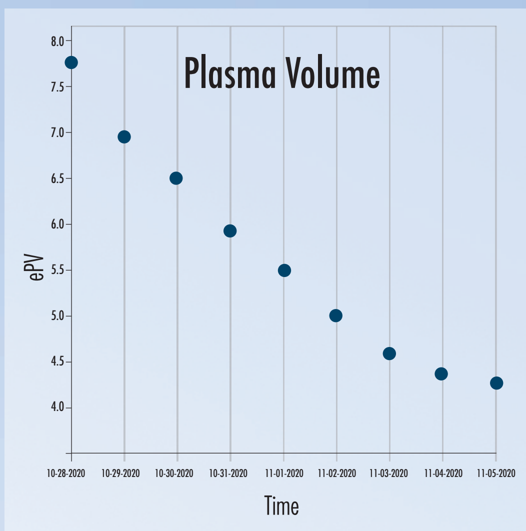

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3ODVP DYROXP HWDWXVLV YLWDO LQ WUHDWLQJ FULWLFDOO LOO SDWLHQWV EXW LV H[WUHP HO GLI¿FXOW DQG costly to obtain, particularly as a POC test. Plasma volume assessment affects almost HYHU DVSHFW RI D SDWLHQW¶V FDUH LQFOXGLQJ JLYLQJ ,9 ÀXLGV GLXUHWLQJ WDUWLQJ YDVRSUHVVRUV initiating renal replacement therapy, deciding on transfusion requirements and intubation and extubation.[1,2]

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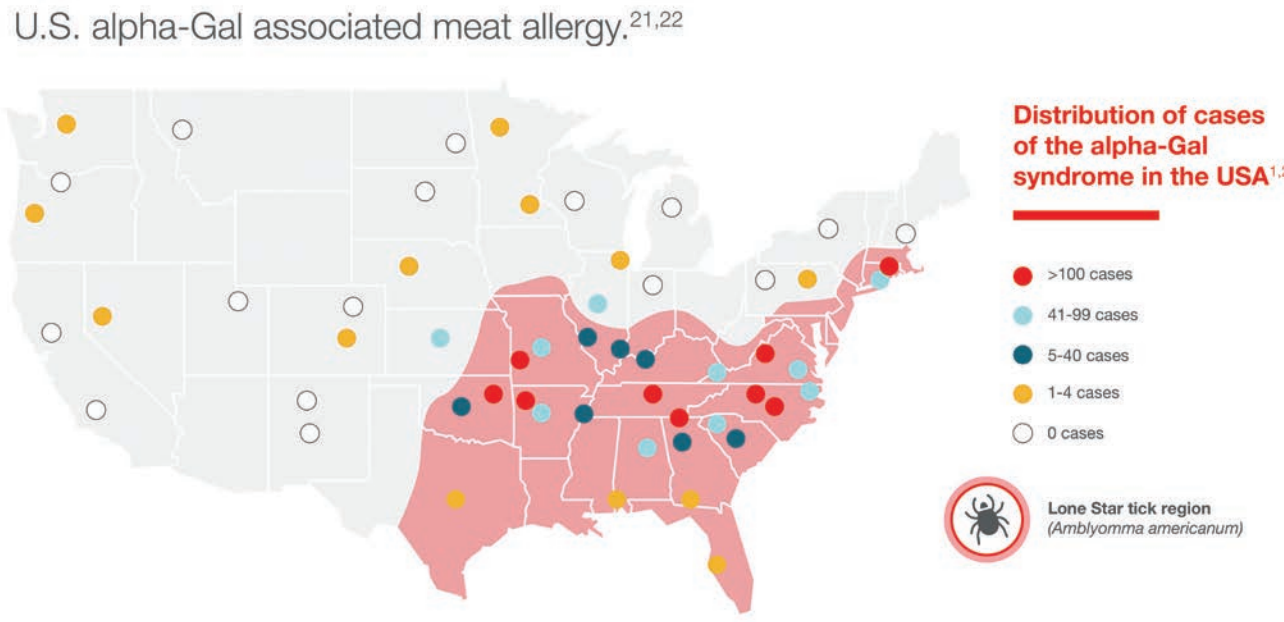

Figure 3. Examples of allergen components available in the U.S.

shedding.[12,13] The protein sequences of many allergen components are unique, but some lipocalins and serum albumins share sequence homology, which can cause cross-reactivity.

Secretoglobins (also known as uteroglobins) most commonly refer to Fel d 1 produced in the saliva of cats. This highly allergenic component is the cause of most allergic clinical symptoms and is structurally unique; therefore, if a patient is symptomatic with cats and is sensitized to Fel d 1, the Fel d 1 is considered a primary sensitizer.[12]

Another unique pet component family is kallikrein. This unique prostatic kallikrein protein is produced in male dogs. This specific primary protein is expressed in the prostate and shed in saliva and dog dander.[1] About 30% of patients who are monosensitized to dog are sensitized to Can f 5; therefore, they may tolerate a female dog or castrated male dog.[12] However, this is not the only significant dog allergy component.

Lipocalins are a significant allergen protein family and can be a primary allergen sensitizer or cross-reactive allergen between the various pets, such as cat, dog, and horse.[1,13] These allergens are expressed in saliva and dander, regardless of breed or supposed hypoallergenicity.[1] See **Figure 2.** Lastly, serum albumins are highly cross-reactive molecules, abundant in dander and saliva, and play little role with clinical symptoms.[1]

Evaluating a patient's pet allergens

Evaluating the patient's unique pattern of pet components allows more precise evaluation of the primary sensitizing allergen causing the patient's symptoms. With protein homology between certain pet families, a whole allergen to dog, cat or horse may be positive, when, in fact, the problem is really cross-sensitization to another animal species. This CRD of primary species sensitization versus cross reactivity information directly impacts patient management.[1]

The pet management consideration informs evidence-based recommendations, such as should there be avoidance of cat, dogs and/or horses? Would a female dog be a better option? Would a cat be a better choice for a family than a dog?

But pet selection or re-homing a pet is not the only consideration. Those sensitized to three or more pet allergen components are at risk for development or worsening of respiratory illnesses. By having this understanding, correct pet avoidance, appropriate medications, and allergen immunotherapy can reduce the bronchial hyperresponsiveness.[11]

Although elimination of pets from the home is considered the most effective avoidance measure, this is often emotionally charged and not a viable option. Novel approaches to reducing the expression of allergenic proteins from pets are emerging. Recently, a cat food that binds with the major allergenic protein Fel d 1, reducing its expression in cats, has been introduced.[14] In addition, other therapeutic interventions for both pets and humans affected by pet allergy have been studied and may hold promise for the future.

For the family inquiring about a pet, these precision diagnostics can lead to the most appropriate pet selection and targeted management for the patient.

Alpha-Gal syndrome

In a different clinical scenario, when a patient has a systemic allergic reaction and medical history is unclear, consideration of alpha-Gal syndrome may be appropriate. Alpha-Gal syndrome is a mammalian (red) meat allergy, typically presenting as a delayed systemic reaction or anaphylaxis, caused by sensitization to a sugar complex protein, galactose-α-1,3-galactose (α-Gal), which is an oligosaccharide present in all mammals, except humans and Old-World monkeys.[15,16]

The vector responsible for sensitization to alpha-Gal is a tick from the family Ixodidae. In the United States, the Lone Star tick (Ambylomma americanum) is responsible for the majority of alpha-Gal sensitization.[17,18] Incidence correlates with geographic distribution of the tick: see **Figure 3**. Transmission occurs with certain tick species that have alpha-Gal present in the intestine from a previous mammalian host that transmits this sugar complex to the human after tick bite.[17]

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Lung
α-1 Anti-Trypsin
KL-6*

Diabetes
Hemoglobin A1c
Insulin
Microalbumin
Microtransferrin*

Inflammation
α-1 Acid Glycoprotein
Anti-Streptolysin O
β-2 Microglobulin
Complement C3
Complement C4
CRP
hs-CRP
Rheumatoid Factor

Immunology
IgA
IgG
IgM
Total IgE

Kidney
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β-2 Microglobulin
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The response to this foreign oligosaccharide results in a type I hypersensitivity and antibody production to alpha-Gal. The delay in response to this sugar complex exposure in mammalian meat is due to absorption of alpha-gal-containing glycolipids binding to lipid particles, which take hours to form and be released into the circulation.[17] Therefore, it may result in a delayed allergic response, following ingestion of mammalian meat.[15]

Diagnosis of alpha-Gal syndrome can be elusive. Alpha-Gal syndrome is often found in adults who had no previous complaints of meat allergy. Symptoms have also been reported after exposure to certain alpha-Gal containing medications and gelatin containing foods.[18] Diagnosis can be challenging, as this is a delayed hypersensitive response to mammalian meat, typically presenting 3-6 hours after ingestion.[15,17,18] Therefore, identification of the offending trigger can be difficult for patients and clinicians to pinpoint.

In the patient scenario previously mentioned, a patient presents to the emergency room late in the evening with a symptomatic allergic reaction to an unknown trigger, and the history is unclear for exposure to allergens. In endemic areas, alpha-Gal is a likely cause, warranting further exploration of a patient's medical history and alpha-Gal sIgE diagnostic testing.

In-vitro testing

In-vitro testing for alpha-Gal syndrome is available with the measurement of IgE antibodies against alpha-Gal, along with immunoassay testing for sIgE to beef, lamb, and pork. Often, a profile for alpha-Gal will also include tryptase and a total IgE. This component profile optimized for clinicians to evaluate a difficult diagnostic presentation is valuable and helps inform both diagnosis and patient management.

While levels of alpha-Gal can decrease over time, subsequent tick bites can increase the levels and the clinical symptoms when mammalian meat is ingested. Avoidance of tick exposure and bites is recommended, and avoidance of mammalian meats is the mainstay of management to prevent future life-threatening reactions.[15,18] Often, patients will tolerate chicken, turkey, or fish without symptoms.[15] They also should use caution with gelatin containing products and certain medications.[18,19]

Other management considerations for alpha-Gal syndrome are an epinephrine auto-injector for possible systemic reactions and consideration about using an oral food challenge to determine risk.[20]

The future of allergic disease diagnosis will continue to be personalized with component resolved diagnostics. This approach and the availability of additional in vitro specific IgE allergen components allows an efficient and confident health system approach to allergic disease management.

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What do cats, dogs, horses, and ticks have in common? Allergenicity and component resolved diagnostics

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TEST QUESTIONS

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

1. **A(n) _____ can identify sensitization.**
   - A. scratch test
   - B. in vitro (blood) whole allergen test
   - C. poem
   - D. in-depth diet history

2. **A whole allergen is made up of a variety of ____.**
   - A. sugars
   - B. proteins
   - C. carbohydrates
   - D. cells

3. **_____ means they have a similar protein structure to other clinically non-relevant proteins.**
   - A. Cross-reactive
   - B. RAST
   - C. Sequencing
   - D. Amino binding

4. **_____ can drive clinical symptoms and are often associated with significant risk for systemic reactions.**
   - A. Pollens
   - B. RAST
   - C. Pets
   - D. Species-specific proteins

5. **_____ are helpful to aid in the diagnosis of allergen sensitization alongside a thorough patient history.**
   - A. Scratch tests
   - B. Specific IgE (sIgE) tests
   - C. Pets
   - D. RAST

6. **In 1865, _____ abraded his skin with a lancet, and applied grass pollen to the wound, resulting in intense itching and a large cutaneous response.**
   - A. Lakiea Wright, MD
   - B. Charles H. Blackley
   - C. Lewis and Grant
   - D. Charles Darwin

7. **The first applied skin prick tests (SPT) were by _____.**
   - A. Lakiea Wright, MD
   - B. Charles H. Blackley
   - C. Lewis and Grant
   - D. Charles Darwin

8. **_____, attributed to type I hypersensitivity allergic reactions, was the last of the five immunoglobulins (IgG, IgM, IgD, IgA) to be discovered.**
   - A. Immunoglobulin E
   - B. Immunoglobulin T
   - C. Immunoglobulin V
   - D. Immunoglobulin B

9. **What does RAST stand for?**
   - A. radial allergy surface testing
   - B. radiological allergy scratch testing
   - C. radioallerimmune system testing
   - D. radioallergosorbent testing

10. **In the third generation of immunofluorescence testing, which _____, allowing for superior surface binding.**
    - A. attaches to the protein spike
    - B. binds allergens to a 3-dimensional polymer sponge
    - C. uses an industrial adhesive
    - D. uses UV rays

11. **_____, components for milk and egg became commercially available in the United States, advancing the immunofluorescence method and instrumentation to allergen component resolved diagnostics.**
    - A. Through extensive Amish research
    - B. Through an international allergy education exchange program
    - C. In 2005
    - D. In 1987

12. **Sandwich IgE assays allow for _____ of the relevant and specific antibodies.**
    - A. solid phase binding
    - B. identification
    - C. the removal
    - D. magnetic repulsion

13. **_____ requires a venipuncture and provides fully quantitative measurement and reproducibility of results.**
    - A. An extensive family history
    - B. Anytime an Epipen is used, it
    - C. Scratch testing
    - D. In vitro blood testing

14. **Component resolved diagnostic testing allows for reimbursement at _____ the whole allergen rate.**
    - A. a third of
    - B. a half of
    - C. three times
    - D. twice

15. **_____ has turned what were once exclusively outdoor allergens into indoor allergens.**
    - A. The use of central air conditioning
    - B. Not taking off shoes
    - C. Keeping windows open
    - D. Domestication of animals

16. **Some lipocalins and serum albumins share _____, which can cause cross-reactivity.**
    - A. sequence homology
    - B. carbohydrates
    - C. spike proteins
    - D. cell membranes

17. **Secretoglobins, also known as_____, most commonly refer to Fel d 1 produced in the saliva of cats.**
    - A. immunogloblins
    - B. Immunogloblin E
    - C. uteroglobins
    - D. pacreaglobins

18. **If a patient is symptomatic with cats and is sensitized to Fel d 1, the Fel d 1 is considered a(n) _____.**
    - A. Ixodidae
    - B. primary sensitizer
    - C. RAST
    - D. allergen target

19. **This unique prostatic _____ protein is expressed in the prostate and shed in saliva and male dog dander.**
    - A. Kallikrein
    - B. Immunogloblin E
    - C. α-Gal
    - D. Ixodidae

20. **_____ is a mammalian (red) meat allergy, typically presenting as a delayed a systemic reaction or anaphylaxis, caused by sensitization to a sugar complex protein.**
    - A. Immunogloblin E
    - B. Kallikrein
    - C. Ixodidae
    - D. Alpha-Gal syndrome

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2. **To what extent was the article well-organized and readable?**
   P ① ② ③ ④ ⑤ E
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Cerebrospinal fluid findings in neonatal Group B streptococcal meningitis

By Marianne Travis, MS; Christy Beneri, DO; Lisa Senzel, MD, PhD

Preserving cellular integrity in cerebrospinal fluid (CSF) specimens can be challenging in laboratory practice. Delays in transporting samples or in processing them can cause cellular degeneration. However, cellular degeneration also can occur on rare occasions even when the specimens arrive at the lab and are processed within acceptable time parameters.

Just such a case occurred with specimens for an infant diagnosed with severe late-onset Group B streptococcal meningitis at Stony Brook University Hospital in New York. Due to widespread cellular degeneration, an accurate CSF cell differential could not be performed. GBS grew in peripheral blood and CSF cultures, confirming the diagnosis.

A potential explanation for this unusual rapid form of WBC degeneration is a recent finding that certain bacterial pathogens can accelerate the process of cell degeneration.

At 27-days-old, the infant arrived in the emergency department with fever, nasal congestion, shortness of breath, and decreased responsiveness. Her rectal temperature and heart rate were 38.7 C and 233 bpm, respectively. Cerebrospinal fluid (CSF), urine, and peripheral blood analyses were obtained, and intravenous therapy with ampicillin, ceftazidime, and acyclovir was initiated.

The infant described in this case was born at 36 weeks gestation (preterm) to a mother who had negative prenatal labs, including Group B streptococcus (GBS). The mother's pregnancy was uncomplicated, including her delivery and postnatal period.

Early- vs late-onset GBS disease

GBS is a primary cause of neonatal septicemia and meningitis, and it is categorized into early-onset and late-onset diseases (EOD and LOD).[1,2] Neonatal Group B streptococcal meningitis is not always attributable to the mother and can occur in infants, even when maternal GBS screens are negative.

EOD affects infants ages 1 to 6 days old via vertical transmission, before or during delivery, when GBS is present in the mothers' genital tracts and anorectal sites.[1,2] Many of the infants developed bacteremia and pneumonia,[3] with low incidence of meningitis.[3,4]

False negative prenatal GBS culture screens contributed to most cases of EOD, primarily due to suboptimal specimens for culture or to maternal conversion from GBS negative to positive after preliminary culture.[5] Although time-consuming with a 36-48-hour incubation period, a vaginal-rectal culture continues to be a gold standard for routine GBS screen, owing to its 98.4% sensitivity, compared to >90% sensitivity for polymerase chain reaction (PCR) with a 1-4-hour turnaround time.[5]

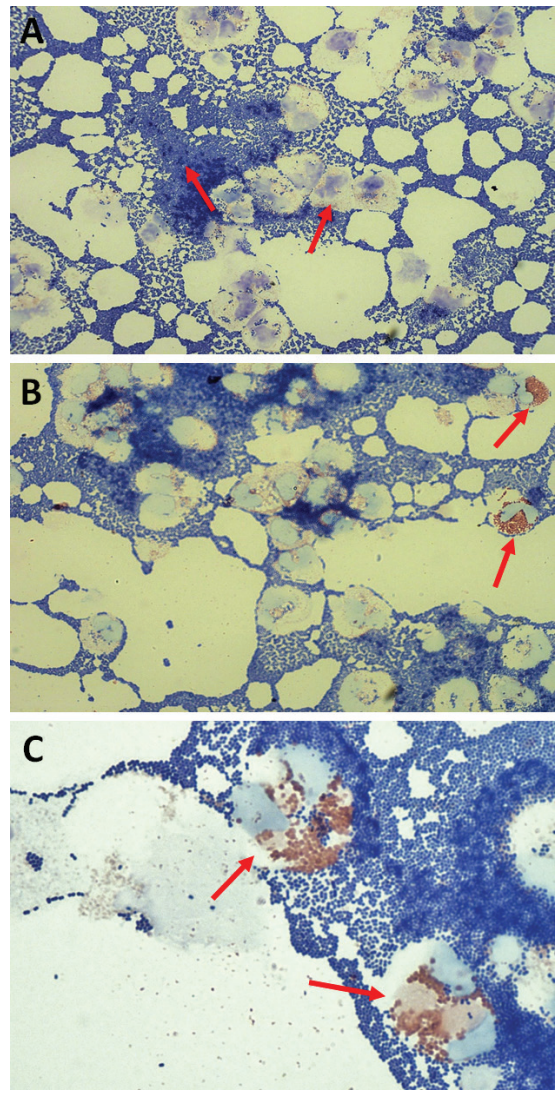

Figure 1. CSF Wright-Giemsa stain of our patient, a 27-day-old female infant with severe LOD-GBS. **A:** Moderate degenerated WBCs (presumably PMNs) and an overwhelming number of GPC, suggesting advanced sepsis (original magnification 500x). **B:** Rare to few semi-intact eosinophils, distinguished by their large, red, acidophilic granules (original magnification 500x). **C:** Degenerated eosinophils with cytoplasmic contents, but with no apparent cytoplasm (original magnification 1000x).

In contrast, LOD occurs in infants between 7 and 89 days of life.[2,6] Multiple potential modes of transmission include: horizontal (spread of infection from one infant to another through shared healthcare givers),[7] vertical (by breastfeeding infected milk),[8] nosocomial (e.g., use of contaminated medical equipment),[7] or genetically driven (i.e., GBS as normal intestinal flora of neonates likely developed virulence factors, which facilitated it to cross intestinal mucosa and blood-brain barrier).[9] LOD is usually associated with bacteremia and meningitis,[3,5] and neurological sequelae are common in severe cases.[2]

Prevention measures, such as a routine prenatal GBS screen and intrapartum antibiotic prophylaxis (IAP), have significantly decreased the incidence rate of EOD from 1.7 to 0.4 per 1000 live births since 1990; however, LOD did not benefit from these interventions, and the incidence rate remains 0.3 to 0.4 per 1000 live births.[5] Availability of an effective vaccine against GBS is a promising tool that would eventually lower the incidence rate of both LOD and EOD.[6]

Clinical and laboratory findings in GBS meningitis

Severe LOD-GBS is diagnosed when meningitis, brain lesion, seizure, death, or need for mechanical ventilation, or catecholamine support, occurred during or after infection.[2] As with other types of

bacterial meningitis, classic laboratory findings in severe LOD-GBS include: CSF turbidity, neutrophilic pleocytosis, elevated protein, decreased glucose (hypoglycorrhachia), and presence of bacterial antigen; observation of bacteria and PMNs on CSF stains; and bacterial growth in CSF and peripheral blood cultures. These findings are indicative of the loss of integrity of blood-brain and blood-CSF barriers during bacterial meningitis, resulting in the influx of WBCs and serum-derived proteins into the CSF. The marked increase in WBCs and proteins can cause CSF turbidity. Glucose is depleted by WBC consumption during phagocytosis and metabolic utilization by bacteria.

In the case described here, the white blood cell (WBC) count in peripheral blood was 1.48 x 103/µL (7.0-17.0 x 103/µL), with a differential of 14% polymorphonuclear neutrophils (PMNs), 7% band neutrophils, 67% lymphocytes, 2% atypical lymphocytes, 6% monocytes, 1% basophils, and 3% metamyelocytes. CSF was turbid, with a protein of 196.5 mg/dL (20.0-120.0 mg/dL), glucose of <2.0 mg/dL (40.0-70.0 mg/dL), WBC count of 159/µL (0-30/µL). In addition, a bacterial antigen was positive for GBS. Many Gram-positive cocci (GPC), moderate degenerated white blood cells (WBCs), and rare to few eosinophils were observed on CSF Wright-Giemsa stain **(Figure 1)**.

The infant was diagnosed with severe late-onset Group B streptococcal meningitis, following observation of seizures, detection of tiny abscess and leptomeningeal enhancement consistent with meningitis by magnetic resonance imaging (MRI) of the brain. She was treated with ampicillin and gentamicin, until blood and CSF cultures cleared, then completed a 4-week course of therapy with penicillin.

Her condition had clinically improved after 15 days of critical care, and she completed the course of therapy at home. She was also discharged on phenobarbital, with continued monitoring for GBS-related sequelae.

	Case 1[10]	Case 2[11]	This case	Normal range
Gestational age	Early term	Full term	Preterm	-
Gender	Male	-	Female	-
Age upon admittance (days)	14	19	27	-
CSF glucose (mg/dL)	13.0	32.0	<2.0	40-70
CSF protein (mg/dL)	344.0	15.0	196.5	20-120
Bacterial antigen	-	-	Positive	Negative
CSF bacterial count	Many	-	Many	0
CSF cell count (/µL)	375	840	159	0-30
CSF cell differential				
Neutrophils (%)	55	47	-	0
Eosinophils (%)	45	24	Rare to few	0
Monocytes (%)	0	27	-	30-50
Lymphocytes (%)	0	1	-	60-70
Basophils (%)	0	1	-	0
WBC count (x 103/µL)	5.2	1.95	1.48	7-17
Peripheral blood cell differential				
PMNs (%)	20	19	14	50-75
Band neutrophils (%)	23	0	7	0
Lymphocytes (%)	2	73	67	25-40
Atypical lymphocytes (%)	0	0	2	0
Monocytes (%)	2	7	6	4-10
Eosinophils (%)	1	1	0	0-3
Basophils (%)	0	0	1	0-1
Metamyelocytes (%)	0	0	3	0
Bacteremia	Yes	Yes	Yes	-
Meningitis	Yes	Yes	Yes	-
Seizures	Yes	None	Yes	-
Brain abscess	None	None	Yes	-

Table 1. Comparison of laboratory profiles of severe LOD-GBS cases with eosinophils in CSF. See references 10 and 11.

CSF eosinophilia in GBS meningitis

The occurrence of eosinophils in CSF of neonates with severe LOD-GBS is rare. We found only two cases reported in the medical literature prior to the case at Stony Brook.[10,11] **(Table 1)**. All three cases exhibited CSF pleocytosis, hypoglycorrhachia, bacteremia, peripheral blood leukopenia, and neutropenia.

In the past, the significance of eosinophils focused on their involvement in parasitic infections and allergic reactions, but current knowledge of their functions suggests that they can be activated by bacteria,[12] engaging in bacterial killing and phagocytosis in inflammatory sites.[13]

Thus, the presence of eosinophils in the CSF of the infant at Stony Brook and the two earlier cases was probably induced by GBS, and their recruitment was most likely facilitated by interleukin-5 (IL-5) and eotaxin-1. IL-5 is a cytokine that regulates eosinophil growth, activation, survival, and migration; eotaxin-1 is a chemokine that stimulates chemotaxis in eosinophils.[14]

Moreover, the occurrence of basophils in Case 2 **(Table 1)** was possibly related to their recently recognized functions as antigen-presenting cells and as regulators of adaptive immunity.[15] Overwhelming sepsis and decreased bone marrow production have potentially caused the reduction of WBCs and neutrophils, as well as the emergence of immature cells in peripheral blood.

Significance of CSF cellular degeneration

Cellular degeneration is a descriptive term for disintegrated, or lysed, cells observed on body fluid stains (e.g. Gram stain, Wright-Giemsa stain). Because degenerated cells lack intact cytoplasm, and cytoplasmic contents are often missing or morphologically distorted, they are difficult to distinguish and are excluded from differential **(Figure 1)**. In cases where cells are excessively degenerated, a differential is not reported, potentially affecting clinical decisions in situations where there is a need to differentiate between bacterial and aseptic meningitis. Sometimes, clinicians may request that the laboratory identify the WBCs and estimate their numbers if possible.

CSF cellular degeneration is known to be caused by delayed transportation and processing time. Removal of CSF from the body may lead to lysis of WBCs as a result of increased pH,[16]

hypotonicity, and decreased concentrations of membrane-stabilizing proteins and lipids.[17]

Neutrophils in CSF have a very short life span *in vitro* (7-h viability). They lyse faster than monocytes and lymphocytes and show a reduction rate of 32% after 1 hour and 50% after 2 hours at room temperature.[18] The present case had a specimen transportation and reporting time of 0.18 h (< 1.0 h) and 0.89 h (< 1.15 h), respectively; therefore, processing delays do not readily explain the approximately 90% degenerated WBCs present on the CSF Wright-Giemsa stain of the patient.

Similarly, Stony Brook had a 12-year-old male patient who had a neurosurgery for hydrocephalus that was complicated by *Streptococcus mitis/oralis* group. There was no delay in specimen transportation and reporting time (0.42 h and 1.10 h, respectively), but the CSF Wright-Giemsa stain showed many GPC and many degenerated WBCs. CSF cell differential was unavailable, as WBCs were too degenerated.

A potential explanation for this unusual rapid form of WBC degeneration is a recent finding that certain bacterial pathogens (e.g. *Streptococcus, Staphylococcus, Listeria, Borrelia, Burkholderia*) can accelerate apoptosis or induce PMN lysis to escape intracellular killing via activation of pro-death Bcl-2 protein family.[19] We hypothesize that the large numbers of streptococci seen on the CSF Wright-Giemsa stains in both of these cases triggered extensive apoptosis, leading to the cellular degeneration seen on the slides. While several methods have been proposed for preserving CSF cellular integrity when processing is delayed (e.g. addition of serum-containing medium or Earle's balanced solution with human serum albumin to CSF specimen, spiking of CSF cells into 5% fetal calf serum or saline, refrigeration of CSF specimen, minimum centrifugation procedure[19]), these steps are difficult to implement in routine clinical practice and may not help for CSF specimens with moderate to many bacterial pathogens that are known to trigger PMN lysis or accelerate apoptosis.

In conclusion, preserving cellular integrity in cerebrospinal fluid (CSF) specimens can be challenging in laboratory practice. In this case, neutrophils in the CSF showed cellular degeneration despite short time to analysis, and occasional eosinophils were present. Neutrophil degeneration in the setting of overwhelming bacteria may be due to accelerated apoptosis. In these cases, it may be appropriate for the lab to provide a descriptive or qualitative report of the WBC morphology for this type of slide. A practical approach to this situation is to refer the CSF stain to a pathologist or lead technologist, because they may be able to classify the degenerated WBCs based on their morphological features, which would help with the clinical interpretation.

Acknowledgements

The authors wish to thank Lorillaine Villasis, MPH; Rebecca Riemann, MT; Bruce Kube, MS; Aderonke Adefisayo, MD; and Eric D. Spitzer, MD, PhD, for their expert technical assistance, clinical input, and review of the manuscript.

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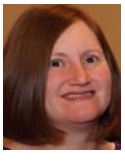

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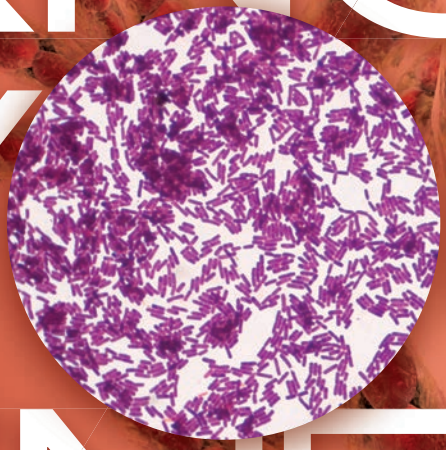

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How flow cytometers are making a difference in clinical labs

By Meredith Salisbury, BA

Flow cytometry was once relegated to sophisticated core laboratories, where only highly trained scientists could operate the complex instrumentation. Now, major advances in flow cytometry have made this technology more accessible and much easier to use — so easy, in fact, that more and more clinical laboratories have adopted flow cytometers for a wide variety of uses.

Even though many flow cytometers are considered "research use only" instruments, their compact footprint and reliable results have made them ideal for quality control steps that take place upstream of clinically regulated pipelines, as well as for laboratory-developed tests in CLIA-accredited facilities.

These platforms allow for extremely high-throughput analysis of cells or small particles. Thanks to the use of fluorescent labels, they can also be used to interrogate many different biomarkers or parameters at a time. Here, we'll review some of the current and coming applications of flow cytometers in clinical labs, as well as key features to look for when choosing the right platform for your lab.

"With their ability to stream thousands of cells per minute, flow cytometers are invaluable for handling any kind of QC process requiring counting, sorting, or basic analysis."

Sample assessment

Most clinical laboratories implement flow cytometers for quality control steps associated with ensuring sample viability or for purification before the sample is prepared for clinical testing. With their ability to stream thousands of cells per minute, flow cytometers are invaluable for handling any kind of QC process requiring counting, sorting, or basic analysis.

Cell counting, for example, is important for ensuring sufficient sample volume for some clinical assays. Assessing viability is also critical for many samples and is frequently needed for blood samples, before any kind of test can be run on them. A purification step that pulls certain cells of interest from a broader population can also be accomplished with these instruments — for instance, separating specific immune cells in preparation for a clinical assay designed to detect natural killer cell activity.

These upstream steps are an excellent fit for RUO flow cytometers and can be performed with the simplest flow instruments available. Though these tasks are not technically part of running a clinical assay, they help to ensure that the downstream clinical results are as robust and reliable as possible.

Beyond sample assessment, flow cytometers have been adopted for more and more clinical assays and other applications. Laboratory-developed tests have deployed these instruments for uses ranging from autoimmune disorders to transplantation. Clinical assays based on flow cytometry are regularly used for detecting primary immunodeficiencies and for monitoring progression markers, such as counting CD4 T-helper cells in patients with HIV. Detecting autoantibodies can help identify patients with autoimmune disorders, while spotting unusual characteristics in blood samples can aid in the diagnosis of leukemias or lymphomas, as well as in the monitoring of a fetal-maternal hemorrhage situation. In some bone marrow transplant cases, flow cytometry is used for analyzing and counting stem cells prior to transplanting them into patients; it may also be used for monitoring the uptake of those stem cells after the transplant.

In addition, flow cytometry may be implemented for clinical studies, where it can be used to monitor vaccine response by tracking antibody presence or to assess immune cell performance or patterns over time.

	Standard flow cytometry	Imaging flow cytometry
Size	Compact, benchtop	Larger, fits under cell culture hood
Ease of use	Simple	More advanced
Traits detected	Fluorescent detection for key markers, antibodies	Standard traits plus cell morphology
Clinical lab use	Often used for QC steps upstream of clinical workflows	More likely to be found in a flow core lab than a clinical lab today

Table: Characteristics of Standard Flow Cytometry vs. Imaging Flow Cytometry

Flow features

For lab personnel considering adopting a flow cytometer, there are a number of factors to consider. Different instruments have varying degrees of simplicity or complexity. If the tool will only be used for the basics — such as cell counting, sorting, and checking viability — then it makes sense to choose the system with best ease-of-use. More sophisticated technology should be reserved for situations where it's really needed. Size is another key attribute; many compact systems are now available for situations where bench space is at a premium.

To ensure robustness and reproducibility, check not only system performance metrics but also the availability of software that includes straightforward modules or apps for each procedure. That reduces the need for training and increases the likelihood that the platform will run the same way every time.

Another option to consider is whether to adopt flow cytometry with imaging. This increases complexity as well as size, so it isn't right for all laboratories. But platforms that

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pair flow cytometry with microscopy for image-based results can be powerful tools for more sophisticated sample analysis. These platforms make it possible to count and sort cells based on morphological differences in addition to biomarkers that can be tagged with fluorescent labels — a feature that can be useful with red blood cells and many other sample types.

Looking ahead

As more clinical laboratories adopt flow cytometers, their applications continue to expand. In the near future, flow cytometers may allow lab technicians to monitor a patient's cancer by analyzing extracellular vesicles (EVs). There are already platforms available that have the resolution required to detect these vanishingly small particles and even to interrogate the protein or DNA cargo they may carry. While this application is primarily used in research labs right now, soaring interest in using EV analysis as a non-invasive cancer monitoring technique could lead to clinical adoption relatively soon.

Another near-term application for flow cytometers is micronuclei testing. Micronuclei populations in a cell are the result of exposure to toxic compounds, including radiation, and their analysis is likely to have strong clinical relevance. At the moment, identifying micronuclei is challenging, and doing so at the throughput needed is very difficult. Scientists are already working to develop this application with imaging-based flow cytometers.

As these and other protocols are developed for flow cytometers, what's clear now is that these instruments are already quite useful in a clinical lab environment and appear likely to become even more important in the years to come.

Meredith Salisbury, BA, is a freelance journalist covering the life sciences field. She has extensive background reporting on genomics, molecular diagnostics, next-generation sequencing and a broad range of technologies.

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Next steps to eliminate race-based reporting of the estimated glomerular filtration rate (eGFR)

By Harvey W. Kaufman, MD, and Lee H. Hilborne, MD, MPH

In 2018, then University of Washington medical student Naomi Nkinsi, was "shocked" by the dual reporting of estimated Glomerular Filtration Rate (eGFR), which is used to assess kidney function by race, specifically African American or non-African American. Other medical students joined Nkinsi's campaign to remove racial factors from medical calculations/algorithms. (This article uses Black, non-Hispanic for the group previously referred to as African American, but the U.S. Census continues to use both terms.)

In response, specifically to the eGFR calculation, the National Kidney Foundation (NKF) and the American Society of Nephrology (ASN) created the Task Force on Reassessing the Inclusion of Race in Diagnosing Kidney Diseases to examine the issue and provide recommendations.

In April 2021, the NKF-ASN Task Force issued an interim report.[1] The interim report is the second step in a three-step process:

1. Clarify the problem of race-based equations and examine the evidence to replace them.
2. Review challenges relative to identifying and implementing alternative methods.
3. Recommend the best approach for replacing the existing equations.

The NKF-ASN Task Force responded quickly, despite the lack of a clear understanding about the outcome of its work. It is imperative to collectively settle on a solution that best serves all patients based on data, equity, and healthcare outcomes. Nephrology is leading the charge to examine the use of variables defined by race in clinical algorithms. Similar challenges exist in other disciplines, including cardiology, cardiothoracic surgery, endocrinology, obstetrics, oncology, pulmonary medicine, and urology. Indeed, the eGFR calculation is only one of at least 13 clinical algorithms that uses a race variable.[2]

How did we get here?

Structural racism that affects health must be distinguished from physiological and pathophysiological determinants of health. "Black, non-Hispanic" is a social construct and not a biological one. (An example of a physiological difference is the relationship between skin pigmentation and ultraviolet light absorption that is critical for vitamin D synthesis.) Binary choices based on social constructs are particularly challenging in populations where an individual's heritage can be heterogenous. Nevertheless, observational studies identified kidney function differences based on the social construct of racial identification.

Directly measured GFR is limited to research applications. Indirect measures are more practical in routine practice, including assessment of serum creatinine and urea nitrogen that are inversely proportional to kidney function. These tests have existed for over a century and are among the most commonly performed clinical laboratory tests. In reporting results, clinical laboratories recognize differences in serum creatinine based on age and sex that may reflect, in part, differences in muscle mass; thus, age- and sex-specific reference intervals are common. As noted, observational studies also demonstrated differences based on race, but causation and how to integrate those observations into diagnoses, categorizations, and treatment were not well understood.

Clinical researchers sought a more unified approach to assess kidney function and explored algorithms that corrected for recognized differences in adults based on age and sex. The Jelliffe equation, published as a letter to the editor in 1973, used serum creatinine, a body surface factor of $1.73m^2$ that became embedded in subsequent equations, along with age and sex. Another early proposed equation that gained utility was the Cockcroft–Gault equation, based on data from 249 white males that measured creatinine clearance ranging from 30 to 130 mL/min. The equation assumed women have 85% of the kidney function of men without any direct data from the original all male cohort. The empirical correction factor for females was later validated to be between 84% and 88% of the male value. Subsequent research, including the Third National Health and Nutrition Examination Survey (NHANES), found that the Cockcroft–Gault equation may be insufficient in Black, non-Hispanic individuals because of higher serum creatinine in Black, non-Hispanic adults compared to White, non-Hispanic, adults.

Recognizing these shortcomings, clinical researchers added a race coefficient to address the observation that patients who self-identified as African American had significantly higher eGFR values compared to the gold-standard of directly measured GFR — leading to the Modification of Diet in Renal Disease (MDRD) Study equation. Despite no inherent physiological difference, race was used as a substitute for unidentified factor(s) that are more common in people who self-identify as Black, non-Hispanic. Importantly, the African American coefficient in the regression models in the MDRD Study equation was a statistically significant improvement when compared to the standard of directly measured GFR. The MDRD Study equation was further validated in the African American Study of Kidney Disease. Andrew Levey, then editor of the *American Journal of Kidney Disease*, was instrumental in promoting the MDRD Study equation as reflective of the observational differences. The dual race-based eGFR reporting became and remains a cornerstone in the International Kidney Disease: Improving Global Outcomes (KDIGO) and US. Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines.

Discussion ensued as to the underlying cause for observed racial differences. Years later, the original investigators updated the analysis; the equation known as the CKD-EPI creatinine equation still included a race coefficient.[3] This revision was based on analysis of pooled data from 10 studies and included almost 3,000 patients who self-identified as Black, non-Hispanic. The CKD-EPI coefficient for African Americans compared to non-African Americans was 1.16 versus 1.21 in the MDRD Study equation.[3] (The higher eGFR when using the race coefficient suggests "more normal" kidney function in Black, non-Hispanic adults than in matched non-African Americans for identical serum creatinine and age/sex.)

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Where are we now?

Black, non-Hispanic adults are three times more likely to suffer from kidney failure; they constitute over 35% of U.S. dialysis patients yet comprise only 13% of the U.S. Census population. Given that Black, non-Hispanic adults have higher rates of kidney failure, delays in care due to under-estimation of risk would exacerbate this gap. With dual eGFR reporting, Black, non-Hispanics have delayed referral to nephrologists. Indeed, in a cohort study that compared eGFR calculated with and without the race coefficient, achieving a clinical threshold for kidney transplant referral and eligibility was delayed because of the higher eGFR calculated for Black, non-Hispanic individuals.[4,5] However, proponents of the CKD-EPI creatinine equation point out that ignoring the observed differences between African American and non-African American eGFR data would lead to over-diagnosis and over-treatment.[6] Specifically, eliminating the race coefficient is associated with a systematic error in the evaluation of Black, non-Hispanic adults by underestimating reported GFR compared to direct measurement of GFR throughout the range of values.

The observed renal differences based on race impact other aspects of nephrology; they are not limited to the eGFR equation. The Kidney Donor Risk Index (KDRI), implemented by the National Kidney Allocation System in 2014, summarizes the likelihood of graft failure after deceased-donor kidney transplant. It includes a factor for race. The inclusion of this factor is based on the empirical observation that Black donors' kidneys perform worse than non-Black donors' kidneys, regardless of the recipient's race. Genetic factors (e.g., the Apolipoprotein L1 gene [*APOL1*]) could replace race in KDRI; however, current turnaround times make implementation challenging because of need to provide test results to inform transplant center decision-making during the finite hours that a deceased person's donor kidney is viable.[7]

What's next?

The NKF-ASN Task Force is working to quickly resolve issues related to the inclusion of race in eGFR and other calculations. It is prudent to await their final report before implementing widespread changes, because some already-suggested approaches are confusing and potentially misleading.[8,9] Clinical laboratories dropping the African American eGFR coefficient before the final report could be unresponsive to the professional guidance from NKF-ASN. Already suggested options include discarding the non-African American value or providing a weighted result. Dropping only the African American value "would induce a systematic underestimation of measured GFR in Blacks, with potential unintended consequences at the individual and population levels," Salman Ahmed and researchers wrote in an article in the *Journal of General Internal Medicine*.[10]

Mallika Mendu and colleagues examined the impact of removing the race coefficient from the CKD-EPI calculation of eGFR on how Black, non-Hispanic patients are classified.[11] More than 30% of Black, non-Hispanic patients would be reclassified as having a more severe stage of CKD, with approximately 25% at stage G3 reclassified to stage G4 CKD based on eGFR. Such a change would result in the referral of many more Black, non-Hispanic patients to more advanced kidney care, e.g., specialists and potential preparation for dialysis. This shift may help some individuals appropriately receive more timely or intensive interventions; it may also lead to "over-treatment" or have unintended consequences for others. Importantly, 3.1% of Black, non-Hispanic individuals with a CKD-EPI creatinine-based eGFR of >20 mL/min/1.73m^2 would meet the criteria for kidney transplant priority (eGFR <20 mL/min/1.73m^2) if the race coefficient were removed. Among Black non-Hispanic patients with a CKD-EPI eGFR <20 mL/min/1.73m^2, only 19.2% were referred for kidney transplantation. Thus, overcoming inequities in access to kidney transplantation cannot be entirely addressed by modifying eGFR reporting.

Vishal Duggal and colleagues found that the prevalence of CKD among U.S. Black, non-Hispanic adults would double if the race coefficient were removed. The change could affect up to 40% of Black non-Hispanics using common medications for which dose adjustments are recommended based on kidney function. Lower doses of drugs cleared by the kidneys could impact clinical outcomes if insufficient drug doses are prescribed; conversely, they could decrease drug toxicity if current dosing is excessive.[12]

The ultimate question may not be what eGFR equation best predicts measured GFR in different populations, but which equation leads to the best health outcomes in each biologically defined population. How can serum-based eGFR and the urinary albumin-creatinine ratio test results be applied differently based on identifiable additional risk factors, including age and sex? The Kidney Failure Risk Equation (KFRE), validated across multiple populations, is one approach. It was developed by Nav Tangri and colleagues and predicts kidney failure risk at 2 and 5 years.[13] The KFRE performed well even without the race coefficient.[12]

Jennifer Bragg-Gresham and colleagues assessed the impact of excluding the race factor in the CKD-EPI equation using data from NHANES (1999 to 2018) and data on Black veterans from the Veterans Affairs (VA) Health System (2018).[14] Based on NHANES data, the mean eGFR for Black, non-Hispanic adults decreased from 102.8 mL/min/1.73m^2 with the race coefficient to 88.1 mL/min/1.73m^2 without it. Using VA data, the mean eGFR decreased from 82.9 to 71.6 mL/min/1.73m^2. The prevalence of eGFR <60 mL/min/1.73 m^2 increased from 5.8 to 10.4% using the NHANES data and from 15.5 to 26.3% using the VA data. These changes demonstrate the magnitude of the impact that dropping the African American coefficient would have using real-world data. It is important, therefore, to understand the repercussions of any possible change before one is implemented.

Introducing a new equation without a race coefficient poses challenges, because most patients will see an eGFR that is different than the one previously reported using the CKD-EPI creatinine equation, especially near decision values. The revised calculation will meaningfully impact many individuals, including both positive and negative changes in, for example, eligibility for clinical trial enrollment, medication management (e.g., diabetes, oncology, antibiotics, anticoagulants), contrast imaging, nephrology referral, dialysis eligibility, kidney transplantation recipient and donor eligibility, medical nutrition-therapy education, and even reimbursement for health services and life insurance. W. Greg Miller notes that the eGFR calculation uncertainty has a greater impact than the race coefficient in the estimation,[15] yet race-based adjustments shift patients into different categories on both an individual and population basis compared to the absence of adjustments.

Professional organizations, including the NKF are likely to provide additional guidance to clinical laboratories about how to communicate and implement the expected changes. This guidance includes whether clinical laboratories report, at least for some time, both the CKD-EPI equation values and the new eGFR value to allow clinicians to compare results. Will existing clinical trials require CKD-EPI-based calculations? Will prior studies need to be re-calibrated to reflect the new eGFR calculation?

Alternatives to race self-identification to describe meaningful underlying physiological and pathophysiological differences must be explored.[16] One approach could be to identify the genetic factors that increase risk for Black, non-Hispanic individuals (e.g., apolipoprotein L1 [*APOL1*], a minor apoprotein component of HDL cholesterol).

APOL1 is involved in protecting against *Trypanosoma brucei rhodesiense* infection, a parasite transmitted by the tsetse fly that causes sleeping sickness. Two *APOL1* coding sequence variants in *APOL1* (G1 and G2) confer resistance to the parasite yet are associated with kidney disease. People with one variant have *Trypanosoma brucei rhodesiense* protection, but those with two have increased risk of kidney disease (i.e., a recessive trait). Many African Americans are descendants of people of West African nations, which have a high prevalence of *APOL1* variants. For example, the Yoruba people of Nigeria have a 40% and 8% prevalence of G1 and G2 risk alleles, respectively. The G1 and G2 risk alleles are found in over 30% of African Americans. Consequently, descendants from West Africa also have a high prevalence of *APOL1* risk alleles, as well as *APOL1*-associated kidney diseases. The risk alleles are found in nearly half (47%) of the hypertension-attributed end stage renal disease in Black, non-Hispanic individuals.

Cystatin C is a viable biomarker to complement serum creatinine and eGFR, because it is less influenced by diet and muscle mass.[17] Currently, NKF KDOQI clinical practice guidelines suggest cystatin C testing for patients with a CKD EPI creatinine-based eGFR of 45-59 mL/min/1.73m^2 in the absence of significant albuminuria (if confirmed for 3+ months, CKD stage G3aA1). Given cystatin C is independent of race, this test may be an attractive alternative. There are CKD EPI equations for cystatin C, either alone or when combined with creatinine. Concerns about substituting or supplementing creatinine with cystatin C testing include higher cost, limited availability, still incomplete assay standardization, and absence of clear payer payment policies.

Conclusion

The clinical laboratory and nephrology communities serve critical roles in assessing kidney function. Progress with eGFR was marred with a response to observed differences between a calculated eGFR from the gold standard of measured GFR for Black, non-Hispanic individuals. The unintended consequences of including race in the calculations are impossible to ignore. Today's heightened awareness of racial issues, with the strong voice of Nkinsi and others raising concern, brings us to the NKF-ASN Task Force and its interim report. The future final report will define a path that will exclude the race coefficient from eGFR reporting. The change will positively and negatively impact patient care. We should all await that report. When issued, laboratorians should quickly implement the recommendations to assure a unified approach to eGFR reporting. This change will not be the final chapter, because other factors (e.g., social determinants of health) influence interpretation of eGFR and CKD progression. Improving kidney disease health equity clearly requires additional interventions beyond the future race-independent eGFR calculation.

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Preparing for the pandemic impact on diabetes testing

Diabetes care was delayed by many patients during the pandemic

By Marisa L. Williams

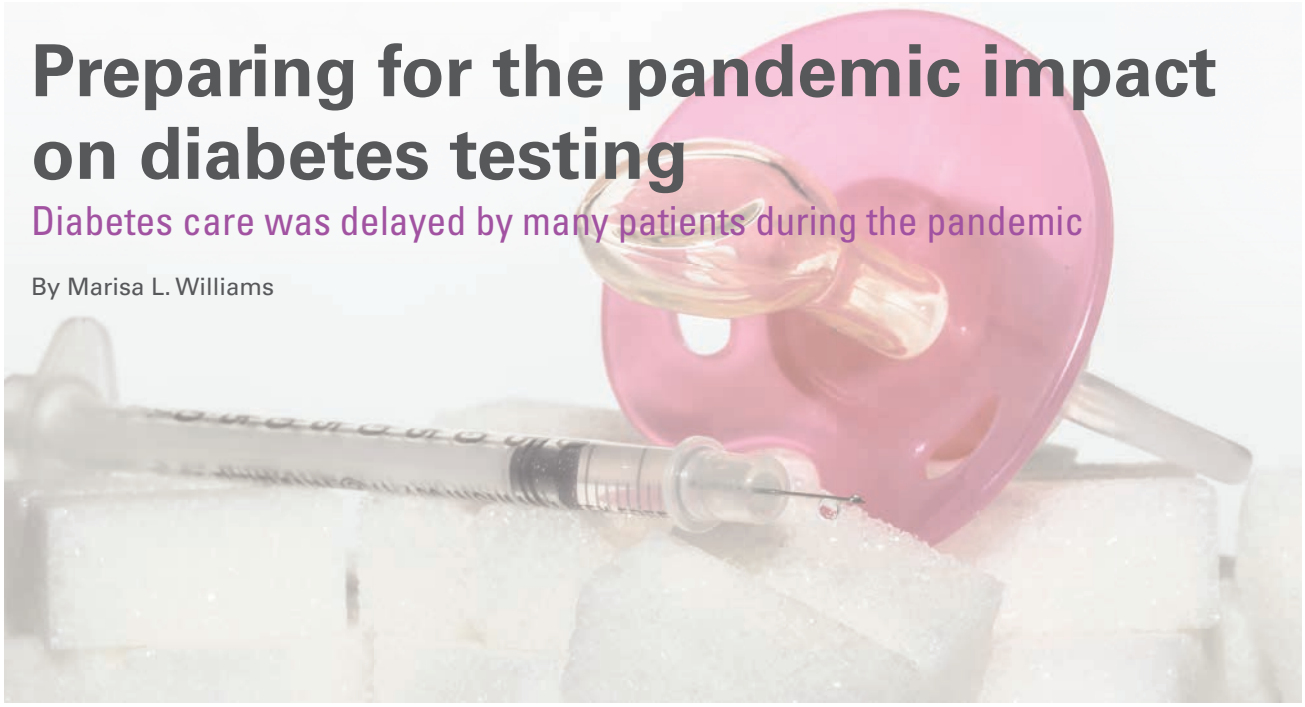

Image by Meine Reise geht hier leider Ende. Ein Neustart beginnt auf from Pixabay

During the peak of the COVID-19 pandemic, the Centers for Disease Control and Prevention (CDC) reported in September 2020 that concerns about the virus impacted an estimated 41% of adults who delayed or avoided medical care, including urgent and emergency care (12%), as well as routine care (32%).[1]

This avoidance was reported most prevalently amongst unpaid caregivers for adults, people with underlying medical conditions, Black adults, Hispanic adults, young adults and the disabled.

People with chronic disease, such as diabetes, were at higher risk than others for COVID-19, resulting in hospitalizations. According to a study by Matthew C. Wagner, PhD, and Thomas Lohmann, MD, which appeared in the November 2020 issue of *Medical Laboratory Observer*,[2] 28.3% of COVID-19 deaths had diabetes mellitus as a comorbidity. Patients with poorly controlled or uncontrolled diabetes had a higher rate of morbidity, and the rate of people hospitalized with COVID and diabetes rose as high as 85.9%.

In *MLO's* State of the Industry (SOI) survey on disease management (page 38 of this issue), *MLO* editors asked laboratory professionals about the types of tests they are conducting to diagnose and monitor diabetes. The most popular response was HbA1c at 78.5%; fasting plasma glucose test, 75.8%; random plasma glucose test, 67.8%; oral glucose tolerance test, 55.7%; and 17.4% of labs replied diabetes testing was not applicable to them.

The *MLO* editors also asked labs about the method of testing that they used for HbA1c, and the most popular answer, at 40.3%, was immunoassay. With nearly a quarter of labs (24.8%) responding that this test was not applicable at their location, the remaining responders revealed enzymatic HbA1c testing was the next most popular, 17.4%; followed by cation exchange HPLC, 14.1%; capillary separation, 4.7%; and boronate affinity chromatography, 4%.

The SOI explores additional areas where laboratorians conquered complications, potentially due to lack of screening and other issues of disease management during the pandemic.

COVID-19 impacted diabetes control

University of Michigan[3] recently reported about the vast undertreatment of diabetes in a global study, citing only one in ten people in certain low- and middle-income countries were getting the comprehensive care they needed.

For further insight on whether people with diabetes were getting the medical services they need, *MLO* asked a Promedica lab in Michigan about its local diabetes testing during the pandemic.

"People who do have health issues were not put by the wayside," assured Wendy Magoon, Registar Phlebotomist at Promedica Laboratory in Newport, MI. "People with diabetes continued to get testing during the pandemic, but they did not see the doctor in person as often. There were much more telehealth visits, but the coverage did continue."

Wendy Magoon

However, when it came to a new diagnosis, the same could not be said. "Patients who did not have the diagnosis of the illness, or thought they could wait until the pandemic was over, waited longer than people who were already diagnosed to be tested. People who were already diagnosed with diabetes were much more pro-active during the pandemic."

Monitoring delays lead to diabetic complications

Lack of monitoring can result in dire consequences for diabetics. Delaying proper foot care for diabetics can result in nails growing into the skin, known as onychogryphosis, or which may require a fungal test.

Ulcers, which may require a blood test to screen for infection, and neuropathy can impact mobility and blossom into necrosis, possibly resulting in limb amputation if not treated in time, as nerve and blood vessels are damaged by hyperglycemia, reducing blood flow, and causing tissue death.[4]

Consequences of unmonitored diabetes can be compounded by risk factors like smoking, which doubles the risk of heart

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disease for diabetics.[4] Additional complications include high blood pressure, which contributes to kidney diseases and eye complications, such as diabetic retinopathy, which may involve fluorescein angiography, an optical coherence tomography test, and regular injections into the eye, potentially leading to complications like developing glaucoma and/or cataracts, which may require surgical intervention.

The National Institutes of Health (NIH)[5] reported diabetics have a greater LV cardiac mass, which may be related to an increased adipocyte release of cytokines, notably Leptin and Resistin, which have hypertrophic effects on cardiomyocytes. Diabetics have been noted to have a slightly diminished diastolic function, which may be due to increased triglyceride synthesis, leading to increased myocardial triglycerides. This increased accumulation has been noted with lipotoxicity and altered calcium hemostasis in myocardium, which both negatively impact diastolic function.

A slight systolic function impact could be from impaired contractile reserve and myocardial sympathetic innervation. Interstitial fibrosis with increased collagen deposits observed in diabetics could contribute to diminished cardiac function.[5] With an increased risk of cardiovascular disease and a higher incidence of myocardial infarction, the one-year mortality rate of diabetics after myocardial infarction is nearly 50%.

When having open heart surgery, being diabetic is a risk factor for sternal nonunion,[6] which occurs when the sternum does not heal back together properly after a sternotomy or blunt chest trauma, resulting in clicking, pain, a gap that can sometimes be felt, and range of motion issues. Computed tomography (CT) scans help identify the location of fractures, fragments and wires, and bone density may be tested to determine optimum wire tension to prevent loss of fixation.

Beyond diabetes, other risk factors include obesity, chronic obstructive pulmonary disease, osteoporosis, malnutrition, radiation to the chest wall, and steroid use, as they inhibit healing. This can also be caused by technical errors when closing the sternum, paramedian (off-midline) sternotomy, being on prolonged ventilatory support, having a decreased cardiac function, fracture pattern, fracture gap, and the harvest of bilateral internal mammary arteries for grafting, which results in bone deterioration and mechanical failure. Sternal nonunion is associated with significant morbidity and is a precursor to osteomyelitis, mediastinitis and deep sternal space infections.[6]

Diabetic Ketoacidosis

"Diabetic Ketoacidosis is a serious complication of Type 1 Diabetes Mellitus; however, it can also affect individuals with Type 2 Diabetes, and is a danger zone for diabetics," said Jenna Coen, Reagents Marketing Manager at Randox Laboratories Ltd.


Jenna Coen

"There are known limitations with testing procedures for Diabetic Ketoacidosis when using nitroprusside-based methods, as they lack sensitivity and present greater risk of false negatives. The nitroprusside method used in semi-quantitative dipstick tests, for example, only detects acetone and acetoacetate, when D-3-hydroxybutyrate is the most abundant ketone produced during ketosis, and as such, the measurement of this analyte is much more sensitive and specific. This is something that healthcare and laboratory professionals need to be fully aware of when treating or diagnosing patients."

Precision and quantitative detection of D-3-Hydoxybutyrate is also used for diagnosing serious clinical conditions, such as sepsis, childhood epilepsy, or gestational diabetes.

"Raising awareness of the advancements in diagnostic methodologies and understanding what is already available in the market is so important for healthcare and laboratory professionals, to help overcome the growing challenges in ketone testing amidst what has been deemed as a 'Silent Diabetes Pandemic,'" said Coen.

Gestational diabetes (GDM)

The National Institute for Health Care Excellence recommends[5] screening for gestational diabetes if patients have risk factors such as obesity, a large weight gain, previous gestational diabetes, or a family history of diabetes.

Gestational diabetes can be treated several ways, depending on the severity of the gestational diabetes. The first mode of treatment is diet, and that's usually set up through a dietitian with experience with pregnancy. If that is not successful, pills are the next option, such as Metformin.

The length a patient may stay on medication depends. While there is an increased risk of miscarriage in patients with Type 2 diabetes, for patients with polycystic ovarian syndrome (PCOS) and insulin dependence, some obstetricians may prefer to maintain the Metformin through the first trimester to decrease the risk of miscarriage. If management with pills is not successful, then injectable exogenous insulin may be initiated.

If a patient has PCOS and is insulin resistant, there is a higher risk of miscarriage. The theory is that by decreasing the insulin resistance with the Metformin, it decreases the risk of miscarriage.

Around 24-28 weeks of pregnancy, a screening should be done,[7] which may consist of the fasting plasma glucose (FPG), HbA1c or 75 g oral glucose tolerance test (OGTT), with the FPG and HbA1c ideally repeated twice to confirm overt diabetes. Note that HbA1c can be used for diabetes screening, but it should not be used for screening GDM, due to the very low sensitivity.

During the COVID-19 pandemic, OGTT's were not performed as often, due to high exposure risks, resulting in some different guidelines for using HbA1c, FPG, or RPG as an alternative during the pandemic.[7] Currently, the threshold for overt diabetes is an HbA1c greater than or equal to 6.5%; however, recent studies revealed this should potentially be lowered to greater than or equal to 5.9%.[7]

Patients with gestational diabetes should get a glucose tolerance test at six weeks post-partum to make sure the diabetes has resolved, and they are not Type 2 diabetic. If they have Type 2 diabetes, then standard diabetes care should be followed.

Additionally, gestational diabetics are at risk of polyhydramnios, increased amounts of amniotic fluid surrounding the baby, which can cause uterine irritability and is associated with an increased risk of umbilical cord prolapse.

Mothers that develop gestational diabetes tend to have larger babies, especially if they're blood sugars are out of control. The babies are exposed to elevated glucose levels from their mothers; thus, the baby secretes more insulin to keep its sugar levels normal. With the increased insulin secretion, babies gain more weight.

Macrosomia[8] is the term for a larger than normal baby, which is commonly defined as a baby larger than 4000 grams or 8.8 pounds (some sources note 4500 grams, 9.9 pounds, for babies of gestational diabetics). This increases risk of additional birth complications, such as shoulder dystocia, when the baby's shoulders get stuck inside the mother;

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clavicle fractures; damage to the nerve that sends signals to the arm, known as brachial plexus injury; uterine rupture; and vaginal tearing.

The babies can develop hypoglycemia after birth,[8] as they continue to create excess insulin. Since they are not exposed to their mother's high sugar levels anymore, their sugars crash. Needing additional glucose, which may be simply sugar water in a bottle, some get so weak, they cannot even suckle and may reqire an IV with glucose supplementation to raise their glucose levels to normal.

Babies with fetal macrosomia have a risk of childhood obesity and metabolic syndrome.[8]

How labs are testing for diabetes

In a paper by H. David McIntyre,[9] et al.,"Testing for gestational diabetes during the COVID-19 pandemic," that appeared in *Diabetes Research and Clinical Practice,* in November 2020, the authors showed how testing for gestational diabetes shifted during the COVID-19 pandemic in different countries across the globe.

For example, the United Kingdom did risk factor based testing, going from 75 g OGTT to no OGTT during the pandemic, and post pandemic, they have transitioned to universal screening for fasting glucose and HbA1c. Pre-pandemic, GDM was if fasting venous puncture glucose (FVPG) was greater than or equal to 5.6 mmol/L and/or 2 hVPG greater than or equal to 7.8 mmol/L. During the pandemic, that changed to being GDM if the HbA1c is greater than or equal to 5.7% (39 mmol/mol), and/or FVPG is greater than or equal to 5.6 mmol/L, and/or Random VPG (not preferred) is greater than or equal to 9 mmol/L.

To reduce the risk of diabetes, the World Health Organization (WHO)[10] launched the Global Diabetes Compact initiative, coinciding with the 100th anniversary of the discovery of insulin, helping efforts to scale up diabetes care globally.

Advances in diabetes

With diabetes testing becoming more commonplace, labs have access to new technology that helps automate diabetes diagnostics.

Vendors of chemistry platforms have advanced the level of automation in diabetes diagnostics, and though the HbA1c and glucose tests are popular, more in-depth diagnostics are available, such as a Fructosamine liquid assay, which monitors the degree of glycemia over 1-3 week timeframes. The American Diabetes Association advises that this may be a better choice when the A1C cannot be measured reliably.[11]

Additional automated assays include the Access C-Peptide, which distinguishes exogenous vs. endogenous insulin excess, helping to differentiate between Type 1 and Type 2 diabetes in human serum, plasma, and urine. CSF Albumin is another urine test to detect low or rising albumin levels, which could reflect kidney complications in diabetics.[11]

Automation of the quality control process for diabetes testing also has improved. For example, "the new InteliQ Diabetes quality control helps clinical labs automate their diabetes QC workflow through the load-and-go efficiency of the barcoded, patient-like QC tubes," said Mary Buchanan, Associate Director of Marketing, Quality Systems, Clinical Diagnostic Group at Bio-Rad Laboratories.

"These barcoded tubes eliminate the need for tedious pour-off steps and manual data entry, which are steps typically performed in most diabetes QC workflows today. Eliminating these manual steps reduces turnaround time, minimizes human error, and increases the labs overall efficiency. As an added benefit, the InteliQ Diabetes control is compatible with major instrument platforms, including Siemens Atellica, Roche cobas, and Abbott Alinity among others, providing a flexible solution for every QC lab."

Diabetic advances on the horizon are even more exciting. The Washington University School of Medicine in St. Louis (WUSTL) has teamed up with other organizations to focus on stem cell research and diabetes. In 2016,[12] researchers from WUSTL and Harvard University produced insulin-secreting pancreatic beta cells from skin stem cells of Type 1 diabetes patients. This led to the university publishing an article in the journal *Science Translational Medicine*[13] with Cornell University demonstrating a nanofiber implant, about the width of a few strands of hair, with cells that secrete insulin in response to sugar in the blood, reversing diabetes without drugs.

They are still perfecting the micro-porous implant, as a challenge is protecting the cells without starving them of needed nutrients while they are inside the device. However, this offers a potential future treatment for diabetes that would not require immune suppressing drugs.[13]

As the labs come back to full volume, pre-pandemic testing levels, the ease of automated diagnostics for diabetes will only lead to more promising discoveries on the horizon for diabetes.

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Challenges of a Lab Tech *Issue #1*

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Liquid biopsies easing diagnosis

By Marisa L. Williams

When a doctor says,"you have a lump,"your heart drops, and your mind races to the worst case scenario. For years, to determine if the lump was cancerous, it might involve a biopsy where a chunk of tissue is cut from your flesh, leaving scars that take a while to heal and can cause emotional issues, such as insecurities about body image. Now, there is the advent of liquid biopsies, a minimally invasive diagnostic tool that does not require an operating room, but relies on laboratory professionals detecting genetic mutations in bodily fluids.

History of oncology biomarkers

While some may not be entirely familiar with the the term liquid biopsy, the search for cancer biomarkers traces back to Henry Bence Jones discovering the Bence Jones Protein in 1848, which was the first major milestone of oncology biomarkers,[1] followed by Thomas Ashworth describing circulating tumor cells (CTCs) with microscopic observation of a blood with metastases, leading to metastatic cancer.

It was not until 1965, when Phil Gold, MD, PhD, and Samuel O. Freedman, OC CQ FRSC, discovered Carcinoembryonic antigen (CEA), and in 1970, Richard Albin, PhD, DSc, found the Prostrate-Specific Antigen (PSA) for prostrate cancer. This is the same year that oncogenes reveal the molecular basis of cancer.

In 1981, Robert C. Bast, Jr., discovered the CA 125 antigen to identify ovarian cancer, and CA 19-9, seen in the serum of patients with colon and pancreatic cancer. The human epidermal growth receptor, known as HER2, was found in 1989 by Dennis Slamon, MD, PhD, et al, and in 1991, Curtis Harris, MD, discovered p53 when he was exploring the consequences of un-repaired DNA. Now, more than half of the clinical trials in the States are being conducted with biomarker testing.[1]

Use of liquid biopsy

"Liquid biopsy has great potential to transform cancer patient testing and is rapidly becoming a compliment to the common tissue biopsy techniques,"said Rajeshwari Samanth, Research Consultant at Fact.MR. Most commonly, liquid biopsy is used for cancer, including lung, ovarian, breast and prostate."Commonly used liquid biopsy is EGFR Mutation Test v2 to determine the eligibility of non-small cell lung cancer patients to receive certain EGFR tyrosine kinase inhibitors. Liquid biopsies are particularly used in patients receiving targeted therapies and to check disease progression and attainment of resistance mutations,"such as BRCA1 reversions in ovarian cancers receiving platinum- or PARP-based treatment.

Rajeshwari Samanth

Currently, techniques used include Circulating Tumor DNA (ctDNA) or Cell-Free DNA Tests, as well as Circulating Tumor Cell Tests (CTCs). CTCs are rare, such as one cell in 10^9 blood cells, creating challenges for isolation. More recently, people have been turning their attention to extracellular vesicles (EVs) and ctDNA to potentially create a global tumor genome and transcriptome. EVs are released by dividing and growing new tumor cells, so EVs have cellular proteins on their surface.

Only a third of the cancer biomarker tests currently ordered are liquid, using blood, tears, pleural effusion bronchoalveolar lavage, urine, cerebral spinal fluid (CSF), saliva, ascites peritoneal lavage, seminal fluid, breast ductal fluid, amniotic fluid, vaginal secretions, utero-tubular lavage and more.

Many liquid biopsies search for cancer-specific genetic changes or mutations of DNA. Another approach uses methylation, chemical tags known as methyl groups, on DNA in bodily fluids. These chemical tags do not change the genetic code, but they play a role in activating or deactivating the genes.

DNA methylation patterns can distinguish between cancerous and normal tissues, as well as the type of tissue, be it breast, brain, lung, kidney, or other.[2] Daniel De Carvalho, PhD, of Princess Margaret Cancer Centre in Toronto, led a team that developed the DNA methylation-based liquid biopsy technique, citing it may be useful for tumors that do not shed much DNA, such as kidney and brain cancers. In non-epithelial cancers, such as brain gliomas, identifying a tumor specific membrane protein is challenging, as only those CTCs with target protein are isolated.

Though MRI or other imaging methods can detect a tumor, the test cannot tell what type of brain cancer it is, or if it is metastatic, originating from elsewhere and spreading. Combining these imaging methods with liquid biopsies and other testing approaches may be the key to early detection for things like brain cancer.

Recent advances in liquid biopsies

"Recently in 2020, The Food and Drug Administration (FDA) approved new tests to detect DNA circulating in the blood after being released by cancerous tumors,"explained Samanth."This test is used for multiple cancers and biomarkers. For example, to detect mutations in BRCA1 and BRCA2 genes in patients with ovarian cancer. This is the first approval to combine two technologies — NGS and liquid biopsy — in one diagnostic test. These tests, along with advanced testing technology, help to provide genetic information if cancer drugs may treat them."

Guardant360 CDx uses NGS to seek specific EGFRmutations in metastatic non-small cell lung cancer, and FoundationOne Liquid CDx searches 324 genes for mutations, including EGFR, that are implicated in cancer.

A far-field nanoplasmon-enhanced scattering (FF-nPES) can be used as an assay for isolation-free characterization of EVs in serum. This may eventually be used for analysis of EV epithelial cell adhesion molecule (EpCAM) expression to detect pancreatic ductal adenocarcinoma patients.[3]

Serum EV-miRNA-21, -92a, and -222 are potential biomarkers of bevacizumab-treated metastatic colorectal cancer. Pancreas-associated pathologies have identified KRAS and TP53 mutations as being of interest for future studies.

According to the Department of Health and Human Services (HHS), one study demonstrated as much as 90% of prostate cancer mutations could be detected in blood samples with exome sequencing of immunopurified CTC patients with metastatic prostate cancer.[3] In CSF, cell-free DNA and specific mutations in histone H3 could be dectected by ctDNA in brain tumor patients, while glioma patients showed lower levels of Alu methylation, and another study revealed all patients with intracranial tumors adjacent to a cisternal space, as well as those with high grade glima, had detectable ctDNA in CSF.

However, there is a reluctance to give a patient a lumbar puncture when they have an intracranial mass, not wanting to induce a

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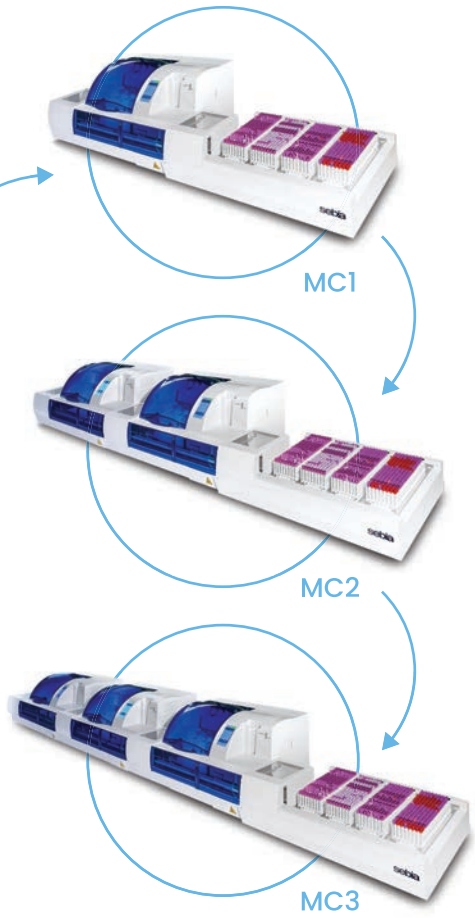

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herniation syndrome. The BRAFV600E mutation, which lies within exon 15 of chromosome 7, has also been identified with brain tumors, but this can be detected at both the DNA and mRNA levels, being detected in plasma using a novel plasma-based ddPCR assay.

Samanth shared that liquid biopsies do have limitations. For example, there is a need for an initial histologic analysis that is done by tissue biopsy, and at present, liquid biopsy is not used as a replacement for the tissue biopsy. The big elephant in the room is the lack of valid data for use of liquid biopsies in clinical trials, and test sensitivity can often be challenging when CTCs are minimal in a sample.

Still, liquid biopsies only appear to be gaining momentum, as Labcorp recently announced that it will begin liquid biopsy testing for lung cancer.[4] In 2018, Quest had been one of the companies to help fund Clinical Genomics in their creation of COLVERA,[5] a liquid biopsy now available in all 50 states that test for aberrant methylation in GCAT1 and IKZF1, which is found at high frequency in colorectal cancer tumor tissue.

Tips for lab professionals

While liquid biopsies are non-invasive or minimally invasive, painless, precise, using a real-time approach, which could potentially reduce cost and diagnostic time, while overcoming the heterogeneity of tumors,[6] they are not completely replacing tissue biopsies quite yet.

Samanth warned, "The presence of sample analyte is often low, leading to higher false negative results, and this requires significantly greater technical efforts to interpret the data and conclude the results."

Storing can also create issues, as well as freezing and thawing protocols, so kits and other consumables should be checked from the manufacturer and stored at recommended conditions only. Macromolecule yield can also be influenced by collection method, processing time, level of hemolysis, and preservation agent exposure. With EVs, the method of vesicle counting can also produce different results.[3]

Samanth added, "Sample labelling also plays an important role for proper patient identification with all other details and history of disease," so be sure to take the time for proper identification of samples.

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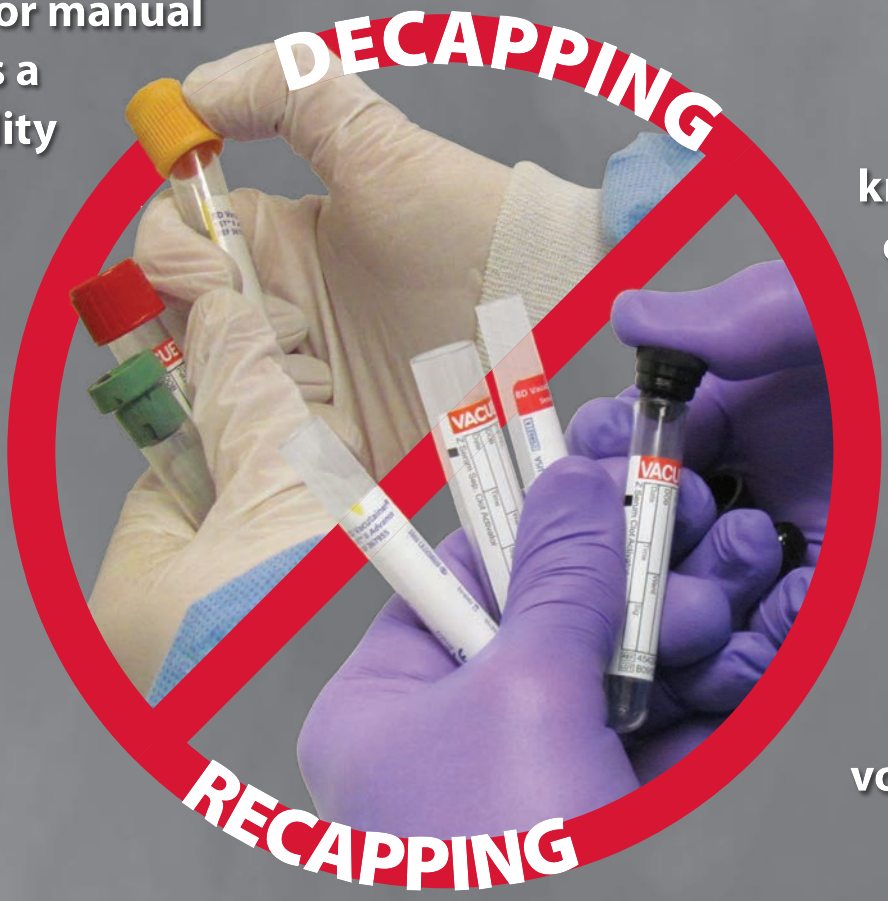

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Labs move into a new phase of the COVID-19 pandemic

by Linda Wilson

As the number of people who are vaccinated for COVID-19 increases, the volume of testing for the virus decreases. That means clinical labs are readjusting their testing portfolios to meet the diagnostic needs of their communities during this phase of the pandemic.

And they continue to grapple with supply shortages, particularly for items involving plastic, such as test tubes. Test kits for some non-COVID-19 pathogens also are difficult to get.

Greg Ingle

"For the vast majority of labs testing for COVID — unless it is an inpatient hospital — their specimen volume has dwindled to 10% of whatever their peak was. Nobody is getting tested," said Greg Ingle, CEO of Clinical Lab Consulting, which provides consulting services to clinical labs, does contract research work for diagnostic companies, and owns and operates a 50,000 square-foot molecular lab in Dayton, OH.

At the peak of the COVID-19 pandemic last winter, the company tested 8,000 specimens a day for SARS-CoV-2 at its lab in Dayton. It now tests less than 100 samples a day. "Half of my clients test for COVID, and their stories are incredibly similar," Ingle said. "I have never seen a more dramatic drop in anything in my entire life."

Medical City Dallas also has seen a drop in volume — although not quite as spectacularly. It performs all SARS-CoV-2 testing in Texas on behalf of its parent company, Hospital Corporation of America (HCA). At Medical City Dallas' molecular lab, COVID-19 testing volumes have dropped from 700-800 tests a day in November 2020 to 350-400 per day in May 2021.

Positivity rates also are declining for HCA's patients in Texas. The rate of positivity peaked at about 30% and now is at about 5%, says Harvey Jones, MS, MPH, C(ASCP) SC, Director of Laboratory Services at Medical City Dallas.

Medical City Dallas and Clinical Lab Consulting are not alone; many labs are adapting their

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testing strategies to fit the current phase of the pandemic.

To find out how labs are approaching diagnostics for COVID-19 and other infectious and chronic diseases, *Medical Laboratory Observer* queried readers through a State of the Industry (SOI) survey. Conducted in April and May 2021, it is the third of four SOI surveys and associated articles that *MLO* plans to release in 2021.


Jodie DeMenge

Most of the survey respondents work in lab administration. The breakdown was 54% who identified themselves as lab managers, administrators, or supervisors; 21% as lab directors; and 5% as section heads or department managers. Nearly 58% of them work in hospital labs, compared with 13% in independent labs, 10% in physician's office labs, 7% in government labs, and 6% in group practice labs. The remander work in other settings.

Survey respondents were distributed across different sizes of labs. Thirty percent work at labs with 1-10 employees, 13% at labs with 11-20 employees, 15% at labs with 21-50 employees, 21% at labs with 51-100 employees, and 20% at labs with more than 100 employees.

COVID-19 TESTING

Most respondents, 76%, have been doing SARS-CoV-2 testing. One quarter, or 25%, have focused on SARS-CoV-2 testing on one analyzer, while 18% have used two analyzers, 14% have used three analyzers, and 19% have used more than three analyzers.

As might be expected, the use of multiple analyzers was more common with larger organizations than smaller ones, the survey found. Similarly, hospital labs comprised the bulk of all locations utilizing three or more analyzers.

Using multiple analyzers has been a popular strategy among lab managers for obtaining access to hard-to-source supplies. "Allocation has probably been our number one barrier to performing COVID testing," says Jodie DeMenge, MT (ASCP) CM, Laboratory Technical Manager – RCH Laboratory for Monument Health, Rapid City, SD. "We did bring in more and different molecular testing platforms into the lab, which I think a lot of healthcare systems did."

During the peak of the pandemic, five-hospital Monument Health also relied on an algorithm the lab team developed to prioritize which samples to process in-house, such as those from high-risk patients or staff members. Other specimens could be sent to reference labs, if necessary.

Now that testing volume is decreasing, Monument Health, like other organizations, is deploying different strategies. It is relying more heavily on rapid molecular tests at the point of care, rather than sending samples via courier to the main lab, where they would be processed on a high-throughput platform.

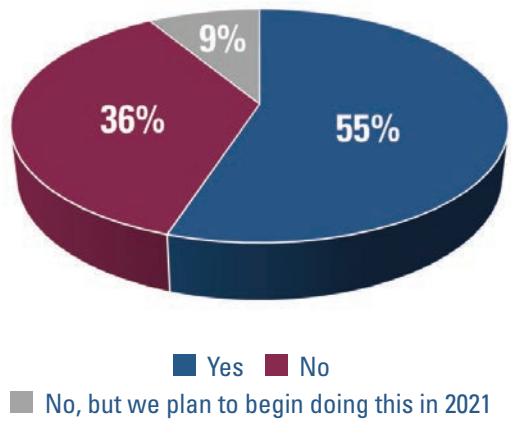
During the peak, testing demand was so high that the system's outpatient locations simply could not keep up with the volume using the rapid testing method, which requires providers to process samples within an hour, DeMenge explains.

The system operated drive-thru testing sites and processed more than 5,000 samples per week during the peak, using five different platforms.

Testing for SARS-CoV-2 is not necessarily a break-even endeavor, according to respondents to *MLO's* State of the Industry survey. A total of 28% said reimbursement for SARS-CoV-2 was covering their costs, while 16% they were not covering their costs; another 36% were not sure. A total of 20% said the question did not apply to them.

Testing volumes for inpatients with COVID-19 also have declined throughout the United States. There are simply fewer of them to test for medical issues associated with COVID-19, such as acute respiratory distress syndrome (ARDs), shock, and organ failure.

Is your lab using syndromic testing (multiplex) to detect SARS-CoV-2 and other infectious diseases, such as Flu A & B, RSV?



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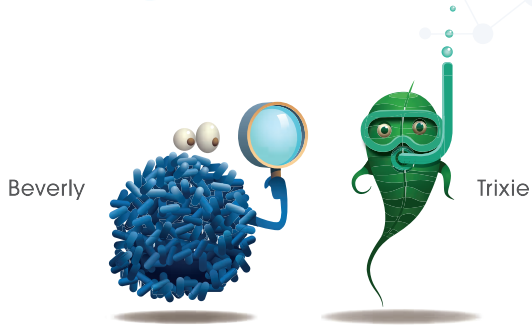

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For example, Medical City Dallas had five inpatient units dedicated to COVID-19 patients during a surge in the pandemic from December 2020 through January 2021. At that time, the staff was treating as many as 110 COVID-19 patients concurrently. The hospital has shut four of those units down, leaving space at one unit available if it admits patients with COVID-19 in the future.

When they do have patients with COVID-19, Medical City Dallas and other hospitals process a variety of tests to assess the medical status of COVID-19 inpatients.

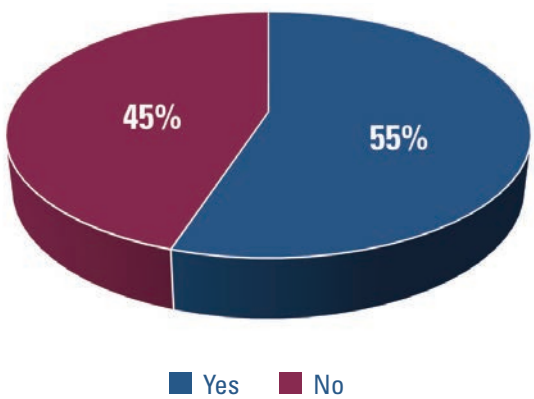
Among *MLO's* survey respondents, the percentage of them who conduct these types of tests was 53% for D-dimer, 50% for C-reactive protein, 30% for fibrinogen, 10% for IL-6, 6% for FDP and 4% for cystatin C (CysC).

PREPARING FOR FLU SEASON

As was the case nationwide, Medical City Dallas did not see many cases of flu during the 2020-2021 season. However, when it became necessary to test for multiple viruses using a syndromic panel, the hospital sent out specimens to reference laboratories for analysis, because it did not have access to the reagents necessary to process the tests in-house.

However, Jones expects to manage those assays in-house during the upcoming season. The organization has a variety of syndromic testing options, including a multiplex panel with 22 biomarkers, which is primarily used for very ill pediatric patients at Medical City Children's Hospital.

Are you doing serological testing in-house for antibodies related to COVID-19?



At CentraState Health Care system, Clinical Laboratory Director Linda Pacetti, BS, MT (ASCP)SM, MBA, expects the system in Freehold, NJ, to process about 60 syndromic tests a month during the upcoming season. However, physicians affiliated with the system usually turn first to a single test to detect influenza A and B, because it is faster and cheaper than a multiplex test. "If they need something else, they will order it," Pacetti says, adding that the system could process as many as 300-plus flu tests a day during a very active flu season.


Linda Pacetti

Monument Health reserves its large, expensive panels for critically ill patients. Physicians treating other patients choose from a combination test for COVID-19/ flu AB/RSV, or single-pathogen assays for each of those viruses.

More than half of respondents to the State of the Industry survey (55%) also use syndromic testing to detect SARS-COV-2 and other respiratory diseases; another 9% said they plan to begin offering it in 2021.

SEROLOGY TESTING

Developers launched various iterations of serology testing for COVID-19 antibodies with great fanfare, beginning with qualitative tests, then followed by quantitative offerings. But uptake of the tests among labs has not been universal.

HCA, including Medical City Dallas, opted not to ramp up serology testing in-house. "If there's a need, we send it out," Jones says, adding that there has been very little demand for the test from the hospital's physicians.

The situation is just the opposite in New Jersey, where CentraState Healthcare System has been processing qualitative serology tests since May 2020. It is in the process of validating a quantitative test, and Pacetti regularly fields queries from patients who ask when the new test will be available.

The problem is that patients who are fully vaccinated want to find out if they are "immune" to SARS-CoV-2, Pacetti explains, but the new quantitative test will not be able to provide them with that level of comfort. They will simply find out if they have antibodies. "It's a very slippery slope on how you report it. The physicians can order it, but we want to make sure they interpret it correctly." Indeed, she

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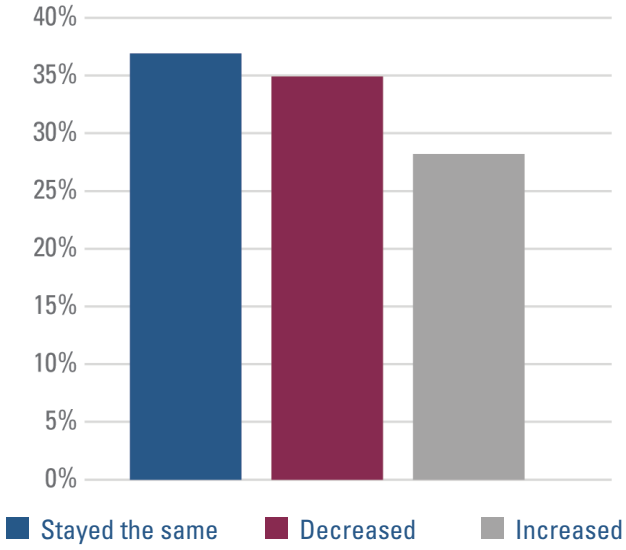
How does the level of staffing in your lab in 2021 compare with pre-pandemic 2020?



says, the lab's medical director has been providing information on test-result interpretation to providers in advance of the new product's debut at the health system.

The U.S. Food and Drug Administration (FDA) issued a similar message in an announcement about using serology testing. "Antibody tests can play an important role in identifying individuals who may have been exposed to the SARS-CoV-2 virus and may have developed an adaptive immune response. However, antibody tests should not be used at this time to determine immunity or protection against COVID-19 at any time, and especially after a person has received a COVID-19 vaccination," the FDA said.[1]

MANAGING STAFF DURING COVID-19

Although always a challenge, lab managers struggled to find enough staff members to cover testing demands during the pandemic's peaks.

At Medical City Dallas, staffing issues were caused by numerous factors, such as a surge in employees who retired, combined with those who found new jobs while they were on furlough from the hospital. Still, other staff members could not work, because they were at home supervising their children while schools were closed.

"We're back to regular staffing now, but it took us the rest of the year to recover," Jones said.

But other labs — 35% of survey respondents — are not as fortunate; they have fewer staff members than they did in 2020, while 37% have the same amount and 28 percent have more.

SUPPLY CHAIN ISSUES

Other vestiges of the pandemic's onslaught remain, particularly in the supply chain.

As diagnostic companies focused on producing supplies for SARS-CoV-2 testing, they often scaled back production of other items that use the same manufacturing lines.

For example, Monument Health struggles to get test kits for chlamydia and gonorrhea, so it reserves the small number of test kits that it has in stock from its usual source for the emergency department. It also has sourced kits to run on another vendor's platform as a secondary solution. But because it is a high-throughput analyzer, and the hospital's volume of tests is small, using it less cost effective, DeMenge explained.

For other outpatients — about 2 or 3 per day — the lab has gone "back to more to traditional lab work," specifically, a wet prep, DeMenge said. In this situation, the lab advises physicians to combine the results from a wet prep, which is less sensitive than PCR, with their clinical judgement, based on a patient's symptoms.

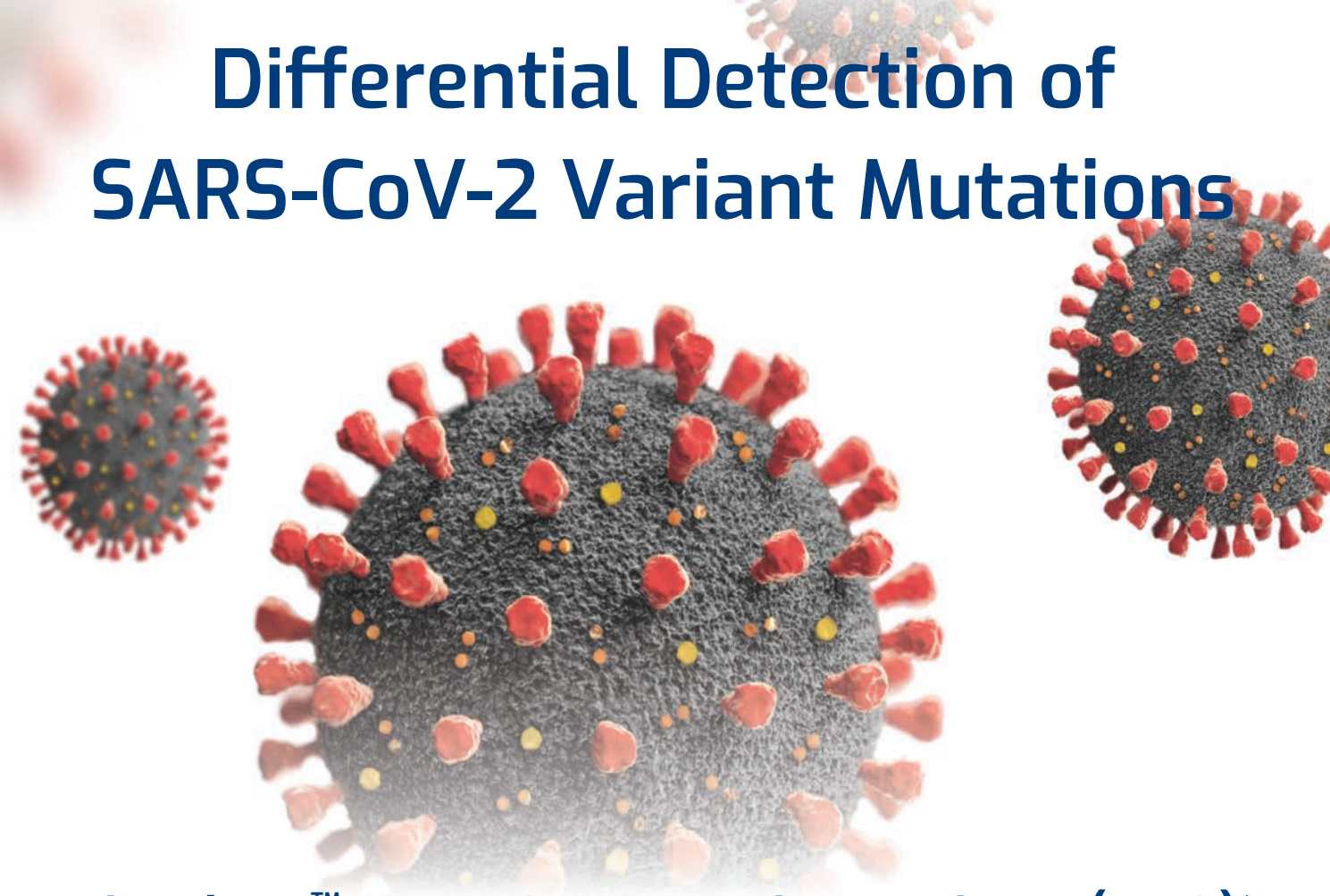
CentraState Healthcare System also has grappled with shortages; most recently for the blue-top test tubes used for coagulation studies, such as for PT/INR and PTT tests, which measure blood-clotting rates. "We go through 200 a day," Pacetti says.

To manage the shortage, CentraState Healthcare System is reserving the bulk of its supply for the critically ill, such as inpatients with COVID-19, as well as patients on anticoagulation therapy who require regular monitoring of their blood-clotting rates.

Shortages also force providers to evaluate how they buy and use those supplies, which can lead to improvements in test utilization, Pacetti says. For example, "We are doing all of these tests, and 97% of them are normal. Maybe you don't need to test the PT/INR and PTT on every patient in the ED."

Gloves are another example. They have been hard to source throughout the pandemic, and those in extra-small and extra-large sizes continue to be in short supply, Pacetti said.

Differential Detection of SARS-CoV-2 Variant Mutations



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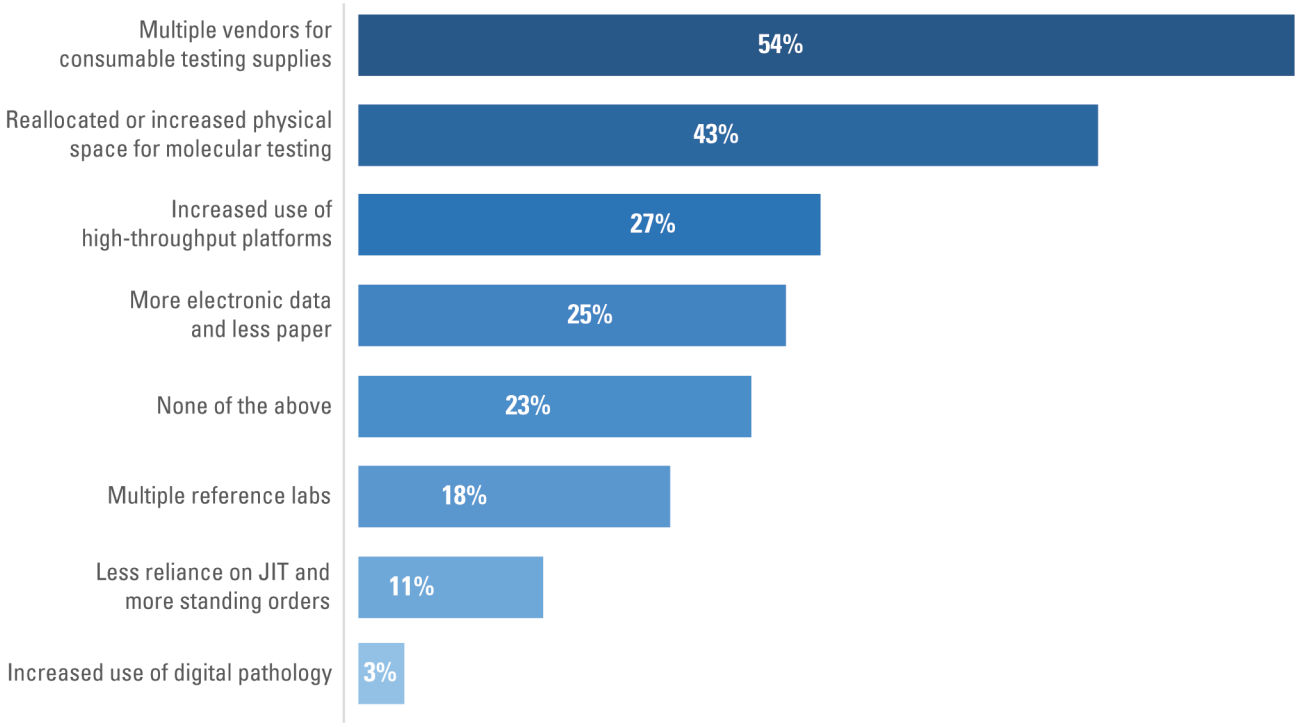
Which changes in diagnostic processes implemented in response to the pandemic are now permanent?



As a result of the shortages, staff members, who often have brand preferences, have learned to make do with whatever supplies the hospital finds.

PERMANENT CHANGES

In addition to changes in test utilization and supply-sourcing strategies, labs have implemented other changes in standard processes.

According to respondents to *MLO's* SOI survey, some of those changes are likely to become permanent, such as using multiple vendors for consumable testing supplies (54%), reallocating or increasing the physical footprint for molecular testing (43%), increasing the use of high-throughput platforms (27%), and using more electronic data and less paper (25%).

Of those strategies, tapping into multiple vendors for consumables was a strategy that many sizes and types of labs deployed and plan to make permanent, MLO's SOI survey found.

In terms of hospital labs specifically, half of those surveyed plan to keep the reallocated and/or increased space they have assigned to molecular, while slightly less than one third of them plan to maintain their increased use of high-throughput analyzers, and about one fifth plan to continue relying on multiple reference labs for send-out tests.

Other changes sparked by the pandemic may lead to additional changes in the future. For example, Monument Health is mulling over the idea of developing its own tests for infectious diseases. There are two main advantages to lab-developed tests (LDTs), compared with proprietary tests developed by vendors: more sources for consumable supplies and flexibility to develop tests tailored to the specific needs of the lab and its customers. "I think we would consider that in the future," DeMenge said.

Clinical Lab Consulting plans to focus on other areas of molecular testing. Says Ingle, "We are deeply entrenched in molecular pathogens, such as UTI and SDI." The company also is involved in wound care and pharmacogenomics. "COVID was a diversion for us," he says.

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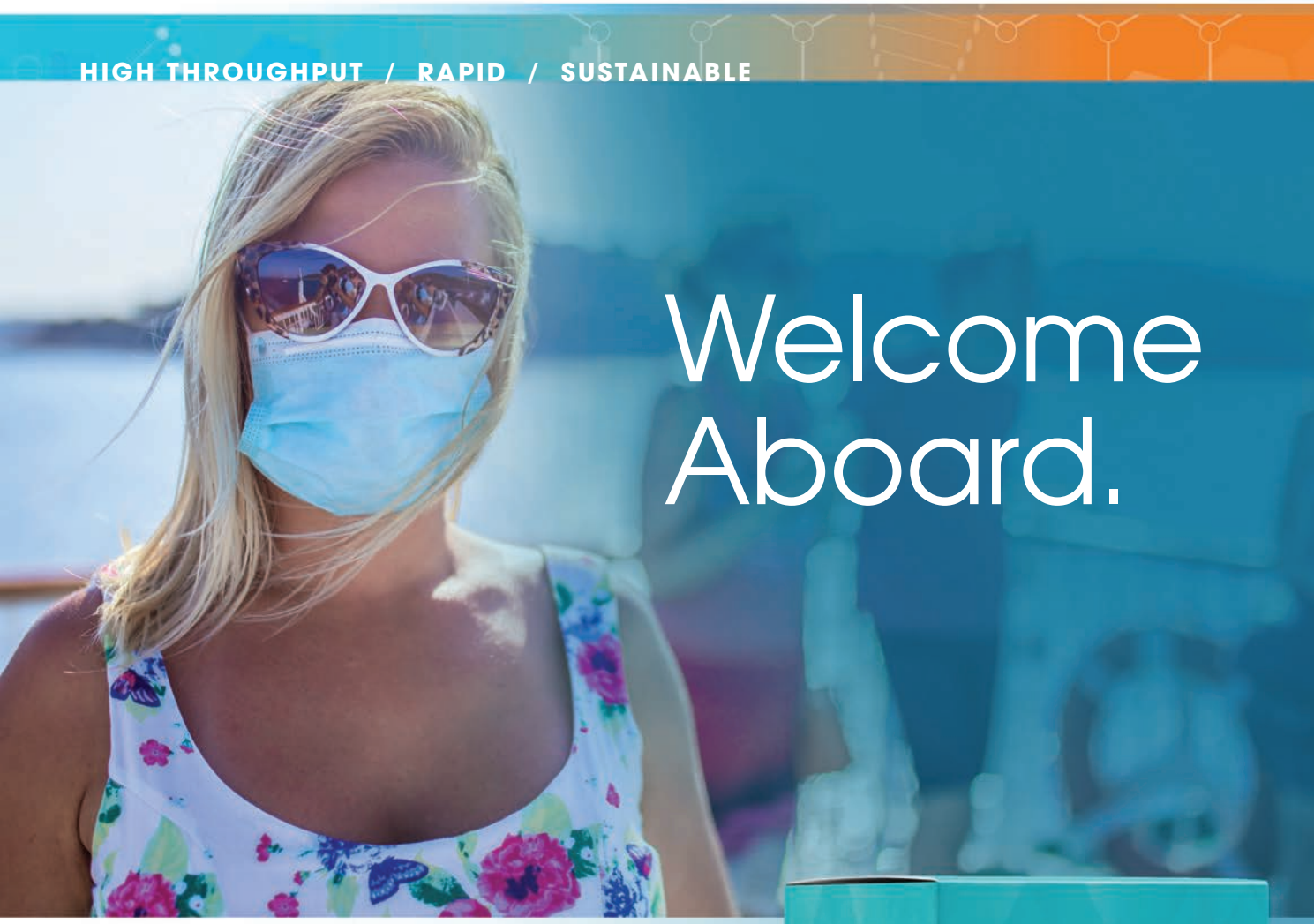

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STATE OF THE INDUSTRY

Transforming cancer diagnostics with NGS

by Linda Wilson

Hospital-based labs are beginning to deploy next-generation sequencing (NGS) for cancer diagnostics in-house, rather than sending samples out to specialized laboratories.

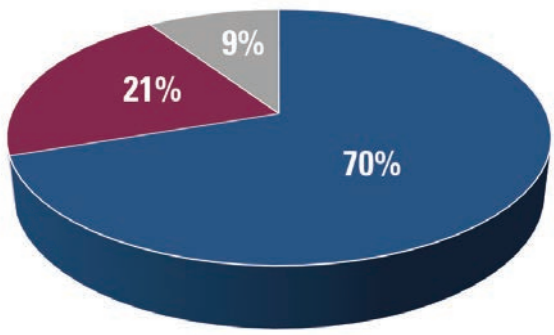
NGS allows labs to uncover the genetic makeup of tumors, helping oncologists determine if targeted therapies would be appropriate for their patients' cancer. These therapies may be more effective and less toxic than standard chemotherapy and radiation.[1]

Despite the promise of targeted therapies, they do have limitations. Cancer cells can become resistant to the treatments over time, leading oncologists to use several therapies together or to pair them with standard treatments, such as chemotherapy.[2]

Nonetheless, the number of targeted therapies is continually increasing, making the case for bringing NGS in-house more compelling. For example, the U.S. Food and Drug Administration (FDA) approved 20 new therapies in 2020, according to an article in the May issue of Medical Laboratory Observer.[1] Overall, there are hundreds of indications for targeted therapies, as the FDA has approved some medications for multiple types of cancer.[2]

A small number of labs are responding to the need for genetic analysis of tumors with in-house NGS capabilities. In *Medical Laboratory Observer's* 2021 State of the Industry Survey on disease management, the editors asked respondents about their plans for NGS in cancer diagnostics. A total of 9% of respondents said their lab conducts NGS for cancer, with more than half of those respondents saying they work at hospital labs or labs more of than 100 employees. Another 21% said they would like to start offering the service. Of those, more than half of them were from hospital labs, but they were spread across size categories.

Does your lab conduct next generation sequencing (NGS) for cancer in house?



- No, we are not using NGS for cancer and we have no plans to start
- No, we have not used NGS for cancer yet in any significant way, but want to start
- Yes, we are utilizing NGS for cancer

REDUCING TURNAROUND AND COSTS

Sentara Healthcare is one example. To improve test turnaround times (TAT) and decrease costs, the 12-hospital system decided to launch its in-house NGS service in 2017.

Before implementing NGS, Sentara did some sequential, single-gene analysis in-house, but samples often were also sent out to specialized labs for tests that the staff did not do.


David Seidman

It was a time-consuming process. "You could be looking at a week and up to a four-week turnaround to get a complete result back to a clinician. One of our major drivers was to be able to reduce that amount of time to result, and next-generation sequencing allows us to do that," explains David Seidman, PhD, MB (ASCP)CM, Scientific Director of Molecular Diagnostics and Serology at Sentara Healthcare, based in Norfolk, VA.[2]

In-house NGS was not only a faster option for Sentara, but also a more cost effective one.

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In the case of non-small cell lung cancer, for example, the cost of performing a sequential three-gene analysis is more than the cost of using NGS to sequence 50-plus genes.

"We are able to sequence a larger panel and provide a larger data set of results that also provides a physician with additional options for therapies and clinical trials that our patients can go on than we could obtain from just the single-gene analysis," he says.

Currently, Sentara uses a commercial NGS panel for solid tumors. Most of the specimens that the lab sequences are non-small cell lung cancer, because there are therapies available that specifically target certain biomarkers in those tumors. "There are just more actionable targets for non-small cell versus other cancer types, so the panel contains genetic targets that would provide that actionable result for non-small cell," Seidman explains.

Working with oncologists, the lab developed a reflex ordering process. If a pathologist determines that a biopsy is non-small cell lung cancer, he or she creates an order for NGS automatically and forwards the tissue block to molecular diagnostics. This saves time that would otherwise be used waiting for an oncologist to read the pathology report and then place the NGS order.

The goal is to provide patients with the results of the NGS test — as well as a treatment plan — at their first visit with an oncologist.

Although Sentara performs the bulk of in-house NGS testing on non-small cell lung cancer, it also sequences other solid tumors, such as for breast, colon, liver, and skin cancers.

The health system also would like to expand into other areas by adding new sequencing panels in the future, including for the analysis of tumor mutation burden and hematologic malignancies. Sentara currently sends out these types of tests to other labs.

EARLY NGS ADOPTER

Meanwhile, Penn Medicine, an academic medical center based in Philadelphia, has been using NGS in cancer diagnostics since 2013 at its Center for Personalized Diagnostics, which runs NGS panels in hematologic malignancies, lymphoma, and solid tumors.[3]

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The lab also has a small panel, with a subset of 60 genes, that it uses if a sample is not adequate for a larger sequencing panel. "It can accept very degraded DNA, or if the DNA is a decent quality, it can accept very minute amounts of DNA," says Robyn Sussman, PhD, Molecular Development Assistant Director for Precision and Computational Diagnostics.


Robyn Sussman

At the time the center launched in 2013, it started with tests for solid tumors and hematologic malignancies, which it developed itself. "We were only sequencing AML (acute myeloid leukemia), brain tumors, lung tumors, and melanoma," says Jennifer Morrissette, PhD, FACMG, Associate Professor and Laboratory Director for the CytoGenomics Laboratories at Penn.

"We decided to start with a small group of malignancies, because we weren't really sure how well the clinical teams would receive genomic data for their patients."

As it turns out, they found it to be clinically useful, and the program has grown substantially since then; it now sequences about 6,000 cases (not unique individuals) a year.

In 2020, the lab moved its panel for hematologic cancers to a hybrid capture-based technology. It plans to move its solid tumor panel to the technology this year.

APPLYING MULTIPLE METHODS

Sussman said hybrid capture-based technology allows the lab to sequence more gene targets than is possible with amplicon-based sequencing techniques. However, turnaround times for either method generally range from seven to 21 days, Morrissette added.

For patients with certain advanced cancers, that is too long to wait. Morrisette says most oncologists aim to have a rapid treatment plan or start treatment within days of a diagnosis. Oncologists want to know "whether to put them (patients) on a targeted therapy, immunotherapy or to go with the standard therapy," she adds.

To solve this problem, the lab is in the process of evaluating a commercially available amplicon-based, end-to-end solution, which is faster than many other amplicon or hybrid capture technologies. It should turnaround results in 24 to 48 hours. While specialized training is still required, this NGS platform is relatively turnkey, so staff members who do not have a significant background in genomic assays can run the tests, Morrissette says. The lab plans to use this technology primarily for lung cancers.

The plan is to test the tumors with the quick method first — which focuses on genetic targets for which there are targeted therapies available — and follow up with more extensive testing afterward, if needed.

The lab also is evaluating whether to extend rapid testing to leukemia and other malignancies for which therapeutic decisions need to be considered in a matter of days post-diagnosis.

For all other cancers, Penn Medicine plans to stick with the larger panels.

In brain cancer, for example, the lab follows brain cancer patients with initial studies to provide information about the pathologic diagnosis. However, it also analyzes subsequent specimens, because the treatments can cause additional mutations, which could then become a target for a specialized treatment. In the case of gastrointestinal tumors, the sequencing studies will inform oncologists if certain treatments would be contraindicated. The tests also will detect targetable mutations.

Overall, Morrissette notes that cancer diagnostics is an exciting area of medicine to be involved in because it is rapidly evolving with new therapeutics, which can improve patients' clinical outcomes. Detecting mutations that may enable physicians to treat some cancers as chronic diseases is the goal, she says. "Imagine if you can start extending the lives of more cancer patients by a few decades; that really matters."

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Immunoassay analyzers for labs of all sizes

By Marisa L. Williams

Immunoassay analyzers vary in the size, throughput and technology. As labs are currently scaling the number of analyzers to align with their throughput needs, some of the leading immunoassay analyzers are compared here.

Rosalyn Yalow, PhD, and Solomon A. Berson, MD, won the Nobel Prize in 1977 for their discovery of Radioimmuneassay (RIA), and the technology grew with Cesar Milstein, PhD, and George J. F. Kohler, PhD, winning the Nobel Prize in 1984 for the production of monoclonal antibodies. Since then, a plethora of immunoassays have launched.

The first Curian HpSA assay was for active infection H. pylori testing, which is being followed by additional assays for Campylobacter, C. difficile GDH/Toxin, and Shiga Toxin 1&2.

Randox's Evidence MultiSTAT has a Stroke Biochip for rapid stroke diagnosis in about 12 minutes, and the LumiraDx Platform is a rapid microfluidic test designed on similar principles as lab analyzer systems in a portable, affordable solution.

For high volume labs, EUROIMMUN's LabWorkstation enables fully automated and standardized processing, while the PerkinElmer GSP Instrument features a refrigerated compartment for up to 13 cassettes, buffers, tracers, and antibodies.

Considerations when purchasing

Arvind Kothandaraman, General Manager of Specialty Diagnostics at PerkinElmer, suggested labs look beyond current needs, "particularly in a post-pandemic world. Lab leaders will benefit from choosing instruments that require minimal human intervention and training for processing samples, while allowing the option to scale up as needed to meet future demands. Automated immunoassay analyzers enable lab staff to save time, standardize processes and reduce errors. Lab leaders should also consider the support and service options available from various manufacturers, as the one they choose will play an important role in facilitating uninterrupted processing of critical assays."

The LumiraDX platform and the Curian have no required maintenance. Others models display needed tasks on their screen, and some manufacturers offer maintenance contracts.

Christopher Liddle, Marketing Executive of Randox, advises, "When purchasing an immunoassay analyzer, lab professionals should consider the following: (1.) Automation – an analyzer which offers greater automation reduces the workload of professionals, while reducing the possibility of human error. (2.) Return on Investment – if the analyzer in question can allow the laboratory to function at a faster rate or with greater efficiency, then the laboratory will be able to improve productivity and in turn, increase profitability. (3.) Improved Precision – when purchasing a modern analyzer with the latest technology in comparison to an older product, the newer software will likely allow for more precise results, which will improve the validity of output data within the laboratory."

For example, Sarah Berger, Senior Manager, Marketing Communications for Diagnostics at Meridian Bioscence, said Curian's platform can bring "testing closer to the patient for improved clinical efficiencies. The fluorescent technology on Curian provides increased sensitivity and eliminates subjectivity associated with other testing methods."

Experiences with immunoasay analyzers

Testing for PCT, or procalcitonin, a biomarker that distinguishes bacterial infections from other inflammatory reactions, effectively helps monitor sepsis. John Boreyko, PharmD BCIDP, Infectious Disease Pharmacist at Duke Regional Hospital, said this testing was incorporated, "to aid us in the reduction of both initiating inappropriate antibiotics and deescalating therapy in culture-negative septic patients. bioMérieux was with us the entire time after our business plan was accepted and was instrumental in helping educate our chemistry lab on using the equipment, validating the equipment and helping us to meet CAP guidelines."

Daniel Feinstein, MD, Medical Director Critical Care at Novant Health, explained, "VIDAS procalcitonin [is] an integral part in antibiotic stewardship and allow[s] physicians to de-escalate and even discontinue or shorten the duration of antibiotics with a lot more confidence, in an algorithmic fashion."

During Spring 2020, Andre B. Gvozden, MD, a Pediatric Specialist, was introduced to the SARSCoV-2 Antigen Test on the LumiraDx Platform as part of a clinical study. Over the last 10 years, Gvozden has evaluated lots of different point of care instruments/platforms for respiratory pathogens, urine and serum testing. During the use of the platform at his practice, Gvozden reported that he, "experienced zero glitches, which has not been the case with previously assessed point of care tests. With other tests, it can be frustrating to spend $20-30 on a test, only to end up with an invalid result or error."

Avoiding that frustration, patients responded positively to the faster testing, trusting "a result which comes from their doctor," explained Gvozden. "This gives a sense of confidence."

Considerations when upgrading

Liddle gives three examples of when a lab may consider upgrading their immunoassay analyzer, listing a financial reason first. "(1.) Alternative Products Offer a Better Return on Investment – If a new product is on the market which offers features that would enhance the laboratory's testing capabilities, then it would be beneficial for the lab to invest in an upgrade in order to further improve its productivity.

"(2.) Increased Wear & Tear – If the analyzer has been used over a long period and is now suffering from increased wear and tear, resulting in growing downtime which negatively impacts performance, then a laboratory should consider an upgrade to prevent this downtime and constant maintenance requirements from impacting their performance. (3.) Outdated Software – Many older analyzers will not have the software available in more modern analyzers. Outdated software may increase processing time and act only to negatively impact throughput time. Therefore, the laboratory may want to upgrade to an analyzer which offers modern technology to increase throughput time and overall efficiency."

According to Brian Duchateau, PhD, Vice President of Scientific Affairs, LumiraDx, Waltham, MA, USA, "Rather than upgrading an immunoassay analyzer, labs should consider where it makes sense to decentralize testing with next generation POC analyzers that combine speed, ease-of-use, and lab-comparable performance."

Greg Stock, Vice President of Commercial Operations, EUROIMMUN US, advised, "Laboratory professionals should always keep in mind what the future might bring to their laboratory. Purchasing for today's needs may not position the laboratory appropriately for future growth and expansion."









Model	Randox Evidence MultiSTAT	LumiraDx Platform	PerkinElmer SuperFlex Chemilu-minescent Immunoassay	EUROIMMUN IF Sprinter	Meridian Bioscience Curian	bioMérieux VIDAS 3	EUROIMMU Sprinter XL	EUROIMMUN LabWorkstation	PerkinElmer GSP Instrument
Volume lab	Small Volume Lab	Small Volume Lab	Small Volume Lab	Small Volume Lab	Small - Medium Volume Lab	Medium Volume Lab	Medium Volume Lab	Large Volume Lab	Large Volume Lab
Throughput	One patient sample per test	STAT testing, 12-minute results	1-12 samples at a time	Up to 96 samples	Incubates one; analyzes multiple	Up to 36 tests/ hour	2 models: 160 or 240, 30 IFA slides or 6 ELISA plates	750 substrate fields and more than 700 samples	Up to 3,600 samples a day
Load reagents and consumbales	Reagent, tip loaded before automation	Yes	Yes	Yes	n/a	Yes without pausing	Yes	Yes	Yes
Technology	Biochip array technology	Microfluidic immuno-flurescence assay	Chemilu-minescence	Indirect immuno-fluorescence (IFA)	Fluorescence technology detection	Enzyme-linked fluorescent assay (ELFA) technology	IFA and ELISA	IFA and ELISA	Time resolved and prompt fluorescence, absorption
Integrate automation and IT	Yes, touch screen, barcode scanner	Yes, Integrates with IT.	Yes, Fully-automatic platform	Yes, bidirectional	Yes	Yes, automated, bidirectional	Yes	Yes	Yes
Size	585 (H) x 535 (D) x 570 (W) mm	210 mm x 97 mm x 73 mm	3 x 3 x 3 ft	25 in x 27 in x 29 in	4.9 in x 4.5 inches x 4.6 in	24 × 29.5 × 25.5 in./5.2 sq. ft.	47in x 33in x 28in	114in x 32in x 34in	1960mm x 1310mm x 760mm
Weight	48kg (106 lbs)	1100 g	250 lbs	Approx. 110 lbs	1.43 lb (.65 kg)	154 lbs	Approx. 331 lbs	Approx. 992 lbs	610 kg
Assay protocols	Cartridge-based, automated	Immuno-flourescence sandwich assay	COVID-19, diseases, cardiac disorders (CE-IVD, RUO)	Autoimmune and infectious diseases	Ready-to-use reagents in single-test format	Assay dependent; compatible assays run simultaneously	Autoimmune and infectious diseases, hormones	Autoimmune and infectious diseases, hormones	Included with the instrument software
Maintanence	Tasks recorded and displayed on screen	Not required – no serviceable parts	Preventative maintenance and calibration	Preventative, service contracts available	Preventative, service and remote monitoring	Weekly 10–15 min. maintenance	Preventative, service contracts available	Preventative, service contracts available	Maintenance wizard + preventive
Specialty testing	21 drugs, immuno-assay, stroke	SARS –CoV-2, D-dimer	Infectious disease, COVID, cardiac (CE-IVD, RUO)	Immuno-fluorescence tests	stool: H. pylori, Campylobacter, C. difficile GDH and Shiga 1&2	Critical care & infectious disease assays, sepsis, PCT	Indirect immuno-fluorescence and ELISA	Automated, high capacity and efficiency	Automated, high capacity and efficiency

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LABORATORY INNOVATOR

Using digital microfluidics to screen newborns

By Linda Wilson

Richard West, MBA, is founder and CEO of **Baebies**, which focuses on newborn screening using digital microfluidics. Previously, West and a partner sold Advanced Liquid Logic, a Duke University spinout that developed a lab-on-a-chip technology, to Illumina for $96 million.

Why did you decide to develop a digital microfluidics platform for newborn screening? What problems in newborn testing did you want to address?

We have a lot of collaborations with top-notch academic institutions via grants from the National Institutes of Health (NIH). This is typically where we identify the problems that need to be solved. Multiple neonatologists talked about the problem of getting sufficient diagnostic information to make good clinical decisions. They are often forced to define a therapeutic course based on insufficient information because adult diagnostics — the only tools available — typically require samples that are too large. An extremely low birthweight infant may only have 50 milliliters of blood. Taking 10% of this to get the needed diagnostic information is impossible or at best risky. This is particularly acute for monitoring applications — where multiple measurements need to be taken — and for conditions where multiple tests requiring multiple samples are required to make the right call.

What role does microfluidics serve in the overall continuum of testing technologies for newborn screening?

Microfluidics makes a lot of sense in newborn screening, because by miniaturizing an assay, we can get more information from a small amount of sample. If we combine microfluidics with multiplex detection technologies, we can do even better. Newborn screening is an interesting target because of the huge gap between what we screen for today in U.S. public-health newborn-screening programs — about 40 conditions or so, on average — and the 200 conditions that have therapies approved by the U.S. Food and Drug Administration (FDA). And the gap is growing larger.

From a public health perspective, what criteria do public health officials evaluate when deciding whether to add a genetic disease to newborn screening?

I'll refer you here to the Advisory Committee on Heritable Disorders in Newborns and Children. This is a federal advisory committee that recommends to the state public health programs what conditions should be included in the Recommended Uniform Screening Panel or RUSP. These are public health dollars being spent, for the most part. At the bottom line, it is about gaining a significant benefit at relatively low risk. A newborn screening test costs just a few dollars, but we need to test every baby, before symptoms appear, to effectively screen for these disorders. Each state has its own process for adding new conditions to their own screening panels, which typically includes an advisory panel at the state level that has clinical and community input and a legislative process to pay for it.

Are there additional diseases that you think will become common in newborn screening in the future? If so, what are they?

There are about 7,000 genetic disorders chronicled. And again, with all of the investment made in rare disease therapies over the last couple of decades, there are many more conditions that have FDA-approved therapies than are screened for at this point. We'll start with those conditions that have effective therapies where early awareness of the condition — through screening — will make a big difference in the child's life. With the advent of gene therapies — and the ability to permanently cure an affected baby — it's even more important to address the condition early, before permanent damage is done, which typically means before symptoms are apparent. We need tools that can address many diseases in a single test. I am hopeful that such tools are on the near-term horizon, but it will take investment, and perhaps a fresh approach to the problem. Newborn screening is over 50 years old in the United States and has gone through dramatic technology advancements a few times since then. It's time for another technology leap forward. But then, we also have to address resources for follow-up, cost of therapy, and a number of other issues.

How do you expect the role of digital microfluidics in diagnostic lab testing overall (not just newborn screening) to evolve over the next three to five years?

Digital microfluidics enables miniaturization of assays, so we can make the most of the sample volume that we can afford to extract. This is true of any microfluidics technology. Where digital microfluidics is different — and better — is when we perform multiple test types, not only on the same instrument, but on the same sample, in a rapid, multifunctional syndromic test panel. Today's point-of-care diagnostic instruments — and the large analyzers in the clinical lab, for that matter — are all aligned with a specific test type. Many perform only a single test. If you need diagnostic information across test types, that means you must collect multiple sample tubes, test them on different machines, and maybe even send them to different labs. Then you have to integrate the data for the clinician. This takes time and presents challenges to the phlebotomists, the labs, and the clinicians — not to mention the patients. Syndromic testing is increasingly adopted in infectious disease diagnostics, while multi-functional syndromic testing, though not seamless, is largely confined to central laboratories. With the advanced technological flexibility of digital microfluidics, multifunctional syndromic testing can be performed in distributed settings, saving time, cost and precious lives.

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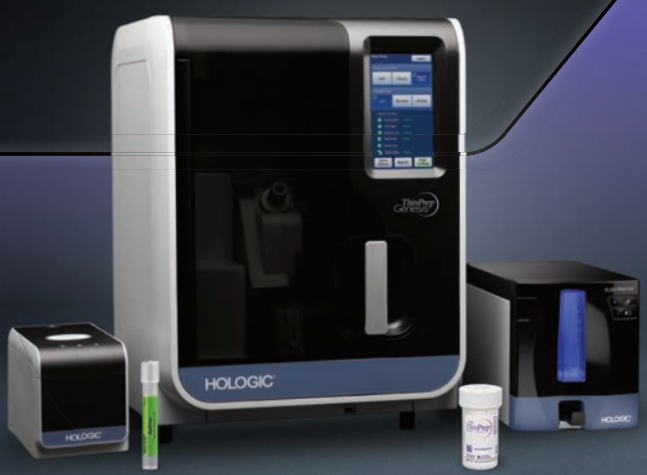

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