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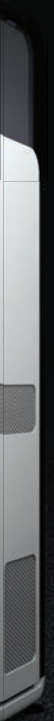

WOMEN'S HEALTH

- CT/NG
- *Mycoplasma genitalium*
- *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
- Flu A/B/RSV
- Paraflu
- AdV/hMPV/RV
- SARS-CoV-2†
- SARS-CoV-2/Flu A/B†
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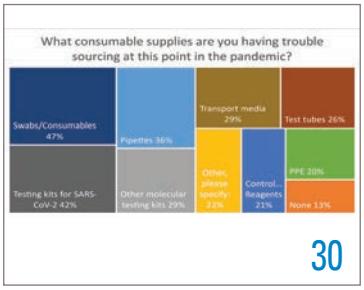

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COVID-19 is the backdrop of AACC's annual meeting

By Linda Wilson
Senior Editor

The American Association for Clinical Chemistry (AACC) held its Annual Scientific Meeting & Clinical Lab Expo"live"in Atlanta in late September.

Despite the pleasant weather (warm, sunny, and not too humid) and a clean and airy convention hall, the COVID-19 pandemic hung over the proceedings. To get inside, attendees needed not only proof of vaccination but also a negative result from a COVID-19 test within the past 72 hours. Diagnostic test manufacturers provided both on-site testing services (LumiraDx) and self-administered tests for attendees to use after returning home (Quidel).

Despite those precautions, attendance was noticeably less than in previous years. Educational sessions, even the plenary sessions, had plenty of extra seats. In the exhibit hall, there was ample room to walk around, and I saw staff members in exhibitors' booths hanging out alone.

COVID-19 also dominated the formal and informal conversations. Here are a few examples:

- In a plenary speech, Margaret Liu, MD, Chairperson of the Board of the International Society of Vaccines and CEO of Pax Therapeutics, said that it is important to fight SARS-CoV-2 using a proactive and coordinated approach globally. That means achieving high rates of vaccination in all countries and following safety measures because these actions will suppress new viral variants of SARS-CoV-2. However, the current global response to the pandemic has been just the opposite, she said, comparing it to the board game Whac-A-Mole."This is something we do with the influenza vaccine. We look at what is popping up," she said.

Speaking about COVID-19 vaccines specifically, Liu said,"I don't think we should despair too much when we read about neutralizing antibodies declining because it doesn't necessarily correlate with protection."In addition, to antibody titers, the current COVID-19 vaccines also induce"helper"T-cells, which assist other immune cells, and cytotoxic T cells, which shutdown infected cells, she noted.

- Numerous other attendees that we talked with discussed serology tests that detect antibodies produced in response to COVID-19. The issue: scientists do not know what level of antibodies equates to adequate protection."My levels may go down but could still be good,"noted Dan Scungio, MT(ASCP) SLS, CQA(ASQ), aka Dan the Lab Safety Man and Laboratory Safety Officer at Sentara Healthcare.
- Jon Harol, President of Lighthouse Lab Services, discussed the Biden Administration's plan to require businesses with 100 or more employees to mandate either vaccination or weekly testing, which is expected to impact about 100 million employees. Harol questioned whether the U.S. has enough rapid tests to meet this requirement. At present, about 56% of the population is fully vaccinated, including about 68% of people 18 years old or older, according to the Centers for Disease Control and Prevention (CDC). While the mandate likely would lead many unvaccinated employees to become vaccinated, a significant number of employees would likely choose weekly testing, Harol predicted.

I hope that the AACC's meeting in 2022 ends up with its usual level of turnout for many reasons. Of course, the most important reason is that robust attendance would likely mean that we've finally made significant progress against SARS-CoV-2.

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

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Fast Facts

COVID-19 Vaccinations

A report from the U.S. Department of Health and Human Services (HHS) reveals COVID-19 vaccinations prevented infections and reduced mortality rates of seniors between January and May 2021.

265,000
COVID-19 infections prevented

107,000
hospitalizations prevented

39,000
deaths prevented among Medicare beneficiaries

352,000
lives were lost during the first nine months of the COVID-19 pandemic

1/5 reduction
in COVID-19 infections among Medicare beneficiaries, as well as COVID-19 related hospitalizations and deaths

nearly 80%
of deaths were among people 65 and older who were also Medicare eligible

11-12%
decrease in weekly COVID-19 hospitalizations and deaths among Medicare beneficiaries for every 10% increase in county vaccination rates

Largest
vaccination-related % decrease in SARS-CoV-2 infections, hospitalizations, and deaths in American Indian and Alaska Native Medicare beneficiaries

5,600
deaths prevented of Medicare beneficiaries in nursing homes

Source: https://www.hhs.gov/about/news/2021/10/05/new-hhs-report-vaccination-linked-to-a-reduction-of-over-a-quarter-million-covid-19-cases.html

CDC updates infection prevention recommendations

The Centers for Disease Control and Prevention (CDC) has updated its guidance for infection prevention and control during the COVID-19 pandemic.

Specifically, the agency says that fully vaccinated people in counties with low to moderate community transmission do not need to wear respirators or masks while inside healthcare facilities. However, the CDC says the "safest practice" is for everyone to wear a face covering, or what the agency refers to as "source control."

In addition, fully vaccinated healthcare personnel could choose not to wear source control or physically distance when they are in well-defined areas that are restricted from patient access (e.g., staff meeting rooms or kitchen). However, they should wear source control when they are in areas of the healthcare facility where they could encounter patients (e.g., hospital cafeteria or common halls and corridors).

Source control options for healthcare personnel include:

- A NIOSH-approved N95 or equivalent or higher-level respirator
- A respirator approved under standards used in other countries that are similar to NIOSH-approved N95 filtering facepiece respirators (note: these should not be used instead of a NIOSH-approved respirator when respiratory protection is indicated)
- A well-fitting mask

The agency clarified recommended intervals for testing. Asymptomatic healthcare personnel with a higher-risk exposure and patients in close contact to someone with SARS-CoV-2 infection, regardless of vaccination status, should have a series of two viral tests for SARS-CoV-2 infection. In these situations, testing is recommended immediately (but not earlier than 2 days after the exposure) and, if negative, again 5–7 days after the exposure. However, testing is not recommended for people who have had SARS-CoV-2 infection in the last 90 days if they remain asymptomatic; this is because some people may have detectable virus from their prior infection during this period.

Cognitive impairment is highly likely for severe COVID-19 patients

In a study of nearly 150 patients hospitalized for COVID at the beginning of the pandemic, researchers found that 73% had delirium, a serious disturbance in mental state wherein a patient is confused, agitated and unable to think clearly.

Patients with delirium tended to be sicker, with more comorbidities like hypertension and diabetes, and appeared to have more severe COVID-related illness as well, said study author Phillip Vlisides, MD of the Department of Anesthesiology at Michigan Medicine.

Using patient medical records and telephone surveys following hospital discharge for patients hospitalized in the intensive care unit between March and May 2020, the team attempted to identify common threads amongst patients who developed delirium.

The disease itself can lead to reduced oxygen to the brain as well as the development of blood clots and stroke, resulting in cognitive impairment. In addition, inflammatory markers were greatly increased in patients with delirium. Confusion and agitation could be a result of inflammation of the brain.

Care teams often were unable to perform standard delirium reduction techniques, such as exercises designed to get a patient moving or allowing visitors or objects from home to orient patients while in the hospital.

Furthermore, there was a correlation between the use of sedatives and delirium — patients with delirium were sedated more often and frequently at higher doses. "It is common to use IV sedatives in the ICU, particular for patients on a ventilator. However, from talking to nurses, we found that patients with severe COVID were inherently more delirious and agitated at baseline, perhaps prompting more sedative use."

The study also found that cognitive impairment can persist even after discharge. Almost a third of patients did not have their delirium marked as resolved in their chart upon leaving the hospital and 40% of these patients required skilled nursing care. Almost a quarter of patients screened positive for delirium based on assessment by their caretaker. For some patients, these symptoms lasted for months. This can make managing the recovery process after hospitalization that much more difficult.

Gut bacteria might be an indicator of colon cancer risk

A study published in the journal *Cell Host & Microbe* reported that the increased presence of certain bacteria in a gut biome indicates a greater likelihood that colon polyps will become cancerous, according to a news release from the University of Washington.

In his research, William DePaolo, PhD, Associate Professor at the University of Washington School of Medicine,

tracked 40 patients who had undergone routine colonoscopies and had biopsies taken near the polyps to identify bacteria present at relatively higher levels compared with those of patients who were polyp-free. All the patients were between the ages 50 and 75, and 60% were women.

"The rising incidence of colorectal cancer is a major health concern, but little is known about the composition and role of microbiota associated with precancerous polyps," the study states.

DePaolo's research team found that a common bacteria, non-enterotoxigenic Bacteroides fragilis, was elevated in the mucosal biopsies of patients with polyps.The research also found distinct microbial signatures distinguishing patients with polyps from those without polyps and established a correlation between the amount of B. fragilis in the samples and the inflammation of small polyps.

Upon closer examination, DePaolo found that the B. fragilis from patients with polyps differed in its ability to induce inflammation compared to the B. fragilis from polyp-free individuals.

Also, when people think of the microbiome and its role in disease, they often think of compositional changes where a potentially dangerous bacteria takes over, he added.

"What our data suggests is that, in order to survive within an environment where metabolic and inflammatory changes are occurring, a normally healthy gut and related bacteria may adapt in such a way that causes it to contribute to the inflammation rather than suppress it," DePaolo explained.

Only 5% of the polyps in the colon actually turn out to be cancerous, he said. He said polyps seem to develop in the same areas of the colon repeatedly — and he theorized that in fact new screenings for colon cancer could look for key bacteria inhabiting the gut — and the amounts of this particular strain of B. fragilis — before pre-cancerous polyps even develop.

The next step, he said, is to expand the study to 200 patients to determine whether a fecal sample might be used as a surrogate for the mucosal biopsy.

Oral antiviral reduced risk of hospitalization, death for COVID-19 patients

Merck and Ridgeback Biotherapeutics announced that the companies' investigational oral antiviral medicine significantly reduced the risk of hospitalization or death in at-risk, non-hospitalized adult patients with mild-to-moderate COVID-19.

In an interim analysis of a Phase 3 study, the antiviral medication, molnupiravir, reduced the risk of hospitalization or death by approximately 50%. Approximately 7.3% of patients who received molnupiravir were either hospitalized or died through day 29 following randomization (28/385), compared with 14.1% of placebo-treated patients (53/377) through day 29. No deaths were reported in patients who received molnupiravir, as compared to eight deaths in patients who received a placebo.

At the recommendation of an independent data monitoring committee and in consultation with the U.S. Food and Drug Administration (FDA), Merck said the companies stopped recruitment into the study early due to these results.

Merck also said it plans to submit an application for emergency use authorization (EUA) to the U.S. Food and Drug Administration (FDA) as soon as possible based on these findings and plans to submit marketing applications to other regulatory bodies worldwide. If authorized, molnupiravir could be the first oral antiviral medicine for COVID-19, Merck says.

Vitamin D and impact on overall health

By Andrea Diebel, MS; Ilaria Valente, PhD; and Claudia Zierold, PhD

Vitamin D is an essential nutrient that is well known for its role in maintaining bone health. Additionally, vitamin D is important in immune function and has been recognized for other health benefits, including reducing the risk of chronic diseases. The most important role of vitamin D is to regulate two key players in bone mineralization — calcium and phosphorus — but it also plays a critical role in many biological functions. Because vitamin D deficiency is associated with numerous increased health risks, the Endocrine Society continues to recommend vitamin D screening for at-risk groups. Understanding the prevalence of vitamin D deficiency and the impact of vitamin D status on overall health can help clinicians provide the appropriate patient care. [1-6]

Vitamin D comes in two forms: vitamin D2 (ergocalciferol) is of plant origin, and can be found primarily in supplements, but also in foods such as mushrooms and some greens; vitamin D3 (cholecalciferol) is produced in the skin after exposure to UV light of a specific wavelength (280-315 nm) and can be found in some foods, such as fortified dairy, fish oils, and cod liver oil. The difference between vitamin D2 and vitamin D3 lies in their chemical structure (side chain). Both vitamin D2 and vitamin D3 are biologically inactive prohormones that must be converted to the active vitamin D hormone through a two-step bio-activation process.[1,2]

Numerous metabolites are created when Vitamin D is activated.

25-Hydroxyvitamin D (25(OH)D)

25(OH)D, or calcidiol, is the form of vitamin D traditionally measured to evaluate vitamin D sufficiency. It is produced when vitamin D_2 and D_3 pass through the liver and undergo an enzymatic conversion (hydroxylation of carbon 25). The majority of vitamin D (D_2 or D_3) that is synthesized in the skin or ingested is converted to 25(OH)D; thus, the measurement of serum or plasma 25(OH)D has been adopted as an indicator of an individual's vitamin D status.[7,8]

1,25-Dihydroxyvitamin D (1,25(OH)$_2$D)

1,25(OH)$_2$D is the active form of vitamin D. It is obtained upon the enzymatic conversion of 25(OH)D, which occurs primarily in the kidney (hydroxylation of carbon 1). 1,25(OH)$_2$D, also known as calcitriol, maintains mineral homeostasis by enhancing the efficiency of calcium and phosphorus absorption from dietary sources in the small intestine. In the event that dietary calcium supply is not sufficient, 1,25(OH)$_2$D can increase the mobilization of calcium from bone into circulation. Furthermore, 1,25(OH)$_2$D acts on the kidney to preserve calcium by reducing its excretion in urine. 1,25(OH)$_2$D levels are not typically used to evaluate vitamin D status due to the lack of correlation between the metabolite in the blood, and the levels of 25(OH)D or vitamin D. However, 1,25(OH)$_2$D measurement is useful when kidney disease, hyperparathyroidism, sarcoidosis, and other bone and calcium disorders are suspected.[7-11]

24,25-Dihydroxyvitamin D and 1,24,25-trihydroxyvitamin D

To inactivate and excrete 1,25(OH)$_2$D in the urine, it needs to be transformed from a lipid soluble to a water-soluble molecule. The first step in this conversion is carried out by the 24-hydroxylase enzyme, which produces both 24,25-Dihydroxyvitamin D and 1,24,25-trihydroxyvitamin D. A biological role for 24-hydroxylated vitamin D metabolites remains controversial. While inactivation of the active 1,25(OH)$_2$D makes physiological sense, the role of inactivation of the not-yet-active 25(OH)D has been questioned. However, that 24-hydroxylation of 25(OH)D decreases the available substrate, affording a viable regulatory step.[1,2,8,9]

C3-epimer of 25(OH)D

The C3-epimer of 25(OH)D is an isomer of 25(OH)D, having the hydroxyl group on carbon 3 in a different spatial plane. It was initially found in infant blood samples, and it can be found at levels similar to or greater than 25(OH)D. Since its initial detection in pediatric samples, it was also detected in adults, albeit less frequently. When present, the C3-epimer is often detected at concentrations below 10 ng/mL, though in extreme cases levels as high as 30-50 ng/mL have been found. To date, no physiological role has been attributed to this metabolite, but increased interest exists to find why some subjects present with such high circulating amounts while others have none.[1]

Vitamin D assays

Vitamin D status is commonly measured by either commercially available, or lab developed 25(OH)D assays. Methodologies for 25(OH)D measurement include high performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS), radioimmunoassay (RIA), and automated chemiluminescence immunoassay (CLIA).[4] HPLC and LC-MS/MS are chemical assay methods that can measure vitamin D metabolites separately. These methods demonstrate excellent analytical specificity and sensitivity. However, the initial capital investment, the labor and time-intensive nature of development, and implementation of clinical LC-MS/MS assays, along with slow turnaround time are key factors hindering greater adoption of this method. Automated immunoassays are the most popular 25(OHD)D tests used by clinical laboratories for high volume testing. Their key advantages over chemical assay methods include relatively low cost, automated sample handling, quick turnaround time, and ease of use.[12,13]

Discordance in immunoassays has been observed due to differences in 25(OH)D_2 and 25(OH)D_3 recovery, C3-epimer

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LEARNING OBJECTIVES

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

1. Discuss how vitamin D is metabolized.
2. Describe vitamin D metabolites and their role in overall health and testing for levels of vitamin D.
3. Describe the testing technologies used to measure levels of vitamin D and testing rates worldwide.
4. Describe the potential medical complications of vitamin D deficiency.

Guidelines	Deficiency	Insufficiency	Sufficiency
	ng/mL (nmol/L)	ng/mL (nmol/L)	ng/mL (nmol/L)
Institute of Medicine (IOM)[31]	<12 (30)	12-20 (30-50)	≥20 (≥50)
Endocrine Society Practice Guidelines[5]	<20 (50)	20-30 (50-75)	≥30 (≥75)
Scientific Advisory Committee on Nutrition and UK Department for Health[32]	<10 (25)		≥10 (≥25)
Global Consensus Recommendations on Prevention and Management of Nutritional Rickets[33]	<12 (30)	12-20 (30-50)	≥20 (≥50)
National Osteoporosis Society (UK)[34]	<12 (30)	12-20 (30-50)	≥20 (≥50)
Canadian Paediatric Society[35]	<10 (25)	10-30 (25-75)	30-90 (75-225)
Working Group of the Australian and New Zealand Bone and Mineral Society, Endocrine Society of Australia and Osteoporosis Australia[36]	<20 (50)		≥20 (≥50)
Italian Guidelines on Prevention and Treatment of Vitamin D Deficiency[37]	<20 (50)	20-30 (50-75)	≥30 (≥75)

Table 1. Deficiency cut-offs according to worldwide guidelines.

cross-reactivity, and assay precision. In an effort to standardize 25(OH)D immunoassays, the Center for Disease Control and Prevention established the Vitamin D Standardization Certification Program (VDSCP) to evaluate the accuracy and precision of vitamin D tests and monitor their performance over time. The VDSCP also provides technical support to external quality assurance programs, proficiency testing programs, and research studies. The College of American Pathologists (CAP) and Vitamin D External Quality Assessment Scheme (DEQAS) surveys are two programs used to monitor the performance of laboratories using various methods for 25(OH)D testing.[12,14]

Potential complications of vitamin D deficiency

As mentioned above, vitamin D deficiency causes imbalance in calcium and phosphorus homeostasis resulting in bone changes. Mineralization defects in the skeleton can cause several bone abnormalities in children (like rickets), and increased bone and muscle pain with consequent risk of falls in adults.[5] Low levels of vitamin D are clearly associated with greater illness severity, morbidity and mortality both in children and in adult patients.[15]

Beyond bones and muscle disorders, low serum 25(OH)D concentrations have been associated with several pathological conditions. In chronic kidney disease, decline of 25(OH)D levels have been associated with worsening of the disease, secondary hyperparathyroidism, low bone mineral density, muscle weakness and risk of falls, metabolic syndrome and obesity, insulin resistance, left ventricular hypertrophy and atherosclerosis, vascular calcification and arterial stiffness, cognitive impairment and mortality.[16] In pregnancy, low vitamin D levels have been associated with preeclampsia, gestational diabetes, low birth weight and premature birth.[17] Vitamin D insufficiency also has been associated with cardiovascular events like hypertension, coronary artery disease, ischemic heart disease, heart failure, stroke, and type 2 diabetes. However, the causative role of vitamin D was not demonstrated in these conditions.[6]

Deficiency prevalence worldwide and increased testing rates

Vitamin D insufficiency and deficiency has been observed worldwide. Prevalence of vitamin D deficiency, defined as levels of 25(OH)D lower than 12 ng/mL, was reported in 6% of the population in the U.S., 7.4% in Canada, and 13% in Europe. Vitamin D insufficiency is more common and is reported to be 24% in the U.S., 37% in Canada, and 40% in Europe. Low levels of vitamin D have also been reported in India, Afghanistan, Tunisia and Pakistan with a prevalence higher than 20%.[5] Testing frequency has increased significantly in the last 20 years. For example, it increased by 4-fold between 2003 and 2013 in Canada,[18] and 42-fold between 2002 and 2017 in Minnesota.[19] There is not complete agreement in the world scientific community on the cut-off for insufficiency and deficiency, and a summary of various guidelines worldwide is shown in **Table 1**. Screening in the general population is not recommended, as current evidence lacks either against or in favor of testing asymptomatic patients.[20] However, screening for vitamin D levels is recommended for certain groups of at-risk subjects, which includes people with malnutrition, people with a sedentary lifestyle and obesity, elderly patients, patients with renal, gastrointestinal or liver disease, or people taking medications that alter vitamin D metabolism (anti-seizure, glucocorticoids, AIDS medications, antifungals, cholestyramine).[5,21]

Impact of vitamin D status on overall health

Even though vitamin D deficiency has been associated with many diseases, the data are conflicting. In many clinical trials, 25(OH)D measurements at baseline or after supplementation are not taken, which confounds outcome analysis on the effects of supplementation on vitamin D deficiency.[4] Importantly, vitamin D is a nutrient, and unlike a drug, it is difficult to control in randomized controlled trials because confounders such as habitual intake and sun exposure exist.[22] Thus, even though the disease association with vitamin D may exist, cause-effect relationships have been difficult to show. The importance of vitamin D sufficiency may be in early prevention. Studies that analyze available data on the general population — and that focus on productivity, healthcare costs, hospitalization, or mortality, independent of specific disease states — strongly suggest that subjects with adequate levels of vitamin D show less absenteeism,[22] have lower health care costs,[24-26] and lower mortality.[27,28]

Specifically, a study by Plotnikoff et al. assessed more than 10,000 healthcare employees in the Midwest, and found differences in work performance assessed by the Workplace Productivity and Activity Impairment questionnaire.[29] Lower serum 25(OH)D levels were associated with statistically significant lower productivity, with subjects with vitamin D levels >40 ng/mL having the least amount of absenteeism. The

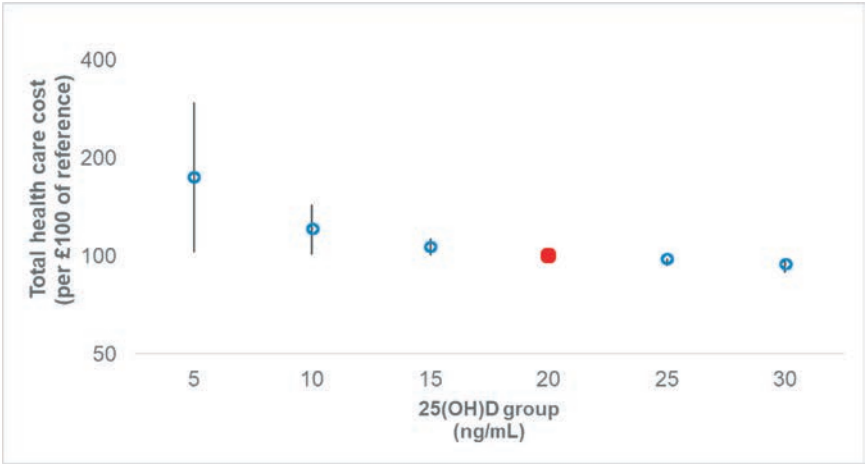

Figure 1. Average total healthcare costs in study participants with 25(OH)D concentrations as indicated using 20 ng/mL as the reference cost (p<0.05). Averages with 95% conference intervals are shown.[25]

authors estimated the potential annual payroll savings at $7.8 million for the employer when its employees achieved 25(OH)D serum levels of 40 ng/mL.[23] Furthermore, in healthy populations in northeastern Germany and in veterans in the Southeastern United States, inpatient and outpatient costs were found to be significantly lower in patients with circulating 25(OH)D levels >20 ng/mL **(Figure 1)**.[24, 25]

The risk of hospitalization was 2-5 times lower in vitamin D sufficient patients as assessed by Kempker et al. in a population of Medicare beneficiaries.[30] Hospital length of stay was also shown to be significantly different between mildly and severely deficient patients (5.2 days vs. 13.3 days) in patients from a surgical intensive care unit.[28] Cost assessment of hospitalized patients also showed significantly higher costs for the vitamin D-deficient patients.[28] Finally, a meta-analysis performed on data from more than 20,000 individuals from the general population in Europe demonstrated a very low risk of mortality in patients with 25(OH)D levels >30 ng/mL. The all-cause mortality risk increased significantly as vitamin D deficiency was more severe.[27]

Many data on disease association with vitamin D exist, and vitamin D is often believed to be some sort of wonder drug. However, it is perhaps necessary to more strongly emphasize that it is not a drug, but a nutrient, and that its true impact is improved health rather than a cure for disease. There are existing studies that analyze certain variables, such as healthcare costs as surrogate measurement of health. However, more studies are necessary to better understand the true impact of vitamin D on health.

Link between vitamin D deficiency and increased COVID-19 severity

By Andrea Diebel, MS; Ilaria Valente, PhD; and Claudia Zierold, PhD

Vitamin D found its way back into the spotlight in 2021 with researchers, clinicians, and the public seeking protection against COVID-19. With a whopping 450+ scholarly articles published on the relationship between vitamin D and COVID-19 in 2021 alone, we are all scrambling to keep up with the latest findings.

Numerous studies have revealed a wide range of pharmacological and physiological functions of vitamin D, including anti-inflammatory, antioxidant, and antiviral effects. Of particular interest, the relationship between vitamin D sufficiency and lowered risk and severity of respiratory tract infections has also been thoroughly researched.[1] Is it too much to hope that this so-called "miracle vitamin" could be the key to lowered risk and severity of COVID-19?

Demir and colleagues showed promising results in their recent publication Vitamin D deficiency is associated with COVID-19 positivity and severity of the disease.[2] In this retrospective study, very severe vitamin D deficiency (<10 ng/mL) was considerably more common in Turkish adult COVID-19 patients (44%) than in non-COVID-19 patients (31%). Among COVID-19-positive patients, the group with vitamin D levels of >30 ng/mL had significantly shorter hospital stays, lower numbers of affected lung segments, and lower D-dimer and C-reactive protein levels (markers of systemic inflammation). It is also worth noting that an astonishing 94.27% of the COVID-19-positive patients and 93.07% of the non-COVID-19 patients had vitamin D levels below 30 ng/mL, the generally accepted vitamin D sufficiency cutoff. This substantiates the need for continued vitamin D deficiency screening in at-risk groups.

Similar findings have been reported in studies performed in other countries. In a U.S. cohort study of 489 patients, the relative risk of testing positive for COVID-19 was 1.77 times greater for patients with likely vitamin D deficiency when compared to patients with likely vitamin D sufficiency.[3] Similarly, significantly lower vitamin D levels were found in COVID-19-positive patients (median value 11.1 ng/mL), compared with negative patients (24.6 ng/mL) in a retrospective study of 107 Swiss adults.[4]

While these studies suggest that vitamin D deficiency is associated with increased risk and severity of COVID-19, further research is needed to fully understand this relationship. There are various other potentially contributing chronic conditions and behavioral factors that could play a role in COVID-19 severity.

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Conclusions

The prevalence of vitamin D deficiency and insufficiency ranges from 6%-40% worldwide, demonstrating the importance of screening for vitamin D deficiency in at-risk groups using an accurate and reliable 25(OH)D assay. Candidates for screening include people with malnutrition, sedentary lifestyle and obesity, elderly patients, and patients with renal, gastrointestinal or liver disease, or those taking medication that may alter vitamin D metabolism.

The number of people in these groups continues to rise, creating a significant increase in vitamin D testing rates. Even though an association between vitamin D deficiency and chronic diseases has been observed in numerous clinical trials, a cause-and-effect relationship has been difficult to demonstrate because vitamin D is a nutrient, not a drug, and levels can be influenced by many factors. Regardless, vitamin D sufficiency has been shown to reduce absenteeism and lower both healthcare costs and mortality. Vitamin D sufficiency should be approached as a means toward improved health rather than a cure for disease. However, more studies are necessary to better understand the true impact of vitamin D on health.

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CONTINUING EDUCATION TEST

Vitamin D and impact on overall health

NOVEMBER 2021 *[This form may be photocopied. It is no longer valid for CEUs after May 31, 2023.]*

Passing scores of 70 percent or higher are eligible for 1 contact hour of P.A.C.E. credit.

TEST QUESTIONS

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

1. **Vitamin D is an essential nutrient that is well known for its role in maintaining _____.**
   - ◯ eye health
   - ◯ bone health
   - ◯ blood health
   - ◯ kidney health

2. **Vitamin D regulates which two key players in mineralization?**
   - ◯ hydrogen and sulfur
   - ◯ potassium and magnesium
   - ◯ calcium and phosphorus
   - ◯ magnesium and calcium

3. **Vitamin D2 (ergocalciferol) is of _____ origin and can be found in _____.**
   - ◯ animal, steak
   - ◯ animal, fish
   - ◯ plant, banana
   - ◯ plant, mushrooms

4. **Vitamin D3 (cholecalciferol) is produced in the _____ and can be found in _____.**
   - ◯ factory, dietary supplements
   - ◯ skin, fish oil
   - ◯ kidneys, milk
   - ◯ bones, steak

5. **The difference between vitamin D2 and vitamin D3 lies in their _____.**
   - ◯ chemical structure
   - ◯ nucleus
   - ◯ spike protein
   - ◯ physical characteristics

6. **Both vitamin D2 and vitamin D3 are biologically inactive prohormones that must be converted to the active vitamin D _____.**
   - ◯ mineral
   - ◯ hormone
   - ◯ chemical
   - ◯ supplement

7. **25(OH)D, or _____, is the form of vitamin D traditionally measured to evaluate vitamin D sufficiency.**
   - ◯ calcium
   - ◯ dydroxyol
   - ◯ hydroxiziole
   - ◯ calcidiol

8. **Vitamin D2 and D3 pass through the _____ and undergo an enzymatic conversion (hydroxylation of carbon 25).**
   - ◯ stomach
   - ◯ intestines
   - ◯ liver
   - ◯ gallbladder

9. **The majority of vitamin D (D2 or D3) that is synthesized in the skin or ingested is converted to _____.**
   - ◯ 35(OH)D
   - ◯ 25(OH)D
   - ◯ calcium
   - ◯ bone

10. **The enzymatic conversion of 25(OH)D occurs primarily in the _____ (hydroxylation of carbon 1).**
    - ◯ liver
    - ◯ stomach
    - ◯ intestines
    - ◯ kidney

11. **_____ maintains mineral homeostasis by enhancing the efficiency of calcium and phosphorus absorption from dietary sources in the small intestine.**
    - ◯ Calcitriol
    - ◯ Sodium
    - ◯ Magnesium
    - ◯ Potassium

12. **_____ can increase the mobilization of calcium from bone into circulation.**
    - ◯ Sodium
    - ◯ Magnesium
    - ◯ 1,25(OH)2D
    - ◯ 25-Dihydroxyvitamin D

13. **1,25(OH)2D levels are _____ used to evaluate vitamin D status due to the lack of correlation between the metabolite in the blood, and the levels of 25(OH)D or vitamin D.**
    - ◯ always
    - ◯ not typically
    - ◯ never
    - ◯ efficient to be

14. **1,25(OH)2D measurement is useful when _____ are suspected.**
    - ◯ kidney diseases
    - ◯ hyperparathyroidism
    - ◯ sarcoidosis
    - ◯ any of the above

15. **To inactivate and excrete in the urine, 1,25(OH)2D needs to be transformed from a(n) _____ molecule.**
    - ◯ ionized
    - ◯ lipid soluble to a water soluble
    - ◯ water soluble to a lipid soluble
    - ◯ mutated

16. **The C3-epimer of 25(OH)D is _____ of 25(OH) D, having the hydroxyl group on carbon 3 in a different spatial plane.**
    - ◯ a mutation
    - ◯ an ionized form
    - ◯ the protein spike
    - ◯ an isomer

17. **_____ are the most popular 25(OHD)D tests used by clinical laboratories for high volume testing.**
    - ◯ Molecular tests
    - ◯ Blood tests
    - ◯ Urine tests
    - ◯ Automated immunoassays

18. **Mineralization defects in the skeleton can cause _____.**
    - ◯ rickets
    - ◯ bone abnormalities
    - ◯ bone and muscle pain
    - ◯ all of the above

19. **Decline of 25(OH)D levels have been associated with _____.**
    - ◯ secondary hyperparathyroidism and atherosclerosis
    - ◯ left ventricular hypertrophy and cognitive impairment
    - ◯ metabolic syndrome, insulin resistance and obesity
    - ◯ all of the above

20. **Prevalence of vitamin D deficiency, defined as levels of 25(OH)D lower than 12 ng/mL, was reported in _____ of the population in the U.S.**
    - ◯ 29%
    - ◯ 6%
    - ◯ 12%
    - ◯ 36%

Tests can be taken online or by mail. Easy registration and payment options are available through NIU by following the links found at www.mlo-online.com/ce.

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P = Poor; E = Excellent

1. **To what extent did the article focus on or clarify the objectives?**
   P ① ② ③ ④ ⑤ E
2. **To what extent was the article well-organized and readable?**
   P ① ② ③ ④ ⑤ E
3. **How will you use the CE units?**
   ☐ state license ☐ employment ☐ recertification ☐ other

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Guidelines	Deficiency	Insufficiency	Sufficiency
	ng/mL (nmol/L)	ng/mL (nmol/L)	ng/mL (nmol/L)
Institute of Medicine (IOM)[31]	<12 (30)	12-20 (30-50)	≥20 (≥50)
Endocrine Society Practice Guidelines[5]	<20 (50)	20-30 (50-75)	≥30 (≥75)
Scientific Advisory Committee on Nutrition and UK Department for Health[32]	<10 (25)		≥10 (≥25)
Global Consensus Recommendations on Prevention and Management of Nutritional Rickets[33]	<12 (30)	12-20 (30-50)	≥20 (≥50)
National Osteoporosis Society (UK)[34]	<12 (30)	12-20 (30-50)	≥20 (≥50)
Canadian Paediatric Society[35]	<10 (25)	10-30 (25-75)	30-90 (75-225)
Working Group of the Australian and New Zealand Bone and Mineral Society, Endocrine Society of Australia and Osteoporosis Australia[36]	<20 (50)		≥20 (≥50)
Italian Guidelines on Prevention and Treatment of Vitamin D Deficiency[37]	<20 (50)	20-30 (50-75)	≥30 (≥75)

Table 1. Deficiency cut-offs according to worldwide guidelines.

cross-reactivity, and assay precision. In an effort to standardize 25(OH)D immunoassays, the Center for Disease Control and Prevention established the Vitamin D Standardization Certification Program (VDSCP) to evaluate the accuracy and precision of vitamin D tests and monitor their performance over time. The VDSCP also provides technical support to external quality assurance programs, proficiency testing programs, and research studies. The College of American Pathologists (CAP) and Vitamin D External Quality Assessment Scheme (DEQAS) surveys are two programs used to monitor the performance of laboratories using various methods for 25(OH)D testing.[12,14]

Potential complications of vitamin D deficiency

As mentioned above, vitamin D deficiency causes imbalance in calcium and phosphorus homeostasis resulting in bone changes. Mineralization defects in the skeleton can cause several bone abnormalities in children (like rickets), and increased bone and muscle pain with consequent risk of falls in adults.[5] Low levels of vitamin D are clearly associated with greater illness severity, morbidity and mortality both in children and in adult patients.[15]

Beyond bones and muscle disorders, low serum 25(OH)D concentrations have been associated with several pathological conditions. In chronic kidney disease, decline of 25(OH)D levels have been associated with worsening of the disease, secondary hyperparathyroidism, low bone mineral density, muscle weakness and risk of falls, metabolic syndrome and obesity, insulin resistance, left ventricular hypertrophy and atherosclerosis, vascular calcification and arterial stiffness, cognitive impairment and mortality.[16] In pregnancy, low vitamin D levels have been associated with preeclampsia, gestational diabetes, low birth weight and premature birth.[17] Vitamin D insufficiency also has been associated with cardiovascular events like hypertension, coronary artery disease, ischemic heart disease, heart failure, stroke, and type 2 diabetes. However, the causative role of vitamin D was not demonstrated in these conditions.[6]

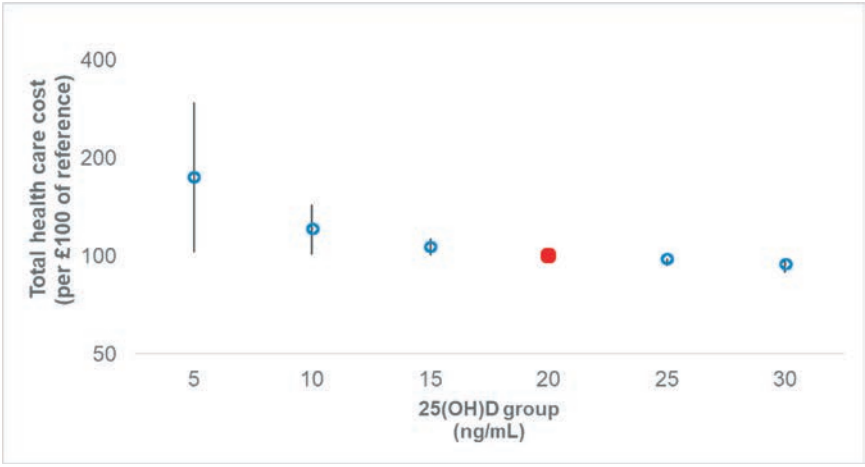
Deficiency prevalence worldwide and increased testing rates

Vitamin D insufficiency and deficiency has been observed worldwide. Prevalence of vitamin D deficiency, defined as levels of 25(OH)D lower than 12 ng/mL, was reported in 6% of the population in the U.S., 7.4% in Canada, and 13% in Europe. Vitamin D insufficiency is more common and is reported to be 24% in the U.S., 37% in Canada, and 40% in Europe. Low levels of vitamin D have also been reported in India, Afghanistan, Tunisia and Pakistan with a prevalence higher than 20%.[5] Testing frequency has increased significantly in the last 20 years. For example, it increased by 4-fold between 2003 and 2013 in Canada,[18] and 42-fold between 2002 and 2017 in Minnesota.[19] There is not complete agreement in the world scientific community on the cut-off for insufficiency and deficiency, and a summary of various guidelines worldwide is shown in **Table 1**. Screening in the general population is not recommended, as current evidence lacks either against or in favor of testing asymptomatic patients.[20] However, screening for vitamin D levels is recommended for certain groups of at-risk subjects, which includes people with malnutrition, people with a sedentary lifestyle and obesity, elderly patients, patients with renal, gastrointestinal or liver disease, or people taking medications that alter vitamin D metabolism (anti-seizure, glucocorticoids, AIDS medications, antifungals, cholestyramine).[5,21]

Impact of vitamin D status on overall health

Even though vitamin D deficiency has been associated with many diseases, the data are conflicting. In many clinical trials, 25(OH)D measurements at baseline or after supplementation are not taken, which confounds outcome analysis on the effects of supplementation on vitamin D deficiency.[4] Importantly, vitamin D is a nutrient, and unlike a drug, it is difficult to control in randomized controlled trials because confounders such as habitual intake and sun exposure exist.[22] Thus, even though the disease association with vitamin D may exist, cause-effect relationships have been difficult to show. The importance of vitamin D sufficiency may be in early prevention. Studies that analyze available data on the general population — and that focus on productivity, healthcare costs, hospitalization, or mortality, independent of specific disease states — strongly suggest that subjects with adequate levels of vitamin D show less absenteeism,[22] have lower health care costs,[24-26] and lower mortality.[27,28]

Specifically, a study by Plotnikoff et al. assessed more than 10,000 healthcare employees in the Midwest, and found differences in work performance assessed by the Workplace Productivity and Activity Impairment questionnaire.[29] Lower serum 25(OH)D levels were associated with statistically significant lower productivity, with subjects with vitamin D levels >40 ng/mL having the least amount of absenteeism. The



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authors estimated the potential annual payroll savings at $7.8 million for the employer when its employees achieved 25(OH)D serum levels of 40 ng/mL.[23] Furthermore, in healthy populations in northeastern Germany and in veterans in the Southeastern United States, inpatient and outpatient costs were found to be significantly lower in patients with circulating 25(OH)D levels >20 ng/mL **(Figure 1)**.[24, 25]

The risk of hospitalization was 2-5 times lower in vitamin D sufficient patients as assessed by Kempker et al. in a population of Medicare beneficiaries.[30] Hospital length of stay was also shown to be significantly different between mildly and severely deficient patients (5.2 days vs. 13.3 days) in patients from a surgical intensive care unit.[28] Cost assessment of hospitalized patients also showed significantly higher costs for the vitamin D-deficient patients.[28] Finally, a meta-analysis performed on data from more than 20,000 individuals from the general population in Europe demonstrated a very low risk of mortality in patients with 25(OH)D levels >30 ng/mL. The all-cause mortality risk increased significantly as vitamin D deficiency was more severe.[27]

Many data on disease association with vitamin D exist, and vitamin D is often believed to be some sort of wonder drug. However, it is perhaps necessary to more strongly emphasize that it is not a drug, but a nutrient, and that its true impact is improved health rather than a cure for disease. There are existing studies that analyze certain variables, such as healthcare costs as surrogate measurement of health. However, more studies are necessary to better understand the true impact of vitamin D on health.

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By Andrea Diebel, MS; Ilaria Valente, PhD; and Claudia Zierold, PhD

Vitamin D found its way back into the spotlight in 2021 with researchers, clinicians, and the public seeking protection against COVID-19. With a whopping 450+ scholarly articles published on the relationship between vitamin D and COVID-19 in 2021 alone, we are all scrambling to keep up with the latest findings.

Numerous studies have revealed a wide range of pharmacological and physiological functions of vitamin D, including anti-inflammatory, antioxidant, and antiviral effects. Of particular interest, the relationship between vitamin D sufficiency and lowered risk and severity of respiratory tract infections has also been thoroughly researched.[1] Is it too much to hope that this so-called "miracle vitamin" could be the key to lowered risk and severity of COVID-19?

Demir and colleagues showed promising results in their recent publication Vitamin D deficiency is associated with COVID-19 positivity and severity of the disease.[2] In this retrospective study, very severe vitamin D deficiency (<10 ng/mL) was considerably more common in Turkish adult COVID-19 patients (44%) than in non-COVID-19 patients (31%). Among COVID-19-positive patients, the group with vitamin D levels of >30 ng/mL had significantly shorter hospital stays, lower numbers of affected lung segments, and lower D-dimer and C-reactive protein levels (markers of systemic inflammation). It is also worth noting that an astonishing 94.27% of the COVID-19-positive patients and 93.07% of the non-COVID-19 patients had vitamin D levels below 30 ng/mL, the generally accepted vitamin D sufficiency cutoff. This substantiates the need for continued vitamin D deficiency screening in at-risk groups.

Similar findings have been reported in studies performed in other countries. In a U.S. cohort study of 489 patients, the relative risk of testing positive for COVID-19 was 1.77 times greater for patients with likely vitamin D deficiency when compared to patients with likely vitamin D sufficiency.[3] Similarly, significantly lower vitamin D levels were found in COVID-19-positive patients (median value 11.1 ng/mL), compared with negative patients (24.6 ng/mL) in a retrospective study of 107 Swiss adults.[4]

While these studies suggest that vitamin D deficiency is associated with increased risk and severity of COVID-19, further research is needed to fully understand this relationship. There are various other potentially contributing chronic conditions and behavioral factors that could play a role in COVID-19 severity.

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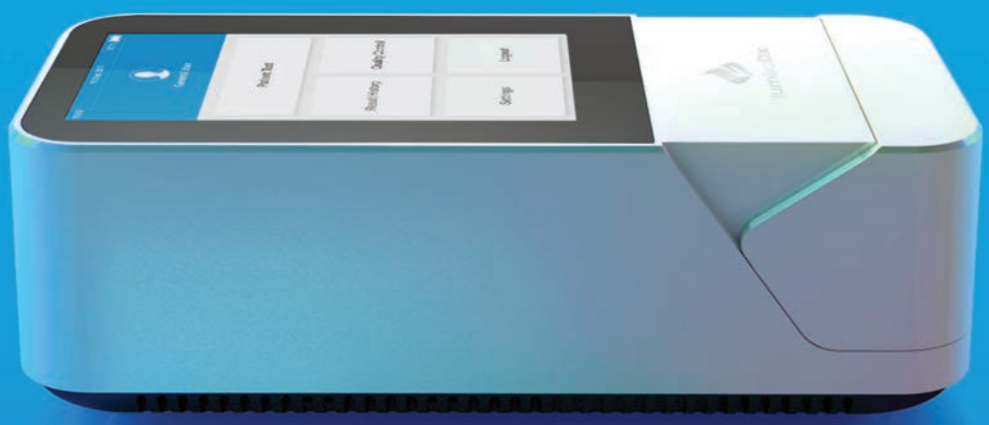

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Conclusions

The prevalence of vitamin D deficiency and insufficiency ranges from 6%-40% worldwide, demonstrating the importance of screening for vitamin D deficiency in at-risk groups using an accurate and reliable 25(OH)D assay. Candidates for screening include people with malnutrition, sedentary lifestyle and obesity, elderly patients, and patients with renal, gastrointestinal or liver disease, or those taking medication that may alter vitamin D metabolism.

The number of people in these groups continues to rise, creating a significant increase in vitamin D testing rates. Even though an association between vitamin D deficiency and chronic diseases has been observed in numerous clinical trials, a cause-and-effect relationship has been difficult to demonstrate because vitamin D is a nutrient, not a drug, and levels can be influenced by many factors. Regardless, vitamin D sufficiency has been shown to reduce absenteeism and lower both healthcare costs and mortality. Vitamin D sufficiency should be approached as a means toward improved health rather than a cure for disease. However, more studies are necessary to better understand the true impact of vitamin D on health.

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TEST QUESTIONS

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1. **Vitamin D is an essential nutrient that is well known for its role in maintaining _____.**
   - ◯ eye health
   - ◯ bone health
   - ◯ blood health
   - ◯ kidney health

2. **Vitamin D regulates which two key players in mineralization?**
   - ◯ hydrogen and sulfur
   - ◯ potassium and magnesium
   - ◯ calcium and phosphorus
   - ◯ magnesium and calcium

3. **Vitamin D2 (ergocalciferol) is of _____ origin and can be found in _____.**
   - ◯ animal, steak
   - ◯ animal, fish
   - ◯ plant, banana
   - ◯ plant, mushrooms

4. **Vitamin D3 (cholecalciferol) is produced in the _____ and can be found in _____.**
   - ◯ factory, dietary supplements
   - ◯ skin, fish oil
   - ◯ kidneys, milk
   - ◯ bones, steak

5. **The difference between vitamin D2 and vitamin D3 lies in their _____.**
   - ◯ chemical structure
   - ◯ nucleus
   - ◯ spike protein
   - ◯ physical characteristics

6. **Both vitamin D2 and vitamin D3 are biologically inactive prohormones that must be converted to the active vitamin D _____.**
   - ◯ mineral
   - ◯ hormone
   - ◯ chemical
   - ◯ supplement

7. **25(OH)D, or _____, is the form of vitamin D traditionally measured to evaluate vitamin D sufficiency.**
   - ◯ calcium
   - ◯ dydroxyol
   - ◯ hydroxiziole
   - ◯ calcidiol

8. **Vitamin D2 and D3 pass through the _____ and undergo an enzymatic conversion (hydroxylation of carbon 25).**
   - ◯ stomach
   - ◯ intestines
   - ◯ liver
   - ◯ gallbladder

9. **The majority of vitamin D (D2 or D3) that is synthesized in the skin or ingested is converted to _____.**
   - ◯ 35(OH)D
   - ◯ 25(OH)D
   - ◯ calcium
   - ◯ bone

10. **The enzymatic conversion of 25(OH)D occurs primarily in the _____ (hydroxylation of carbon 1).**
    - ◯ liver
    - ◯ stomach
    - ◯ intestines
    - ◯ kidney

11. **_____ maintains mineral homeostasis by enhancing the efficiency of calcium and phosphorus absorption from dietary sources in the small intestine.**
    - ◯ Calcitriol
    - ◯ Sodium
    - ◯ Magnesium
    - ◯ Potassium

12. **_____ can increase the mobilization of calcium from bone into circulation.**
    - ◯ Sodium
    - ◯ Magnesium
    - ◯ 1,25(OH)2D
    - ◯ 25-Dihydroxyvitamin D

13. **1,25(OH)2D levels are _____ used to evaluate vitamin D status due to the lack of correlation between the metabolite in the blood, and the levels of 25(OH)D or vitamin D.**
    - ◯ always
    - ◯ not typically
    - ◯ never
    - ◯ efficient to be

14. **1,25(OH)2D measurement is useful when _____ are suspected.**
    - ◯ kidney diseases
    - ◯ hyperparathyroidism
    - ◯ sarcoidosis
    - ◯ any of the above

15. **To inactivate and excrete in the urine, 1,25(OH)2D needs to be transformed from a(n) _____ molecule.**
    - ◯ ionized
    - ◯ lipid soluble to a water soluble
    - ◯ water soluble to a lipid soluble
    - ◯ mutated

16. **The C3-epimer of 25(OH)D is _____ of 25(OH) D, having the hydroxyl group on carbon 3 in a different spatial plane.**
    - ◯ a mutation
    - ◯ an ionized form
    - ◯ the protein spike
    - ◯ an isomer

17. **_____ are the most popular 25(OHD)D tests used by clinical laboratories for high volume testing.**
    - ◯ Molecular tests
    - ◯ Blood tests
    - ◯ Urine tests
    - ◯ Automated immunoassays

18. **Mineralization defects in the skeleton can cause _____.**
    - ◯ rickets
    - ◯ bone abnormalities
    - ◯ bone and muscle pain
    - ◯ all of the above

19. **Decline of 25(OH)D levels have been associated with _____.**
    - ◯ secondary hyperparathyroidism and atherosclerosis
    - ◯ left ventricular hypertrophy and cognitive impairment
    - ◯ metabolic syndrome, insulin resistance and obesity
    - ◯ all of the above

20. **Prevalence of vitamin D deficiency, defined as levels of 25(OH)D lower than 12 ng/mL, was reported in _____ of the population in the U.S.**
    - ◯ 29%
    - ◯ 6%
    - ◯ 12%
    - ◯ 36%

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Different approaches to SARS-CoV-2 testing using mass spectrometry

By Leann M. Mikesh, PhD

Current detection methodologies for SARS-CoV-2 include molecular testing using real-time, reverse transcription — polymerase chain reaction (RT-PCR) (gold standard), or a variety of other amplification-based methods, as well as antigen testing by a variety of immunoassay-based methodologies. Two different mass spectrometry-based methodologies/technologies, will be discussed: matrix assisted laser desorption ionization – time of flight (MALDI-ToF) and liquid chromatography – tandem mass spectrometry (LC-MS/MS). These methods are used for the detection of SARS-CoV-2 nucleic acid (molecular) or protein molecules (antigen).

Mass spectrometry-based testing for SARS-CoV-2

Mass spectrometry-based technologies for COVID-19 testing detect cDNA from SARS-CoV-2 RNA or peptides derived from SARS-CoV-2 proteins. With these methodologies, ideal target candidate viral cDNA or peptides have previously been identified for targeted analysis. Samples are collected and prepared by either RT-PCR or protein digestion as appropriate. The cDNA (currently analyzed by MALDI-ToF) or peptides (currently analyzed by LC-MS/MS) from the sample are then ionized and introduced into the mass spectrometer, where they are detected and analyzed. It is generally accepted that more than one SARS-CoV-2 target region has to be positive for the mass spectrometry assay to be positive.

MALDI-ToF mass spectrometry testing for SARS-CoV-2

MALDI-ToF mass spectrometry is not new to the clinical laboratory. It has been approved for use by the U.S. Food and Drug Administration (FDA) as a bacterial identification mass spectrometry-based diagnostic test for several years. This technology combines ionization of sample molecules by MALDI with a ToF analyzer to identify the molecules of interest.

Currently (as of June 22, 2021), there are three (two are laboratory restricted) mass spectrometry-based tests for SARS-CoV-2 detection with emergency use authorization (EUA) from the FDA.[1] All three testing methodologies are based on a multiplex RT-PCR amplification of SARS-CoV-2 nucleic acids from upper respiratory tract specimens followed by MALDI-ToF mass spectrometry analysis for qualitative detection.

All three of the FDA EUA approved tests also use the same nucleic acid amplification methodology and panel kit for SARS-CoV-2 RNA detection. Prior to mass spectrometry detection, RNA is isolated from upper respiratory samples, converted into cDNA, and specific gene regions are amplified by RT-PCR. In this instance, the specific regions amplified for SARS-CoV-2 testing are regions corresponding to the nucleocapsid gene, open reading frame (ORF)1 and ORF1 ab. Post RT-PCR, excess dNTPs are inactivated before another round of PCR amplification is performed, adding one additional nucleotide to a sequence specific probe bound to the amplified SARS-CoV-2 target region. This generates allele-specific PCR products with different specific masses.

After SARS-CoV-2 targeted regions have been amplified and probe added, MALDI-ToF mass spectrometry is used to detect and determine the masses of the amplified PCR product(s). With this type of mass spectrometer, molecules are ionized by MALDI, then analyzed by ToF. MALDI is an ionization technique that uses matrix added to samples and a nitrogen laser to ionize (acquire a charge) and vaporize samples spotted onto a sample plate. The ionized sample then travels down the flight tube of the mass spectrometer, where the different PCR products' time of travel is related to its mass to charge ratio (m/z), with heavier ions taking longer to reach the detector. The information resulting from this analysis generates a graph (mass spectrum), plotting m/z on the x-axis and relative abundance on the y-axis. The specificity is obtained from the m/z of the ion and allows for the identification of previously determined SARS-CoV-2 RNA targets being identified in the sample.

The targeted cDNA regions tested by the three EUA approved tests contain a panel of five SARS-CoV-2 target assays to remain robust in the presence of novel mutations. The commercially available EUA SARS-CoV-2 test can run 96 to 384 samples at one time. The PCR amplification steps takes about 2.75 hours, depending on thermocycler performance, while the time to analyze 384 samples by mass spectrometry is about 50 minutes. This test also reports a limit of detection (LOD) from nasopharyngeal swab samples to be 2.5 copies/µL with a positive percent agreement (PPA) of 95-100% and negative percent agreement (NPA) of 100%.[2] Similar performance characteristics of the other two laboratory-restricted EUA tests are listed in **Table 1**.

LC-MS/MS Testing for SARS-CoV-2

While there currently is no mass spectrometry-based method for SARS-CoV-2 testing approved by the FDA (EUA or otherwise) other than the previously mentioned MALDI-ToF tests, there are several LC-MS/MS based methodologies for SARS-CoV-2 testing reported in the literature. This technology differs from MALDI-ToF in several ways. First, it tends to be a targeted proteomics approach, allowing for both qualitative and quantitative analysis using tryptic-digested peptides from SARS-CoV-2 proteins as the analytical target (although other targets can also be analyzed). In addition, molecules are first separated using LC before introduction into the mass spectrometer. Also, the mass spectrometers typically used in this methodology combine ionization of sample molecules by either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) with a triple quadrupole mass analyzer (or higher-end hybrid) to identify the molecules of interest. Another way the mass spectrometry methodology differs is that LC-MS/MS uses a targeted approach, such as selected reaction monitoring (SRM) or other closely related methods, where only selected peptides from digested SARS-CoV-2 proteins in a sample are first filtered and "isolated" by the mass spectrometer based on their m/z, targeted for fragmentation, then only specific fragment product ions are detected and analyzed either qualitatively or quantitatively. Of note, if an unknown mutation is present in the targeted peptide, the m/z will differ, and it will not be detected.

The targeted proteomics methodologies are still more research-based, versus clinically adopted methods. They tend to utilize higher-end instrumentation; although, laboratories also utilize the more clinically available instrument, a triple quadrupole. LC-MS/MS methodologies are typically more complex than a basic MALDI-ToF assay with a higher level of method understand-

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[1]Johansson MA et al. (2021) SARS-CoV-2 Transmission From People Without COVID-19 Symptoms. JAMA Netw. Open 4, e2035057–e2035057

Table 1: Parameters and Performance Characteristics of SARS-CoV-2 Mass Spectrometry Methodologies

	EUA commercial test[2]	EUA lab-restricted test [6]	EUA lab-restricted test [7]	Cardozo et al. test[3]	Cazares et al. test[4]	Singh et al. test[5]
Analyte detected	Nucleic acid	Nucleic acid	Nucleic acid	Peptides	Peptides	Peptides
MS method/ instrument	RT-PCR/ MALDI-ToF	RT-PCR/ MALDI-ToF	RT-PCR/ MALDI-ToF	LC-MS/MS (SRM) Triple Quadrupole	LC-MS/MS (PRM) Quadrupole-Orbitrap hybrid	LC-MS/MS (sMRM) Quadrupole-ToF hybrid
Sample run time and testing capacity	Up to 96 or 384 samples/run 3hr 35min (PCR+MS)	NA	NA	96 samples/4 hr (prep) 500 samples/24 hr (MS) 4 samples/10 min (MS only)	7 min/sample (MS only)	2.3 min/sample (MS only)
LOD	2.5 copies of viral RNA/uL	0.69-2.75 copies of viral RNA/uL	1 $TCID_{50}$/mL	2.7-3.2 ng/mL peptide	195 amol peptide (~$2x10^5$ viral particles/mL)	NA
LOQ	NA*	NA*	NA*	4.4-6.0 ng/mL peptide	390 amol peptide	NA*
Performance	95-100% PPA 100% NPA	100% PPA 99.5-100% NPA	98.1% PPA 96.3% NPA	78.2-83.6% sensitivity 93.3-96.8% specificity	NA	90.5% sensitivity 100% specificity

*=qualitative test, LOD=limit of detection, LOQ=limit of quantification, MS=mass spectrometry, NA=not available, NPA=negative percent agreement, PPA=positive percent agreement, PRM=parallel reaction monitoring, sMRM=scheduled multiple reaction monitoring, SRM=selected reaction monitoring, TCID=tissue culture infectious dose

ing and user expertise needed to develop/run the test. However, laboratories that typically run mass spectrometry-based tests, such as drugs of abuse, steroid analysis, or vitamin D assays, are familiar with most of this technology. Laboratories can also reduce testing complexity (and turnaround time) by utilizing automated instrumentation on the front end for sample handling and processing, with automated data analysis on the back end.

Select examples from the literature using this methodology demonstrate the feasibility of this method for use as a clinical diagnostic assay, including the method by Cardozo using an SRM approach and targeting two SARS-CoV-2 peptides from nucleoprotein.[3] Cazares uses parallel reaction monitoring (PRM) for a proof-of-principal demonstration using two peptides from the spike protein and two peptides from the nucleoprotein.[4] Singh targets two SARS-CoV-2 peptides, one from the spike protein and one from the replicase polyprotein 1ab protein.[5]

Some of these assays report running up to 500 samples per day.[3] Others demonstrate a LOD of 195 amol of peptide and LOQ of 390 amol of peptide.[4] The authors estimated that the LOD is equivalent to approximately $2x10^5$ viral particles/mL, which is only 2 to 20 times higher than the lowest range of the observed SARS-CoV-2 titer from saliva ($1x10^5$ to $1x10^{11}$ copies/mL) and nasal ($1x10^4$ to $1x10^{10}$ copies/mL) swab samples,[4] and a factor of ten greater than the commercially available EUA mass spectrometry method. Moreover, they also state sample handling was not optimized and could have impacted detection.[4] Unlike in RT-PCR, proteins cannot be exponentially multiplied; therefore, sensitivity can be lower when compared to this method. However, higher-end mass spectrometers can detect very low quantities of peptides, some in the low amol range, which may compensate for the lack of amplification. Sensitivity using LC-MS/MS methods have been reported in the 78.2% to 90.5% range and specificity in the 93.3% to 100% range.[3,5] **Table 1.**

Conclusion

There are many different approaches that can be taken for mass spectrometry-based SARS-CoV-2 testing. Currently, only three different companies/laboratories using the RT-PCR/MALDI-ToF methodology have EUAs for SARS-CoV-2 testing. These RT-PCR/MALDI-ToF tests detect the genetic component of SARS-CoV-2, while the LC-MS/MS based methodologies tend to detect the protein (or antigen) component of SARS-CoV-2 by targeted proteomics. In the case of RT-PCR/MALDI-ToF, the mass spectrometer is used to detect the amplified nucleic acid product. With the LC-MS/MS method, mass spectrometry is integral to the methodology itself.

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Frontline testing with multiplex respiratory panels

By Tyler B. Dodd, PharmD, BCPS, BCIDP; Heather Nutting, M(ASCP)CM, MT(AMT); Brandon K. Hill, PharmD, BCPS, BCIDP; Tiffeny T. Smith, PharmD, BCIDP

Acute respiratory tract infections (RTIs) have a high burden of disease and can be caused by a variety of pathogens that include viral and bacterial agents.[1] The clinical presentation of a patient with an RTI is often nonspecific and could mimic other non-infectious etiologies (i.e., chronic obstructive pulmonary disease exacerbation). Further complicating accurate diagnosis are the changes in influenza and other respiratory virus activity during the ongoing COVID-19 pandemic. Rates of co-infection with SARS-CoV-2 and other common respiratory viruses ranges from 3-21%; the large gap is potentially due to differences in sample size between studies and variations in quarantine measures between countries.[2,3] More recently, U.S. surveillance summary data showed an atypical seasonal circulation pattern of respiratory viruses.[4] Given this information, the Centers for Disease Control and Prevention (CDC) recently advised that clinicians should be aware of increased circulation, sometimes offseason, of some respiratory viruses and consider multi-pathogen testing.[4]

Rapid identification of causative pathogen(s) with a molecular multiplex respiratory panel (RP) is important to provide a level of diagnostic certainty as well as appropriate clinical management, effective initiation of isolation precautions, and patient cohorting. Furthermore, given the growing concern of antibiotic resistance, the Infectious Diseases Society of America (IDSA) guideline for implementing an antibiotic stewardship program (ASP) recommends the use of rapid testing for respiratory pathogens to reduce the inappropriate use of antibiotics.[5] The use of a multiplex RP as a frontline testing option may offer favorable benefits to overall stewardship efforts, such as decreasing unnecessary antibiotic use and length of hospital stay, while increasing appropriate use of antivirals and patient satisfaction.[6,7]

Impact of respiratory illness testing on antibiotic stewardship

In order to meet SARS-CoV-2 testing demands, institutions found it vital to adopt at least one, but often multiple, respiratory testing platforms, and these institutions varied widely in their respiratory illness testing algorithms. For instance, some laboratories have instituted an algorithm that involves automatic reflex testing to a broader panel if a limited panel (e.g., influenza A/B) does not yield a positive result. Alternatively, some labs chose to be more restrictive and only perform a multiplex RP upon written request by the clinician after the results of the initial limited test(s) have been made available. Yet, another approach includes a combination of both algorithms with automatic reflex testing to a multiplex RP only for specific patient populations (e.g., immunocompromised patients, critically ill, or those admitted with respiratory symptoms).

It is imperative that an institution's respiratory testing algorithm be established with interdepartmental collaboration, and patient care is at the forefront of these decisions.

Antibiotic overuse in patients with viral respiratory tract infections has only been exacerbated by the COVID-19 pandemic. Empiric antibiotic regimens are often initiated while waiting for additional clinical evaluation, and antibiotic continuation may be associated more with concerns of concomitant bacterial pneumonia despite detection of a virus. A recent meta-analysis of 24 studies showed that bacterial co-infection in patients diagnosed with COVID-19 was identified in 3.5% of patients, and despite this, nearly 72% received antibiotics.[8]

However, is there enough clinical value to test all patients who present to the hospital with acute respiratory symptoms with a multiplex RP? A study by Semret et al. evaluated the impact of multiplex RP on changes in antibiotic therapy among hospitalized adults.[9] Notably, patients who tested positive for influenza were nine times more likely to be initiated on oseltamivir if they were not empirically started. Conversely, among patients who were empirically started on antibiotic therapy prior to RP results, antibiotics were discontinued in 39% of patients who tested positive for influenza and 20% of those who tested positive for non-influenza viruses.

In a randomized controlled trial evaluating the routine use of a multiplex RP in adults presenting to the hospital with an acute respiratory illness, Brendish et al. found that patients who were tested with a rapid multiplex RP experienced a reduced length of stay (5.7 days vs. 6.8 days), and more specifically, patients that tested positive for a viral pathogen received shorter courses of antibiotics (6.2 days vs. 8.0 days) compared to the control group.[1] Similarly, Weiss et al. noted an 8.4% drop in admissions for patients with a positive multiplex RP test result from the emergency department, likely due to an increase in provider comfort in discharging a patient with a confirmed viral pathogen.[10]

A study in a pediatric setting showed a rapid multiplex RP was associated with a trend towards decreased antibiotic usage but was unable to demonstrate similar length of stay or admission outcomes in the adult population.[11] Importantly, these studies highlight the need for stewardship tactics involving testing with multiplex RPs including, but not limited to, development of testing algorithms, clinician education and clinical decision support tools.

Aside from the challenge of differentiating between viral and bacterial respiratory infections, another problem is that ASPs face barriers in terms of adequate resources and stewardship personnel, especially during the COVID-19 pandemic. This now prompts the question: how can multiplex panels best be utilized to maximize efficiency for overburdened stewardship programs and to improve patient care and resource utilization?

How to approach testing with multiplex panels

One such approach may be to enact what could be considered passive interventions via clinical decision support software (CDSS) using targeted comments or alerts to clinicians. This strategy would allow ASPs to focus resources on what one might consider higher impact interventions (e.g., prospective review of positive blood culture results) while also guiding physicians with more than a lone multiplex result. This concept has proven useful in several studies when utilized for respiratory and bloodstream infections in varying ways. Banerjee et al. implemented a comment for clinicians providing guidance for resistance marker interpretation and therapy selection for bloodstream infections that led to near identical time to appropriate antibiotic escalation as real-time stewardship intervention.[12]

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Two additional studies, which focused on multiplex RP testing, implemented alerts to identify patients in which antibiotic de-escalation or discontinuation may be feasible.[13,14] The first study utilized positive RP results plus a negative procalcitonin (PCT) result as a prompt for clinicians to reassess the necessity of ongoing antibiotic therapy.[13] This specific strategy resulted in a reduction in antibiotic days of therapy by 2.2 days and is unique in that it directs the provider's attention to not only the positive RP result (which alone does not necessarily rule out the presence of bacterial co-infection), but also to other factors, suggesting the absence of a bacterial infection, such as a negative PCT.[13] In the second study, a CDSS alert was created to alert ASP personnel when patients receiving antibiotic therapy had a positive multiplex RP result.[14] Improvements were made in time to initiation of oseltamivir for influenza-positive patients (11.3 hours vs 3.6 hours), and a slight change in time to discontinuation of antibiotic therapy, albeit nonsignificant, was also observed.[14]

For ASPs with limited resources and other areas of major focus, using rapid multiplex RPs to the fullest extent may seem daunting. SARS-CoV-2 has created an environment where antibiotic overuse may be difficult for prescribers to avoid, given their understandable concerns about patients' risk for concomitant infections and severe illness. Now, more than ever, strategies requiring minimal use of direct stewardship resources are needed to reduce unnecessary antibiotic use for viral respiratory tract infections.

Financial impact of broad multiplex testing

Lastly, it is important to consider the downstream financial effects associated with the broader implementation of a multiplex respiratory panel. While patient outcomes should be at the forefront of these decisions, financial implications cannot be ignored. Reimbursement for RPs can be limited and, in some cases, with patients potentially receiving a bill anywhere from hundreds to thousands of dollars for these tests.[15,16] Physicians are aware of the perceived high cost to patients and appropriately may limit the use of these panels for financial reasons alone.

Interestingly, the IDSA and five other governing bodies drafted a letter to the Centers for Medicare & Medicaid Services (CMS) in the early days of the pandemic, requesting immediate national coverage of multiplex RP tests, highlighting the benefits of routine use of RP when SARS-CoV-2 testing supplies are limited and to aid in diagnosing co-infections.[17] Given that the average cost per test of a multiplex RP panel (varying by manufacturer) is about $120 and likely to continue to decrease, we hypothesize that if the reimbursement were decreased to a figure that still covered the laboratories cost, but minimized the billing implications to the patient, we would use a multiplex RP test more often on patients.

Benefits to patient care and hospital cost savings related to more rapid decision-making about the appropriate course of treatment are important factors to justify broader use of these panels, especially as the pandemic shows no signs of slowing.

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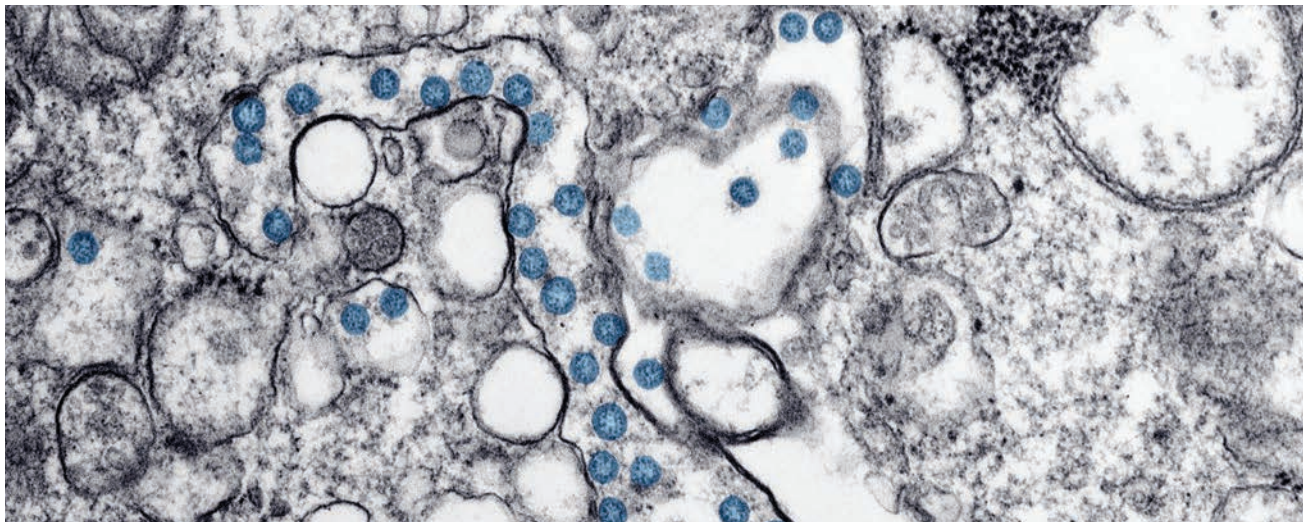

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Realizing the promise of digital pathology

By David McClintock, MD

Shortly after COVID-19 was declared a national emergency in the U.S., the Centers for Medicare & Medicaid Services (CMS) issued a memorandum on March 26, 2020, waiving the pre-pandemic requirement for remote sites to have a CLIA certificate when reviewing histopathology slides for primary diagnosis.[1] The U.S. Food and Drug Administration (FDA) soon followed on April 24, 2020, with a guidance document relaxing enforcement policies that effectively allowed the interim use of digital pathology devices intended for "reviewing and reporting of digital pathology slides from remote locations" without a pre-market notification submission.[2] The guidance covered automated digital image microscopes, whole slide imaging (WSI) systems, image viewing and management software, and digital pathology displays — typically used in healthcare facilities, but also key components for reviewing and reporting of digital pathology slides from remote settings. The FDA cited continuity of patient care, reduction of healthcare personnel contact, and risk of exposure during the pandemic as the underlying rationale for its decision.

Over the past year and a half, these regulatory responses to the pandemic have brought to the foreground some of the benefits of digital pathology for clinical laboratories. For example, automated slide imaging can translate into faster, more accurate results and expedited clinical decision-making, resulting in better patient care. The reduction in manual labor required to handle and distribute slides for remote consultations improves process and cost efficiency. Importantly, digital pathology creates more possibilities for collaboration with colleagues at different locations, both in real time and asynchronously. This makes it much easier to invite second opinions and provide timely access to specialty expertise throughout the world, helping to ensure the highest standard of care.

Beyond operational advantages, digital pathology also holds promise for clinical decision support in a world where artificial intelligence (AI) and computational pathology are becoming increasingly useful and accepted tools within healthcare.

Developing and implementing a digital pathology strategy

When implemented properly, digital pathology has great potential to add value for a healthcare institution through data integration, cost efficiency, and improvements in quality and service. However, digital pathology implementation is complex, and, in most cases, full digitization across all operations and use cases cannot feasibly be achieved in a big bang approach. An important first step is to formulate a strategy based on current and future organizational needs, the regulatory environment, an assessment of resources — expertise, infrastructure, financial — and a vision for the outcome, including workflow metrics.

Defining digital pathology for your organization

Digital pathology has different connotations for different enterprises. To create a manageable, scalable plan, begin by defining digital pathology for your organization and establishing a common vision for what it might encompass. Digital pathology, while oftentimes considered synonymous with whole slide imaging or slide scanning, may include other pathology imaging modalities, such as gross imaging, electron microscopy and telepathology. Determining which of these modalities are needed to best meet laboratory and organizational goals is an important first step. Even within a given modality, laboratories must decide which specific technologies and functions to implement in order to support intended and potential use cases. For example, WSI technology comprises not just the slide scanner, but also the software applications and server hardware to manage and display images, and in some cases, algorithms to assist the pathologist or technologist with image interpretation. There are no universally correct answers, and decisions should be based on your organization's unique needs, goals, and expectations.

At this early stage, it is also important to determine where you will incorporate slide scanning into the laboratory workflow: before microscopic analysis, after sign-out but before archiving, on demand after archiving, or maybe a combination of these options? The right answer will vary according to specific circumstances.

A multidisciplinary team

Successful implementation of digital pathology will require many different types of expertise, including informatics, IT, quality, and clinical laboratory proficiency regarding the day-to-day operations of an anatomic pathology lab. Effective com-

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munication and change management skills are critical, as are input and buy-in from key stakeholders including pathologists, laboratory staff, administration and finance. A steering committee of representative stakeholders can drive the process of enlisting the right expertise.

Regulatory environment

Digital pathology is an emerging field, which means guidelines for clinical use will continue to evolve at local, state, and federal levels. A solid understanding of these requirements at all levels is key. Useful reference materials are available; one example is from the College of American Pathologists (CAP) Whole Slide Imaging Validation Guideline Expert Panel.[3]

Prioritizing initial use cases

The overall goal for implementation of digital pathology will drive selection of initial use cases. During the COVID-19 pandemic, the primary use case driving adoption of digital pathology has been routine WSI for the purposes of remote pathology. This is a logical starting point. If a scaled implementation of a specific use case (e.g., WSI) is preferred, one potentially effective strategy is to start with lower volume anatomic pathology services (e.g., autopsy, renal, transplant, pediatrics) to establish standard operating procedures and resolve preliminary issues, with the goal of onboarding higher volume services over time (e.g., genitourinary, gastrointestinal).

Starting with only WSI will make sense for some; others may choose to invest early in digitizing a broader range of imaging modalities. Other use cases (remote sign-out of intraoperative frozen sections, remote consultations and second opinions, tumor boards, image analysis) can also add value and be considered for early or future implementation. Alternatively, if a major goal is to improve clinical efficiency through the use of image analysis and/or artificial intelligence algorithms, a lab may want to consider the time required for various tasks in pathology to pinpoint where the greatest efficiency improvements are possible.

Infrastructure needs — now and for the future

The next step is building the required laboratory and IT infrastructure for digital pathology, which involves a range of functional considerations that should ultimately align with your defined use cases. Among the key elements of the infrastructure that you will need to evaluate are scanners, storage, and network speed.

A first consideration in evaluating scanners is throughput, with high throughput scanners outputting 60–100 slides per hour and allowing for continuous scanning at a 40x equivalent magnification. Scanners also differ by capacity, which refers to the number of slides an instrument can hold at once. For example, high-capacity scanners can batch anywhere from 100 to 1000 slides per batch. However, keep in mind that the greater a scanner's batch capacity, the larger the physical footprint and capital investment will be for your laboratory. A third consideration is ease of use, or level of automation (e.g., automatic tissue detection) to enable lab staff to load slides and walk away. A final consideration is the extent to which a scanner will support positive patient identification. With barcoded slides and LIS interface/integration, lab staff can match slides to patient records with ease and accuracy. Ultimately, your specific digital pathology plan will guide your selection process for scanning technology that meets immediate needs and offers versatility to scale over time. Plans for more diverse use cases may require an assortment of scanners.

Then there is data storage. Expect your data storage needs to be higher than you originally anticipated. Current general estimates are 1.25 GB per slide at 40x equivalent magnification, doubled for system redundancy. Determine your initial storage needs, followed by the subsequent increases that will occur as you bring new services online. Starting small and building up allows you to detect and resolve issues with minimal impact on laboratory and digital pathology operations overall. Laboratories may reduce storage costs by selecting storage options appropriate for their needs. For example, a laboratory might include active and recently signed out cases in readily accessible but higher cost "hot" storage and then later move older cases for archiving using lower cost, but less rapidly accessed "cold" storage. Choose a data management strategy to meet your storage, security, maintenance, and networking needs.

Planning for the appropriate network speed also is important. Digital pathology consumes substantial bandwidth, typically requiring 1–10 gigabits per second (Gbps) with room for higher performance to support future growth. If the ideal network speeds are not available, discuss with your IT department the possibility of upgrading your network. Suboptimal network speeds will still allow some applications of digital pathology, but not all. For example, images can be downloaded locally or accessed from external storage media (e.g., flash drives) prior to review, obviating the need for fast network connections. Even if not ideal, such workarounds could still support uses as occasional second opinions.

Moving forward

As you identify your digital pathology strategy and key investment needs, an important final step is developing a comprehensive and realistic budget. It should take into account the costs of system components, including storage and networking, required to meet critical performance expectations, such as promised turnaround times and improvement goals. It is equally important to garner pathologists' buy-in and earn their advocacy, starting early in the process and with the guidance of the steering committee. There is no universal path forward. Each organization will encounter its own challenges, pitfalls and burdens, but a clear, data-driven strategy for implementation will guide your organization into the future of digital pathology, with the potential to add value through data integration, cost savings, and improvements in quality and service.

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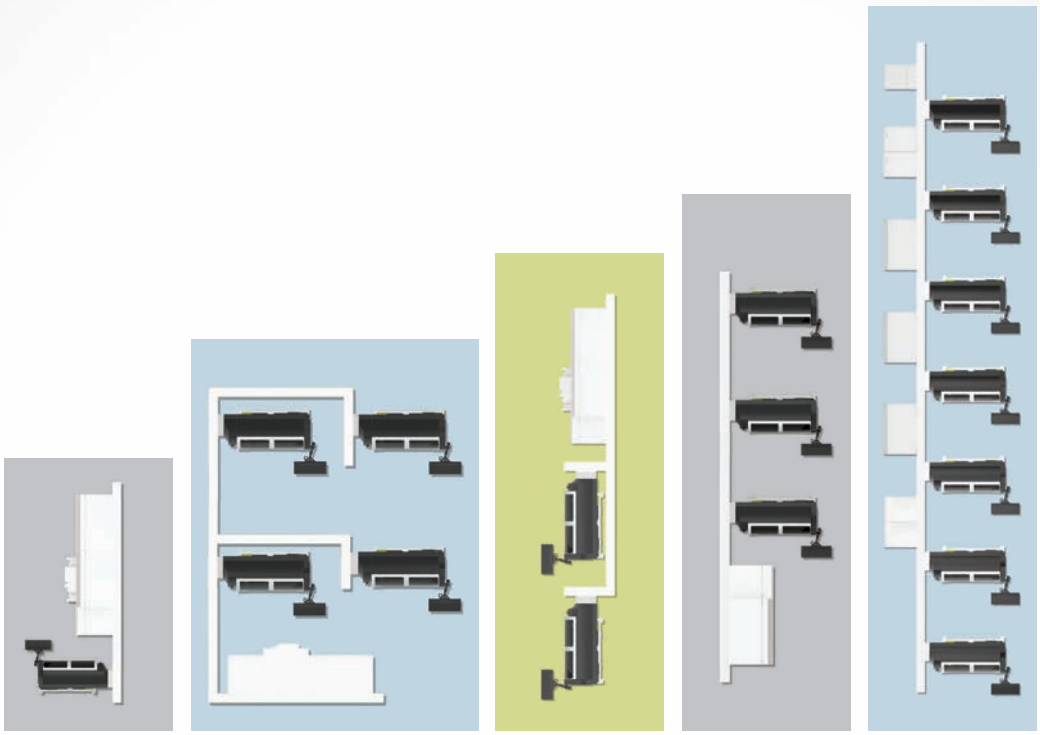

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The role of modern laboratory diagnostics in supporting clinicians' decision-making

Raj Gopalan MD, MSIS

In an age when laboratory testing has become incredibly accurate through use of advanced analyzers and highly automated using robotics and cutting-edge information technology, it is a surprise to learn that the interpretation of lab test results remains prone to human error and variation.

Clinical laboratories have been an integral part of healthcare delivery for many years, and the Centers for Disease Control and Prevention (CDC) states that 70% of today's medical decisions depend on laboratory test results, with more than 14 billion tests ordered every year.[1] Today's modern laboratories are equipped to perform huge volumes of tests with great accuracy and consistency in an extremely short turnaround time. However, appropriate test selection and final interpretation of results continue to be done by clinicians using a manual cognitive process.[2] Moreover, medical students in some institutions today spend as little as 12 hours learning about laboratory medicine over the course of a four-year curriculum.[3] It is no surprise that diagnostic errors account for about 20% of all diagnoses and result in billions of dollars of malpractice claims each year.[4,5]

The astronomical cost of healthcare is pushing the system to the verge of collapse. Do clinical laboratories have a role in controlling skyrocketing healthcare costs in a post-COVID era? Can they help improve individual and population health by enabling early and accurate disease identification, clinical diagnosis, and treatment?[6]

Brain-to-brain loop concept

The brain-to-brain loop concept in the laboratory testing cycle starts with the cognitive component of test ordering, followed by pre-analytic, analytic, and post-analytic phases, and ending in a final cognitive phase of results interpretation by clinicians.[7] The cognitive phase depends on the clinician's training, experience, and diagnostic skills to determine the right diagnosis for the patient based on history, signs, symptoms, and physical examination. After a list of provisional differential diagnoses is formulated, clinicians must tap into their knowledge of laboratory medicine to choose the appropriate set of tests to help rule in and rule out the diagnoses on the list. This process can be challenging. After the test is ordered, the pre-analytical, analytical, and post-analytical phases follow. These phases consist of sample collection, labeling, transport, quality assessment, processing, analysis, validation, and reporting. These phases are predominantly automated using high-precision instruments, robotics, and information technology; thus, they are reasonably consistent and reproducible, even across multiple laboratories. After this thorough process of reporting results, clinicians still face the challenge of interpreting them.

As the number of laboratory tests available for physicians to order has increased to more than 4,000, and when multiple panels of tests are ordered for a patient, each producing numerous individual test results, the interpretation process becomes problematic and challenging. The human brain is poor at metacognition, and intuition cannot scale to solve a multidimensional data problem.[2]

Information technology today has the potential to ease some of these challenges. We have seen its impact on other industries, and certainly laboratory diagnostics is no exception to this trend. Over the last 40 years, the laboratory has transformed from using predominantly manual processes to automated operations. Modern laboratories can process and analyze thousands of samples per day with the utmost efficiency and accuracy. In most laboratories, the result review and reporting process is semiautomated through autoverification, freeing up laboratory staff to focus on exceptions, where their expertise is needed. These advances have reduced turnaround time and greatly increased the accuracy and consistency of laboratory testing.

Even though information technology has had a positive impact on the pre-analytic, analytic, and post analytic phases, it has not made much progress in supporting the manual cognitive processes used by clinicians in ordering tests and interpreting results.

Shortcomings with reference intervals

Clinical laboratories report reference intervals together with laboratory test results to help distinguish health versus disease. While the establishment and use of reference ranges has evolved in some institutions, many still rely on the traditional ranges established more than 50 years ago.[8] These original reference intervals were determined by establishing small groups consisting of 100 individuals drawn from a reference group and deemed as being healthy. These groups were further categorized by gender, age, and a self-declared health status determined by responding to a questionnaire. After a physical exam, group members provided blood samples for analysis, which produced individual results that varied based on demographics, ethnicity, and race. These test results were plotted using a Gaussian distribution, and the range was calculated based on the results that fell within two standard deviations. These reference intervals have been in use for over 50 years, but often, individual test results that fall on the edges of the reference intervals are ambiguous in determining health versus disease. Therefore, the combined indication of multiple test results must be factored in when determining the status of the patient. This process is cognitively challenging for a clinician.

Even though clinical laboratories have transformed over the years in terms of efficiency, accuracy, and consistency, their role in delivering decision support to physicians has not expanded much beyond providing the reference intervals and highlighting the results that fall outside of those intervals.[9] This certainly has helped clinicians in quickly scanning a large number of results and picking the abnormal ones for further review. The biochemical process of any disease is a complex mechanism, and the early signs of a majority of diseases are evident by changes in blood parameters that occur way before the first symptoms appear. Changes in the parameters of commonly ordered routine blood panels can be a clue to underlying disease processes and provide an opportunity to order specific tests to further confirm or rule out potential diseases.

Some other common capabilities of laboratory-enabled clinical decision support include functions such as reflex testing to automatically order additional relevant tests based on abnormal lab results. Delta-checks are also commonly used to help identify a potential change in a patient's health based on a percentage or absolute-value change in specific lab values for blood work done over a period of time. For example, a snapshot of a hemoglobin value of 12 mg/dL may be considered normal for an adult female. But a trend of 15 mg/dL down to 12 mg/dL could indicate internal bleeding. Such trends can be more useful than a snapshot, where an abnormal test result may still be regarded as normal as long as the trends are in a desirable direction. Potential problems can be flagged by the laboratory through use of delta-checks.

Although these capabilities offer definitive value to clinicians, they are basic compared to those that laboratories potentially can offer. In recent times, and especially with the advent of molecular and genetic testing, the number of choices for laboratory testing has skyrocketed. The Choosing Wisely initiative started by the American Board of Internal Medicine (ABIM) to help clinicians order the appropriate tests is endorsed by more than 70 medical specialties. The goal of this initiative is to improve the safety and quality of patient care and reduce harm.[10] With the advent of electronic medical records (EMR), institutional laboratories have access to the initial, provisional diagnoses documented by clinicians. It seems logical that a future service provided by the laboratory would include recommendation of an optimal test battery that could be used to help to rule in the most probable diagnosis and rule out the unlikely ones. In addition, it may be possible to rank-order tests that are less-expensive and covered by the patient's insurance.

AI's value in disease diagnosis

There are also opportunities for labs to support clinicians during the final phase of the brain-to-brain loop, when the test results are reported back to the physician for interpretation. Artificial intelligence and machine learning (AI/ML) technology can be used in this phase to help interpret multidimensional test results. Based on the patient's age, gender, comorbidities, and current presentation, AI/ML technology has the potential to compare the results of the various analytes and provide probability scores that can be used to help rule in or rule out the potential candidates for diagnosis. Another approach may include providing a list of the most likely diseases the patient may have based on the combined evaluation of the patient's lab test results compared to a large population of confirmed diagnosed cases.[11] These opportunities could expand the value that laboratories currently provide to physicians to improve clinical decision support and help rule in or rule out diagnoses with much greater confidence.

Another important role of laboratory medicine is providing clinicians with information to choose the appropriate therapy based on the clinical status of the patient. A prime example includes testing to assess kidney and liver function, then monitor therapy for effectiveness and toxicity. Clinical decision support (CDS) can play a crucial role in tracking blood results and trends to monitor organ functions, thereby ensuring appropriate dosing to maintain patients within the most effective therapeutic spectrum. Thus, CDS creates the opportunity to pull data from multiple modalities, IVD, imaging, and the EMR, integrate that data, and present it visually to the multispecialty care team to enable comprehensive assessment of patients for optimal management decisions.

As we enter the post-COVID era, it is imperative to expand the traditional role of the clinical laboratory beyond its four walls to further benefit hospital units and clinics. In addition to the decision-support information and services the laboratory can provide, there is also opportunity for a more-direct engagement by laboratory specialists. Laboratory directors who are trained physicians specializing in laboratory medicine have a unique role in working with other clinical specialists on care teams to evaluate, diagnose, and treat patients. With the advent of the EMR system and data interchange standards, laboratory directors can access complete medical data of patients and have the expertise to help clinicians with ordering appropriate tests, interpreting results, and guiding further investigations.

In closing, the laboratory is very well positioned to expand the value it offers to clinicians. Advanced information technology and data science tools, such as AI/ML, have a tremendous potential to help identify disease processes at the very early stages of routine blood examinations. This could have a tremendous impact on population health and disease prevention. It could also support improved use of appropriate testing, interpretation, diagnosis, treatment, and therapeutic monitoring. AI/ML-enabled clinical decision support has the potential to propel the modern clinical laboratory to the forefront of diagnostic modalities and, thus, support appropriate intervention to keep the population at larger healthy. Therefore, the crucial role of the clinical laboratory in reducing healthcare costs and preventing the system from eventual collapse cannot be ignored.

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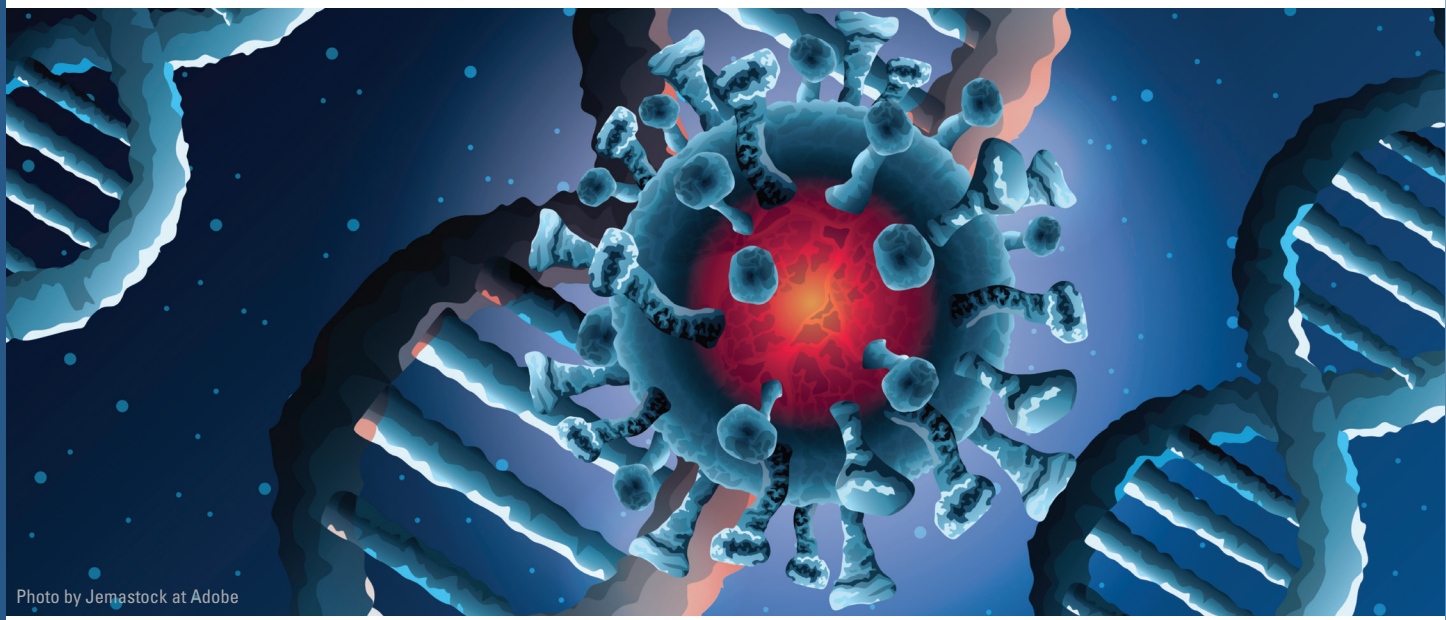

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State of the Industry for molecular diagnostics in 2021

By Marisa L. Williams

In the year that has passed since the first State of the Industry (SOI) on molecular diagnostics, a survey that *Medical Laboratory Observer* sent out to its readership of laboratory professionals, there have been many changes. With the influence of the pandemic, *MLO* asked laboratorians if they were still responding to supply shortages, had purchased new equipment, or made any relevant changes in the area of molecular diagnostics in the lab.

The results of this year's SOI are in, and while various shortages surprised healthcare professionals, it seems the pandemic fueled innovation, as the U.S. Food and Drug Administration (FDA) granted emergency use authorizations (EUAs) to a number of tests that hit the market. Labs received training, and in some cases, reverted back to manual methods when automated components ran short.

COORDINATING TESTING SOLUTIONS

The demand for technology increased during the pandemic, especially for tracking the virus and variants, with labs across the globe sharing their viral data. When labs were asked how their facility handled reporting COVID-19 test results to the various authorities, 69% said they use specific software for reporting results, while 30% send aggregate test results with only the data requested, and 15% send aggregate test results with all data to be reviewed.

Only half of labs surveyed were tracking COVID-19 results. Of the labs tracking the virus, 26% were only tracking positive and negative COVID-19 test results, 12% had another facility tracking their results, 8% were tracking COVID-19 using genomic sequencing to monitor variants, 3% used to track COVID-19 but have since stopped tracking the virus, and only 1% were tracking COVID-19 and variants.

Tammi Ranalli, PhD, Senior Vice President of Molecular Diagnostics at Quidel, described the pandemic's impact behind the scenes in clinical labs. She saw struggles to implement solutions for COVID-19 testing and the race to bring in real-time PCR, observing how lab employees who were using kits with a pipette instead of automation for extraction had a bit

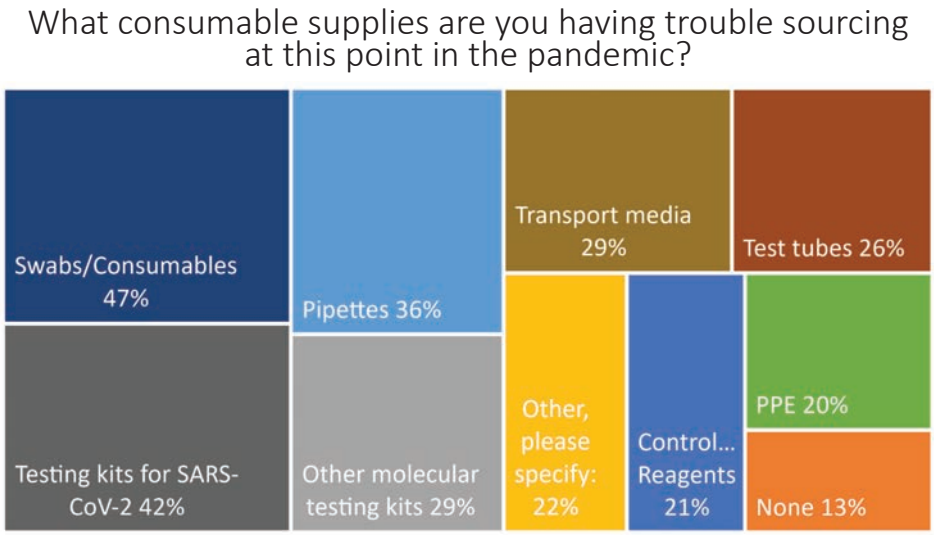

Chart 1: SOI survey results show what percent of labs had shortages of specific products.

of a learning curve, noting some people had to relearn what they had previously abandoned. With lab personnel being accustomed to automated testing processes, "it took more time for training to get reacquainted with how to run the assays, until they became second nature." She explained that the pandemic, "reinvigorated the use of assay kits," for both COVID-19 and other diseases.


Tammi Ranalli

During the months when labs thought COVID-19 might be starting to wane, Ranalli remarked how many labs were expanding in-house testing options with the equipment purchased for COVID-19.

When labs were asked if they had excess capacity with analyzers that they had purchased to handle COVID-19 testing, results were split, with 47% replying yes, and 53% replying no.

Brigitte Fernandes, Vice President of Molecular Diagnostics at Roche Diagnostics Corporation, said, "Laboratory leaders now have the opportunity to maximize newly acquired resources and equipment to offer more testing-menu (options) for patients. There are a lot of unknowns with COVID, but what is known is that patients still need access to routine screening. Expanding on available testing capacity put in place for COVID-19 could allow institutions to expand their testing services."


Brigitte Fernandes

Of the respondents who said they had excess capacity in analyzers purchased for the pandemic, more than half, 51%, planned to add new tests in-house, and 12% wanted to retire some analyzers. "Other" responses included donating the equipment to a mission, reallocating it to other departments, or using it for testing at schools and events. Some respondents simply planned to remain prepared, a smart move after new variants caused an influx of additional testing.

Ranalli, familiar with the seasonal changes and challenges in diagnostics, especially during respiratory season, discussed how some products, such as flu assays, will fluctuate in demand with the seasons, sometimes unpredictably, such as with the Delta variant.

TRAINING DURING THE PANDEMIC

As social distancing during the pandemic influenced training, Ranalli shared how many field specialists turned to training via Zoom or video chat, before they were able to go to facilities in person. Charting unpredictable territory with unexpected waves of changing needs and supply availability issues, labs turned to technology for troubleshooting and training, she said.

On-demand videos helped labs with staff turnover, or when the third shift was not getting the same training as the morning shift. "This opened the channel for communication, refresher courses online and recorded videos, as some labs were using skills they had not used in a while, sometimes out of necessity due to shortages or other issues." Ranalli compared it to "students getting out of school for the summer and needing a refresher in the fall."

SUPPLY SHORTAGES IN THE LAB

The SOI survey also asked if labs had issues maintaining a supply of testing products for COVID-19. More than half, 58%, replied yes, and 42% replied no, they have adequate supply. As for non-COVID-19 products, 70% said they are

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- Of particular interest in the study, StatSensor showed better accuracy than the laboratory Jaffe methodology in the 60-89 mL min/1.73 m^2 range, where individuals with early disease may benefit from renal protective measures.[1]

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1.George J et al. Evaluating chronic kidney disease in rural South Africa: comparing estimated glomerular filtration rate using point of care to iohexol measured GFR. CCLM 2021.

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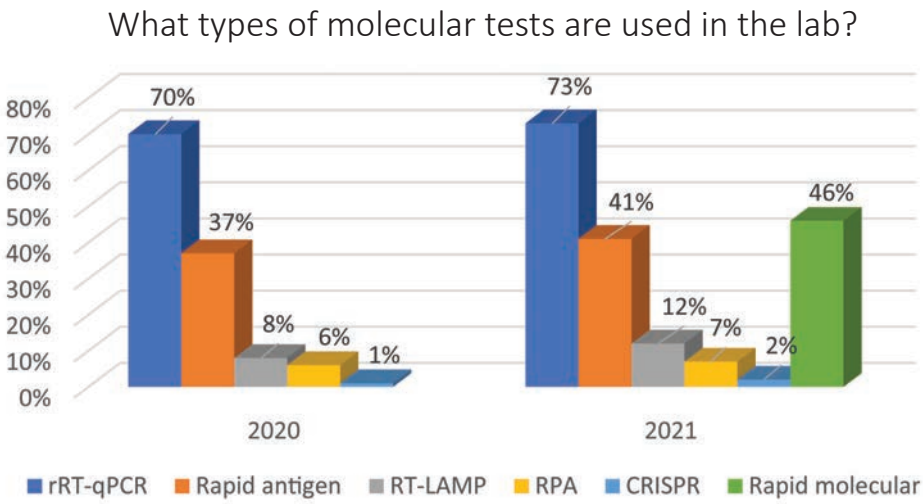


Chart 2: SOI survey results reveal the percentage of labs using various molecular diagnostic testing.

lacking, while 30% said no, supply is adequate.

Swabs and consumables topped the list of supplies hard to secure, with 47% of labs feeling the shortage. Testing kits for SARS-CoV-2 came in at a close second, 42%; followed by pipettes, 36%; molecular tests, 29%; transport media, 29%; test tubes, 26%; controls/reagents, 21%; and personal protective equipment (PPE), 20%.

Labs listed "other" responses: hematology and media items, histology supplies, serology kits, cryovials, syringes, pipette tips and plastics, like bottles; 13% of labs reported no shortages.

The SOI survey revealed 87% of labs had shortages in 2020. Test kits topped the list,76%; swabs and consumables, 74%; transport media, 66%; PPE shortages, 44%; and controls/reagents, 32%.

TEST CHOICE BASED ON AVAILABILITY

The *MLO* SOI survey also asked labs which of the common methodologies they used for COVID-19 testing; 84% responded they only used commercial test kits with an EUA, while 9% used lab-developed tests, and 7% used a combination of EUA and lab-developed tests.

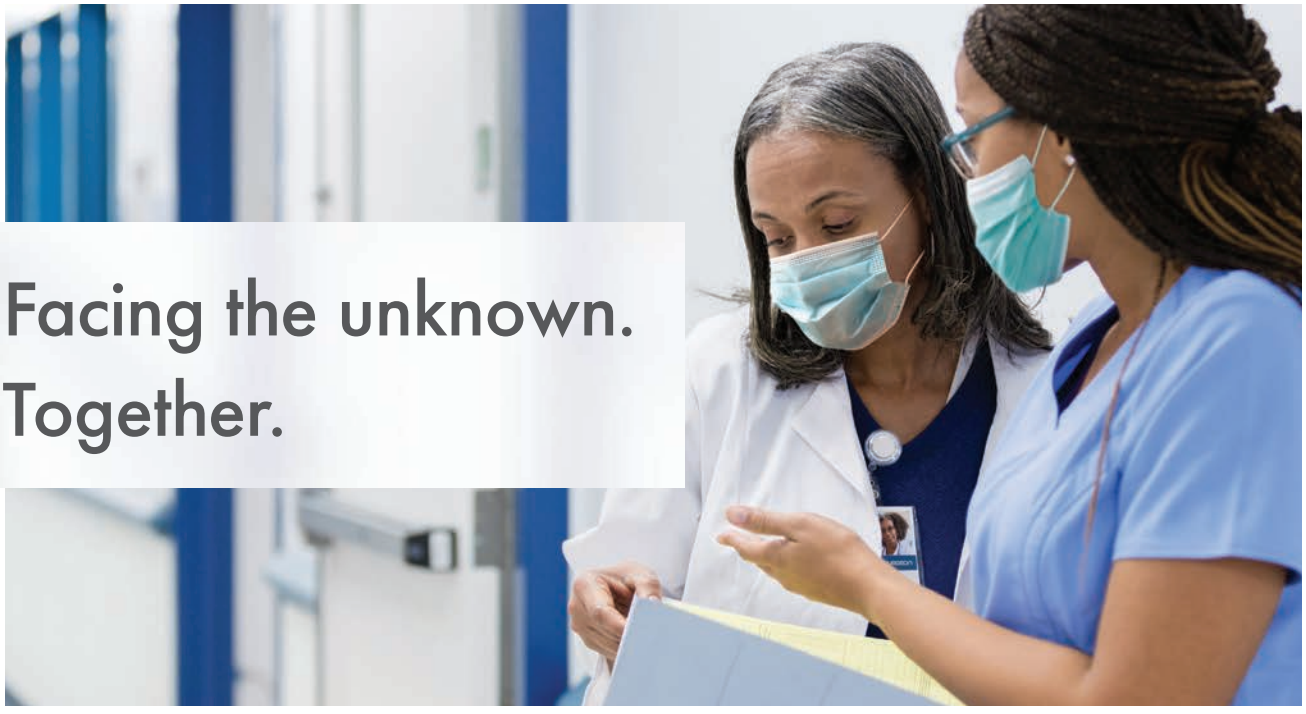
When asked why they chose a particular method, 42% said it was due to test availability, while 34% looked at accuracy and reliability, 9% considered the cost of the test, and 6% considered turnaround time (TAT). Other lab responders said they already had analyzers in place, some were provider-driven choices, and others had an open platform.

DIAGNOSING AT THE MOLECULAR LEVEL

When polled about the types of molecular diagnostic tests used by labs to detect COVID-19, 73% said they used reverse transcriptase quantitative polymerase chain reaction (rRT-qPCR). Rapid molecular tests were next at 46%, followed by rapid antigen tests, 41%; reverse transcription loop-mediated isothermal amplification (RT-LAMP), 12%; recombinase polymerase amplification (RPA), 7%; and CRISPR-based diagnostics, 2%.

Although it was not an option on the survey, Bradley Hart, Senior Director of Clinical Research Marketing, Thermo Fisher Scientific, touted mass spectrometry. "During the past 18 months, there has been a tidal wave of interest to deploy mass spectrometry (MS) towards COVID-19 testing."

He added, "Mass spectrometry has been



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heavily pursued as an alternative supply chain to PCR, which was stretched at the height of the pandemic. Since COVID-19 testing reduced the usage of those instruments for their traditional tests, a capacity opportunity presented itself for the increase in its utilization. COVID MS-based assays were primarily focused on the identification of the nucleocapsid protein; with proteins being stable analytes, this makes for an attractive attribute for a robust clinical assay." Preparation costs are similar to PCR reagents.


Bradley Hart

IMPROVING TEST QUALITY

With new technologies and when testing high volumes, errors can happen. How do labs handle questionable results with molecular tests? A majority, or 62%, of this year's facilities opt to repeat the test with either a different employee, equipment, or test. That is an increase of 5% over 2020's survey results. This year, 19% said they send results to another lab for verification and a second test, a decrease from last year's 26%; 11% verify the procedure followed was correct, up from 7% previously; and 4% check for analyzer operational issues, same as last year.

Describing the steps taken to reduce the number of potential false positive test results, more people this year, 45%, verify all pre-analysis steps are performed correctly than last year's 29%. Not as many, 20%, repeat the test with another method and compare results, compared to last year's 29%; 17% repeat the test with the same sample and new extractions, not far off from last year's 16%; and 15% do nothing, down from 21% last year. Others responded that they use a combination of these options.

Fernandes said that the best way to reduce errors is to automate testing processes. "Pre-analytical sample processing represents one of the most problematic areas in molecular laboratory testing," explained Fernandes. "Highly skilled technicians perform repetitive manual tasks, like vortexing, decapping, and labeling tubes. These time-intensive, manual steps not only cause bottlenecks, but they increase the opportunity for human error and cross-contamination, with up to 75% of all laboratory error occurs during this phase. By automating these manual processes,[1] labs have the potential to eliminate nearly 100% of human errors and could realize ~33% reduction in process steps, which allows highly-trained staff to accomplish more, focus on high medical value projects, and provide more balance to improve morale and job satisfaction."

Donna McGowan, Marketing Manager of Indigo BioAutomation, pointed out, "No matter what instrument is in operation, the data release bottleneck is oftentimes the data. Although assays are built and validated on clean and well resolved peaks, sometimes the samples aren't clean." Algorithms and machine-learning have advanced accuracy, accelerating the release of results, managing sensitivity and specificity.

MOLECULAR USED IN THE LAB

MLO also asked labs about non-PCR diagnostic technologies that they use in their molecular operations. Almost one-third, or 30%, said they use DNA and genetic testing, 22% use next generation sequencing (NGS), 12% use flow cytometry, and 6% of labs use liquid biopsies to screen for cancer genes with bodily fluids. More than half of labs either did not use the indicated technologies or used only PCR testing, while other labs mentioned using Sanger sequencing, as well as RNA analysis.

PURCHASING MDX

"When planning to expand with new or more molecular assays, it's important for labs to reach out to the assay vendor to make sure they can provide clinical evidence, educational and training material," advised Nikos Pavlidis, the Vice President, General Manager of Molecular Diagnostics and Women's Health & Cancer at Becton, Dickinson and Company (BD). "This will help you to better communicate to your customers, department and financial decision-makers the importance and value of the new assay, as well as make clear that your team will be fully prepared to collect samples appropriately and interpret the new results."


Nikos Pavlidis

Noting the challenges with supply chain, Pavlidis suggested, "Before making a choice, make sure that the assay/instrument manufacturer has control and will be able to provide

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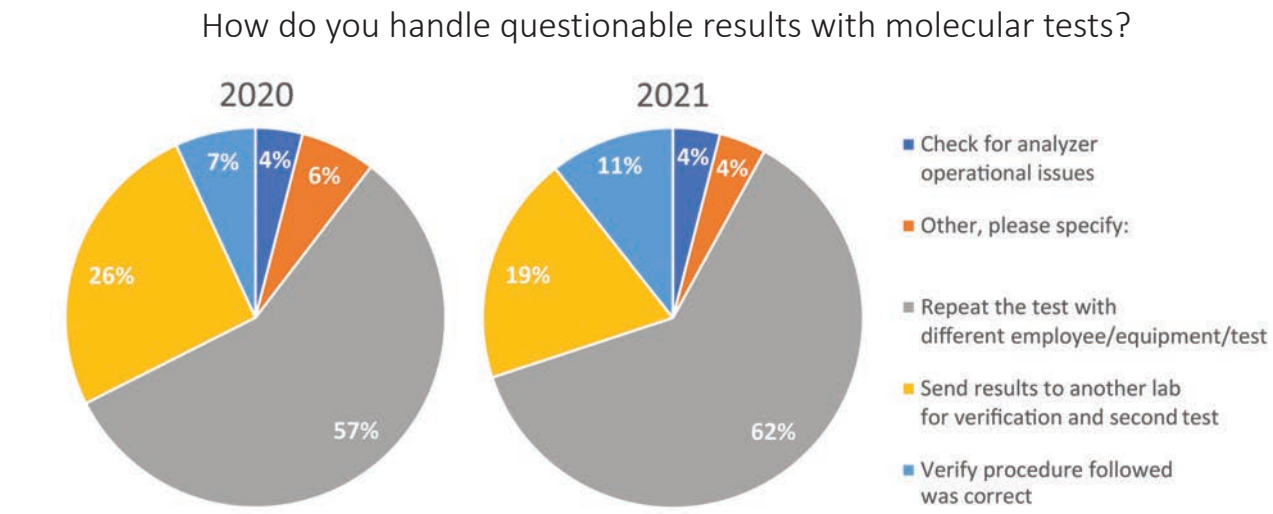

Chart 3: The MLO SOI survey asked labs how they handle questionable results with molecular tests.

the reagents, consumables, instrumentation and services required to maintain and support testing in your lab in a timely manner."

He pointed to collection devices, pipetting tips or other consumables that the lab might need to source from a different supplier. "Having access to an all-inclusive consumable/assay source and a single, fully-automated molecular platform manufactured and serviced by the same vendor could prove to be a benefit in terms of both time and cost."

THE FUTURE OF MDX

Molecular diagnostics drives innovation in a number of diseases. Ned Patterson, President and CEO of Anavasi Diagnostics, explained how multiple genome targets may increase accuracy of COVID-19 variant testing and advances in HIV testing include determining which strain for the best treatment, a growing area of MDX.

"Molecular testing was already on the rise prior to SARS-CoV2 and addressing the pandemic has pushed its growth and rapid adoption even further," said Kim Futrell, MT(ASCP), MSHI, Senior Strategic Marketing Manager, Orchard Software. "Providing timely, highly accurate test results help providers gain insights to patients at the molecular level; that is exactly what we need as the next step toward improving outcomes."

"Consumers have more awareness than before about molecular tests," Ranalli said. "The average person talks PCR and molecular, knowing the differences between molecular, antigen and antibody testing." She hopes the public awareness during the pandemic will inspire career choices in the younger generation.

"Working with inexperienced labs, helping labs grow to plan out the addition of molecular diagnostics in the lab, it was a collaborative effort that brought awareness to virology and better stewardship." She predicted there may soon be a need for long-term COVID-19 testing, post-acute, especially for the immunocompromised.

Hart added that offerings of molecular diagnostics will continue to expand. "We can imagine a single analysis for the detection and confirmation of the common cold, influenza variants, COVID and even tuberculosis. We expect to continue to develop and expand protein assays towards the clinic at capillary and analytical flow rates as fully integrated workflows and even toward rapid analyzers. We expect also to see mass spectrometers that can be deployed closer to the patient. Finally, we truly see infectious disease identification and confirmation as a critical opportunity for the community as we move closer towards making the world a healthier, cleaner and safer place."

Roche's Fernandes said, "Healthcare was experiencing unprecedented change and disruption already, which has been further accelerated by the COVID-19 pandemic. What is expected of healthcare organizations is shifting from delivering services to achieving improved patient outcomes, while reducing costs."

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Achieving equal detection for all patients in cervical cancer screening

By D.Yitz Goldstein, MD

Cervical cancer screening is undeniably one of the most successful medical screening programs ever devised. The ability to identify and treat precancerous lesions through Pap testing has led to a declining incidence in the United States over the past 50 years.[1] Armed with the scientific understanding of the natural history of HPV infection and the strategic guidance of our medical communities, the goal of cervical cancer elimination seems almost attainable.

The addition of high-risk HPV (hrHPV) testing to cytologic sampling has unquestionably ushered in a new age of cervical cancer screening with their combined clinical sensitivity being superior to either Pap or hrHPV testing alone.[2–7] Recently, there have been major changes to guidelines presented by various organizations in their suggested use of hrHPV testing as part of an overhaul of cervical cancer screening and management in the United States.[8–10] These organizations are to be applauded for their efforts and their comprehensive evaluations. However, it remains important to critically assess their findings and conclusions to determine whether their guidance will have a meaningful impact to further reduce the burden of cervical disease in the United States.

Risk-based approach to screening

In 2019, the American Society for Colposcopy and Cervical Pathology (ASCCP) updated its management guidelines to provide greater focus on treating patients based on their risk of disease progression instead of an algorithm based solely on individual test results.[8] To this end, any patient whose current test results and testing history places her risk of having a CIN3+ lesion on a biopsy at 4% or greater should be referred for colposcopic evaluation. Treating individual patient risk and not specific test results ensures that our understanding of the natural history of cervical precancer and HPV clearance rates gets incorporated into a holistic patient assessment based on all available data. The ASCCP risk calculations were developed based on a mathematical model formed from over ten years of data collected from Kaiser Permanente Northern California (KPNC).[11] The population in this integrated healthcare system is generally more closely screened, resulting in lower cervical cancer rates than the broader U.S.[12] Additionally, the patient demographic and socioeconomic breakdowns at KPNC are not wholly representative of the entire country.[13]

The new ASCCP guidelines recognize the importance of differentiating risk based on whether patients have known or unknown screening histories. However, the risk estimates for patients with poor screening history were developed using a KPNC patient's first historical result.[11] This means that the risk estimates associated with never or rarely screened groups were not developed to align with an inadequately screened population but simply a well screened population without preexisting data.

Disparities in data selection

It is not surprising, therefore, that when the KPNC risk estimations were validated for general applicability using three external data sets — namely the Centers for Disease Control and Prevention's (CDC) National Breast and Cervical Cancer Early Detection Program (NBCCEDP), the Roche ATHENA trial and the BD Onclarity trial — it was found that all three external datasets demonstrated significantly higher immediate CIN3+ rates than KPNC. Additionally, the NBCCEDP data, which includes a greater proportion of higher risk patients, found the immediate CIN3+ rate in the poorly screened cohort to be almost three times that of the KPNC data.[7]

The ASCCP acknowledged that there were scenarios where risk discrepancies between the datasets would have provoked different management and changed the intended strategies in those situations. However, it should be clear that the published risk values underrepresent true risk even when compared to other well-structured clinical trials (ATHENA, Onclarity), and particularly as it relates to poorly screened patient populations (NBCCEDP). The practicing clinician should understand that the ASCCP estimated risks are not only produced by evaluation of the well screened KPNC cohort, but that those estimations remain mostly applicable to such a population.

Even accepting the KPNC risk estimates, there remain significant issues with recent guidance in cases where a clinician would come to different management decisions based on different screening strategies due to having either more or less information to assess a patient's risk. The ASCCP accepts a primary hrHPV approach and the updated American Cancer Society

	Screening Strategy	HPV History	HPV Current	Cytology	ASCCP Management	"CIN3+ Risk (KPNC)"	"CIN3+ Risk (NBCCEDP)"
47-year-old patient with documented screening history	Primary HPV	Negative	Negative	-	5 Year Follow Up	0.10%	-
	Cotesting	Negative	Negative	HSIL	Colposcopy	13.80%	33.30%
	Cotesting	Negative	Negative	ASC-H	Colposcopy	2.76%	5.16%
	Cotesting	Negative	Negative	AGC	Colposcopy	0.78%	3.07%
47-year-old patient without a known screening history	Primary HPV	Unknown	Negative	-	5 Year Follow Up	0.14%	-
	Cotesting	Unknown	Negative	HSIL	Colposcopy / Treatment	25.20%	45.10%
	Cotesting	Unknown	Negative	ASC-H	Colposcopy	3.42%	3.16%
	Cotesting	Unknown	Negative	AGC	Colposcopy	1.07%	5.56%

Table 1.

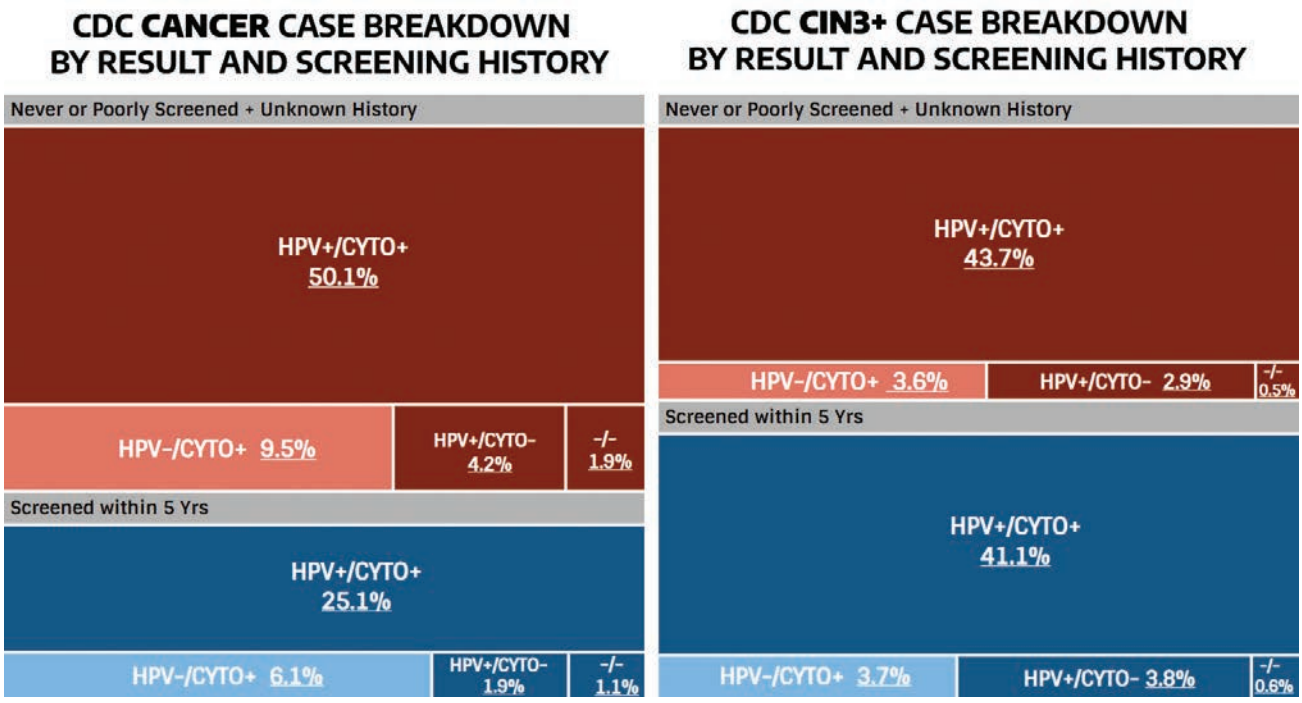

Figure 1.

(ACS) guidelines not only lay out a preference toward primary HPV screening but recommend that other methods of screening, including co-testing, be phased out of future guidelines.[9] However, the KPNC and NBCCEDP data themselves demonstrate that a primary hrHPV screening strategy simply cannot accurately estimate a patient's risk based on HPV testing alone.

For example, a 47-year-old patient with unknown screening history who presents for initial examination and has primary hrHPV screening that is found to be negative would be estimated to have an extremely low risk of immediate CIN3+ based on ASCCP published values, and the recommended management would be for the patient to return for follow-up in five years. However, if this patient had a Pap test that demonstrated either HSIL, ASC-H, or AGC, the risks in these instances would have been deemed by ASCCP as significant enough to recommend colposcopy **(Table 1)**.[11] This is due to the high specificity of the Pap test in these categories, and highlights that risk estimation and guidance is much more accurately assessed, if not entirely dependent, on the additional information obtained through the cytology result.

One might think that these instances of HPV negative cervical lesions constitute a small minority of CIN3+ disease overall, however, the data obtained by CDC's NBCCEDP,[7] published in support of the ASCCP, paint a starkly different picture. In the CDC data **(Figure 1)**, 3,033 instances of CIN3+ disease were identified, and 256 of them (8.4%) occurred in patients who were entirely HPV negative. Additionally, 67 of their 359 invasive cancers (18.7%) were HPV negative as well. In 87% of the HPV negative/CIN3+ cases, and 84% of the HPV negative/cancer cases the accompanying cytology would have prompted either a shortened follow-up period or a colposcopy, given the elevated patient risk based on the additional cytology result. This finding is not unique to the NBCCEDP, and the literature now recognizes that a significant proportion of cervical disease cases test negative for HPV.[3–5,14,15]

While the ACS concedes that there are some co-testing screening strategies that may lead to fewer cancer cases diagnosed and fewer cancer deaths as compared to primary HPV screening,[9] the authors contend that these marginal differences must be balanced against potential harms. The main harm discussed is a potential treatment-related adverse obstetric outcome. However, the cited literature associates this unquestionably concerning outcome not with the performance of cytology, or even a follow up colposcopy, but with procedures involving excisional or ablative treatment.[16] Such techniques would only be necessary following an already positive biopsy obtained through colposcopy and would not be a result of utilizing cytology as a co-test to more accurately assess risk on samples already collected for HPV testing. Additionally, the safety and tolerability of colposcopy has been well established in the literature, even when performed during pregnancy.[17]

The ACS goes on to list physical discomfort and anxiety associated with possible additional colposcopic examinations, which would not have occurred using HPV testing alone, as a potential harm against which their mathematically modeled marginal benefit must be weighed. While it is certain that patients who had a falsely negative HPV test would not have had a colposcopy performed in the absence of an abnormal cytology result, the entire concept of a risk-based approach for referral to colposcopy ensures that only those patients with a significant enough risk of immediate CIN3+ be referred for such a procedure. By definition, the ASCCP management guidelines refer a patient to colposcopy only when the immediate risks to a patient have reached a clinical action threshold that warrants such an evaluation. The risk to the patient is what prompts a colposcopy, not a cytology result, and these thresholds have been precisely determined by the ASCCP to identify levels at which the benefits outweigh the potential discomfort and anxiety associated with its performance.

Role of co-testing

Every major guideline issuing organization recognizes that the single most important factor to the success of a cervical cancer screening program is consistent adherence to an acceptable strategy.[9,18,19] Gaps in any individual testing strategy are compounded by a lack of regular attendance by the screening population. Unfortunately, while it might be convenient to utilize microsimulations from well screened populations for mathematical modeling, the reality is that the burden of this disease does not fall upon that population in the United States. It is

estimated by the NIH[20] that approximately one half of all cervical cancer cases occur in patients who were never screened. In the NBCCEDP data, patients with an unknown or poor screening history made up 42% of patients, yet 66% of all of the cancers were identified within this group.[7]

The realities of inadequate screening and their impact on various communities in the United States are not uniform. Race, ethnicity, and socioeconomic status have all been demonstrated to impact cervical cancer incidence and mortality.[21–23] Clinicians who care for diverse patient populations that have limited or infrequent access to care must be aware not only of the limitations with the current guidelines and the manner in which they were developed, but the real world risks they may pose to their patients as well. It is no wonder that the ACS guidance has prompted such firm responses from the Black Women's Health Imperative, the American Society for Clinical Pathology (ASCP) and the College of American Pathologists (CAP).[24–26]

Any successful screening strategy must ensure that it incorporates an understanding of the population at risk in addition to the benefits and pitfalls of any single testing strategy. The data from the NBCCEDP[7] and many others[3–6] continue to demonstrate that the use of cervical cytology in conjunction with hrHPV can provide an additive benefit for the identification of significant disease in HPV negative lesions. The Pap has provided us with some of the greatest successes in cancer screening in the last century and continues to be a valuable tool in the care of our patients.

While it might be tempting to dream about cervical cancer elimination in this country, the reality is much more sobering. This year, in the United States, more than 14,000 women will be diagnosed with cervical cancer and more than 4,000 will die of this preventable, screenable, and treatable disease.[27] Given the resources and technological advances in cancer screening in this country, each and every one of these lost lives highlights a systemic failure. As we strive for equitable healthcare, we must first recognize the existence of distinct healthcare disparities and how they can lead to incorporation of biases into guidance. To crusade for cervical cancer eradication is to advocate for all of our patients, and specifically the underprivileged who will shoulder the burden of this disease. To win this battle, we must provide these patients with the best opportunity for disease discovery through co-testing; otherwise we are simply exacerbating existing healthcare inequities and will continue to leave them behind.

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Automating hematology analysis

By Marisa L. Williams

Hematology analyzers vary in size with the ease of automation for precise assessment of blood, and in some analyzers, other bodily fluids as well.

When to upgrade

Labs may seek to upgrade based on the needs of the patient population, desire to upgrade to a newer version, to increase testing,or to automate from single sample to autoloader.

Technologies advance, but with budget limits, labs may keep and use instruments longer. Retaining older instruments risks parts and software availability, with increased maintenance. Newer technologies improve workflows, providing more ecologically friendly reagents, and clinically relevant parameters.

When purchasing, labs should consider cost per test, interface time, potential chemical exposure, and high first-pass yields to improve turnaround time.

The technical features

According to product descriptions, Sight and PixCell offer analyzers that combine artificial intelligence with single-use cartridges that do not require calibration or chemical exposure, designed for smaller-sized labs needing quick results.

To lessen exposure to toxic chemicals, Mindray offers cyanide-free reagents, as well as barcoded reagents for their analyzers.

Many hematology analyzers offer automated maintenance. Features vary, as Horiba's product description explains its patented technology, such as DHSS (Double hydrodynamic sequential system) technology for accurate cell-by-cell counting and MDSS (Multi-distribution sampling system) for precision pipetting with tangential flow for mixing of the dilution with the sample in the reaction chamber.

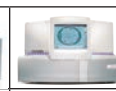
Like Horiba, Beckman Coulter offers analyzers of all sizes. Beckman Coulter's Monocyte Distribution Width (MDW) test is a hematology-based cellular biomarker designed to identify patients with severe infection leading to sepsis, reported automatically with routine complete blood count (CBC) with differential to count and identify the differnt types of white blood cells (WBC) on the DxH 900 and DxH 690T.

"The value of MDW," explained Elliott Crouser, MD, Professor of Critical Care Medicine, Ohio State University Wexler Medicial Center, in a Beckman Coulter testimonial, "is that it provides physicians with an earlier indication of sepsis."

To accompany analyzers

ELITECH's Aerospray Hematology Pro Slide Stainer and Cytocentrifuge pairs with CellaVision DC-1 and DM1200 for microprocessor-controlled slide staining and cell preparation. The product description details how the Cytopro Rotor accessory gives full-featured cytocentrifuge capability.

To accelerate the release of LC/MS and GC/MS results, ASCENT from Indigo BioAutomation is a software for preventing data release bottlenecks. When samples are not clean, ASCENT uses machine learning to resolve the peak management of cases near the baseline, suffering co-elution, or have lost sensitivity and specificity to distinguish the true peak signal within the chromatographic trace.

											
Brand and Model	**Beckman** DxH 560 AL	**Beckman** DxH 690T	**Beckman** DxH 900 Workcell	**Elitech** Aerospray Hematology Pro Slide Stainer/ Cytocentrifuge	**Horiba** ABX Micros ES60	**Horiba** ABX Pentra 60C	**Horiba** ABX Pentra XL80	**Mindray** BC-3600	**Mindray** BC-5390	**Sight** Sight OLO	**Pixcell** HemoScreen
Volume Lab	Small	Medium	Large	Medium	Small	Medium	Large	Small	Medium	Small	Small
Test/hr	Up to 60 samples/hr	Up to 100 samples/hr	100 - 300 samples/hr	>115 slides/hr	50 samples/ hr	Up to 60 samples/hr	80 samples/hr	60 test/hr	60 test/hr	6 samples/hr	10 tests/hr
Test menu	CBC with 5-part differential	CBC, differential, MDW, retic, body fluids	CBC, differential, MVP, PLT, retic, body fluids	Rapid Wright-Giemsa, Wright-Giemsa, May-Grünwald Giemsa, custom stain	16 parameters	20 parameters	20 parameters	CBC, CBC + differential	CBC, CBC + differential	CBC with 5-part differential, 19 parameters	CBC with 5-part differential
Tech	Coulter principle and flow cytometry with dynamic gating	Triple count Coulter principle, VCS flow cytometry with data fusion	Triple count Coulter principle, VCS flow cytometry with data fusion	Micro-processor-controlled	Impedance, photometry	Cyto-chemistry, impedance, optical, DHSS, MDSS	Cyto-chemistry, impedance, optical, DHSS, MDSS	Impedance method	Impedance, flow cytometry, laser scatter & chemistry dye methods	Fluorescent digital microscope and AI	Visco-elastic focusing and machine vision AI
Size	17.3" x 19.7" x 18.1"	32.45" x 29.75" x 36.6"	68.5" x 29.75" x 32.6"	22" x 10" x 21	16.9" x 14.2" x 14.2"	20.3" x 17.5" x 19"	21.5" x 32.3" x 22.4"	16x 18x 18"	22.4x 23.2x 20.6"	9.8" x 12.7" x 11.2"	7" x 10" x 12"
Weight	48.5 lbs	282 lbs	780 lbs	33.5 lbs	30.9 lbs	77 lbs	122 lbs	62 lb	143 lb	21.3 lbs	12 lbs

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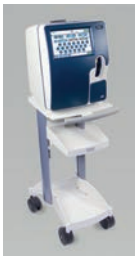

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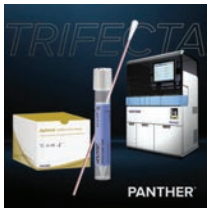

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LABORATORY INNOVATOR

Advancing knowledge about hemoglobinopathies

By Linda Wilson

Sean T. Campbell, PhD, is Assistant Professor of Pathology at Albert Einstein College of Medicine and Associate Director of the Clinical Chemistry Laboratory at Montefiore Medical Center. He sits on an advisory board for Abbott and has research funding from Sebia and Global Blood Therapeutics.

Based on your professional experience and research focus on coagulation assays, rare hemoglobin variants, sickle cell disease, and diabetes, what led you to your current position?

A lot of it was my work on coagulation and hemoglobin variants. The Bronx, NY, has one of the most diverse populations in the world, and with that comes interesting variants every week, as well as the very needed work to support my colleagues in our sickle cell clinics. For coagulation, former Hematology and Coagulation Director Morayma Reyes Gil, MD, PhD, is one of the foremost experts on coagulation. The chance to work with her and pursue research and work in hemoglobinopathies was too good to pass up.

Will you describe your role as the associate director of clinical chemistry at Montefiore?

I have moved from the hematology and coagulation laboratory to chemistry. Now, my clinical work is to ensure the smooth operation of the chemistry laboratories, ensure that all assays and instruments are operating as they should, and to decide what tests to offer to our clinicians. In addition, I also teach residents when they are on service and engage in research on sickle cell disease, hemoglobinopathies, and diabetes.

In the area of sickle cell disease, will you briefly describe the gene therapies now being tested to combat this disease?

There are three main techniques currently under investigation, all related to bone marrow transplantation. Bone marrow transplantation is already used in some cases to treat sickle cell disease by replacing much of the patient's erythropoietic stem cells with those from a healthy donor. This results in a phenotype closer to sickle cell trait, the asymptomatic carrier status. However, this carries all the usual risks of graft rejection. In contrast, editing techniques can be done on the patient's own stem cells, eliminating issues of rejection. Some gene editing techniques deliver a new, wild-type beta gene (or an 'improved' gene with anti-sickling properties) that can be expressed alongside the sickle mutated gene in the patient's stem cells, typically using lentiviral vectors. CRISPR techniques, conversely, focus on editing the chromosomes to reverse the disease. One way under investigation is to edit the sickle cell gene back to wild type, reversing the disease or changing them to a sickle trait phenotype. And a last technique involves editing the genes involved in the production of gamma globulin, the protein in fetal hemoglobin (HbF), with CRISPR. This would induce a phenotype similar to Hereditary Persistence of Fetal Hemoglobin, or HPFH, by increasing production of gamma and lowering production of the mutated beta gene, which can have a highly protective effect in sickle cell disease patients and also result in a sickle-trait like phenotype at high enough levels.

Have there been advances in diagnostic testing to monitor sickle cell disease? If so, what are they?

There has not been a large change in diagnostic testing for sickle cell disease and hemoglobinopathies. The last major advance I know of is the introduction of capillary zone electrophoresis for diagnosis and management, which was brought into the clinical lab about a decade ago. For sickle disease monitoring, this has similar utility to HPLC techniques, which have been used in the clinical lab for several decades. Most of the above treatments can be monitored using either, which would test for new beta or gamma globulins. For the diagnosis of disease, the use of both CZE and HPLC has several advantages, in my opinion. It should be noted that diagnosis of sickle cell disease (or other hemoglobinopathies) should always be done with at least two techniques, as there are many hundreds of different mutations to hemoglobin, many of which look identical to sickle cell disease or trait if only one technique is used. For this reason, the College of American Pathologists (CAP) specifically mandates the use of two different techniques for the diagnosis of sickle cell disease. The HPLC/CZE combination is newer, as CZE is newer, and many labs including my own now use this. Much like treatments for sickle cell disease, much less attention has been paid to the diagnosis and monitoring of sickle cell disease than for other diseases, despite its status as one of the most common monogenic diseases in the world.

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