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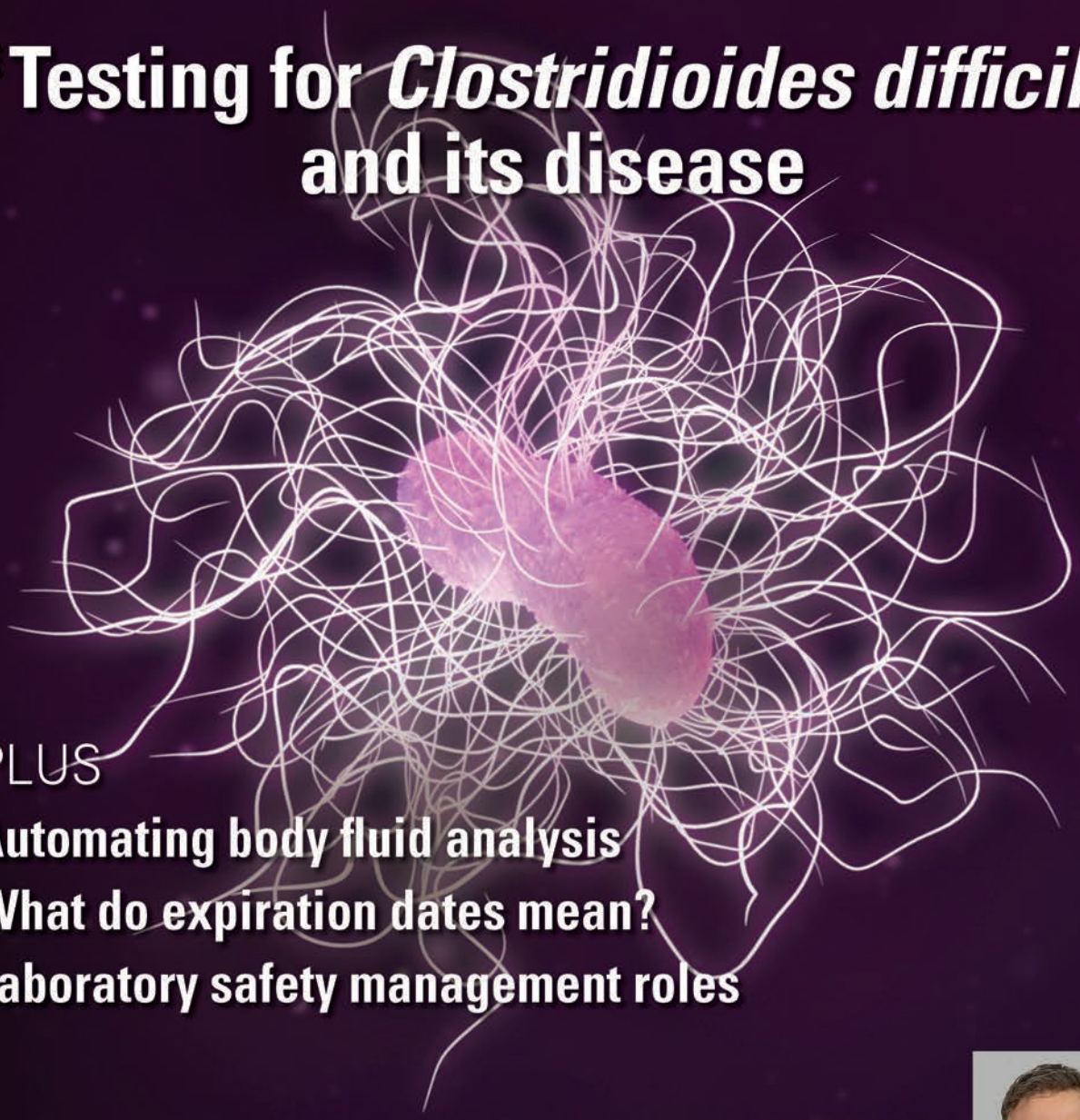
CE Testing for *Clostridioides difficile* and its disease



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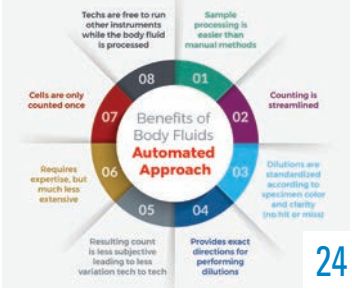

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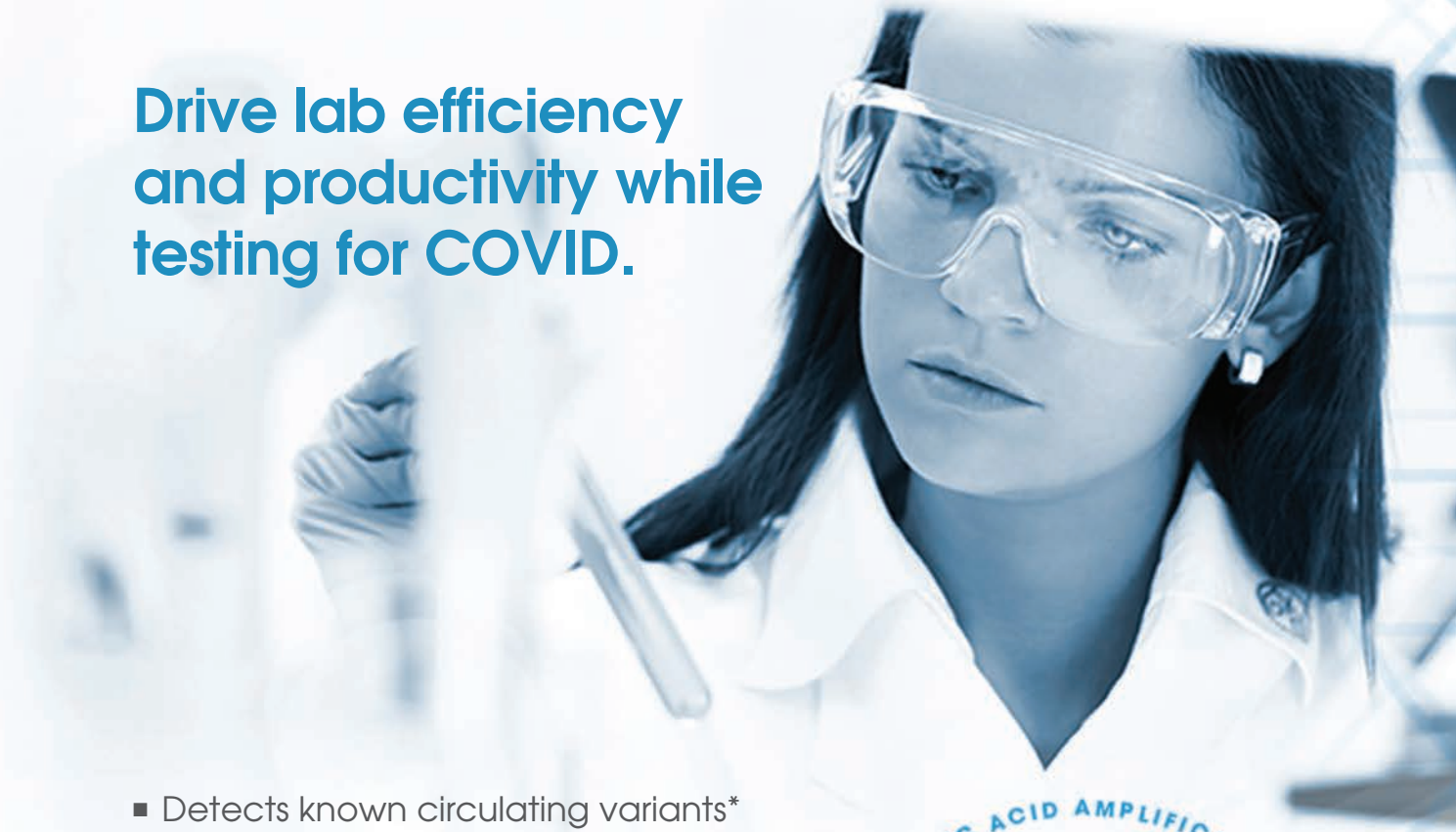

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SD-COM-ART-00130

Omicron enters the COVID-19 battle

By Linda Wilson
Senior Editor

As 2022 dawns, a world weary from battling SARS-CoV-2 faces a new variant of the virus: Omicron (B.1.1.529).

The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have classified Omicron as a variant of concern (VOC). It has a large number of mutations, including 26-32 in the spike protein.

The good news is that most diagnostic RT-PCR and antigen tests do not appear to be impacted by Omicron, according to the WHO. The issue is whether a diagnostic test will produce accurate results if a mutation occurs in the genetic target that the test is designed to detect.

If a test detects multiple targets, it will still detect the presence of SARS-CoV-2, via the other targets, and produce accurate test results. Since many of the widely used tests do, in fact, use multiple targets to detect the presence of SARS-CoV-2 in a specimen, they should continue to produce accurate results, according to the U.S. Food and Drug Administration (FDA), which has been assessing the performance of various tests based on the mutations present in Omicron. The agency also has been posting the results of its assessments online, so clinical labs have the information they need to provide diagnostic testing services.

Most of the sequences of Omicron that labs have performed show a deletion in the S gene. That is why vendors whose tests target the S gene (in addition to other targets) have suggested that their tests could be used to flag specimens that may contain Omicron. Those specimens could then be prioritized for sequencing to confirm the presence of the variant. Sequencing is necessary to make an official determination on the presence of Omicron because the S-gene deletion can also be found in Alpha and subsets of Gamma and Delta, according to the WHO.

Sequencing specimens to track the activity of various SARS-CoV-2 variants is also a central component of surveillance efforts.

As of early December, the WHO said Omicron already had been identified in 63 countries. The WHO also predicts that Omicron will spread more rapidly than Delta did. "It is spreading faster than the Delta variant in South Africa where Delta circulation was low, but also appears to spread more quickly than the Delta variant in other countries where the incidence of Delta is high, such as in the United Kingdom. Whether Omicron's observed rapid growth rate, in countries with high levels of population immunity, is related to immune evasion, intrinsic increased transmissibility, or a combination of both remains uncertain. However, given the current available data, it is likely that Omicron will outpace the Delta variant where community transmission occurs," the WHO said.

The WHO also said the overall risk presented by Omicron is very high. "Preliminary evidence suggests potential humoral immune escape against infection and high transmission rates, which could lead to further surges with severe consequences."

What all of this means for clinical laboratorians is clear: They are likely to spend a lot of their time in 2022, as they did in 2020 and 2021: testing specimens for evidence of 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

Malaria

The World Health Organization reveals that the COVID-19 pandemic has disrupted malaria services, leading to an increase in cases and deaths.

241 million
cases of malaria in 2020, 14 million more cases than in 2019

627,000
malaria deaths worldwide in 2020, 69,000 more deaths than in 2019

47,000
additional deaths were linked to disruptions in the provision of malaria prevention, diagnosis, and treatment during the pandemic

15.3
deaths per 100,000 is the 2020 global mortality rate

95%
of malaria cases occur in Sub-Saharan Africa

80%
of malaria deaths in the African region are among children under 5 years of age

33
countries reported 819,000 children with low birthweight due to malaria infection during pregnancy

Source: World Health Organization - https://www.who.int/news/item/06-12-2021-more-malaria-cases-and-deaths-in-2020-linked-to-covid-19-disruptions

https://cdn.who.int/media/docs/default-source/malaria/world-malaria-reports/978924004049-eng.pdf

Severe COVID tied to high risk of death, mostly by other causes, within year

Survivors of severe COVID-19 — especially those younger than 65 years — may be at more than twice the risk of dying within the next year than those who had mild or moderate illness or were never infected, finds a study in *Frontiers in Medicine*, according to a news release from the Center for Infectious Disease Research and Policy (CIDRAP) at the University of Minnesota.

Another finding of the analysis of electronic health records of 13,638 patients who tested positive or negative for COVID-19 is that only 20% of those who had severe COVID-19 (requiring hospitalization) and died did so because of complications of their infection, such as abnormal blood clotting, respiratory failure, or cardiovascular problems. Rather, 80% were due to different reasons typically considered unrelated to COVID-19.

Of all patients, 178 had severe COVID-19, while 246 were mildly or moderately ill, and the rest tested negative. Among all patients, 2,686 died within 12 months of their COVID-19 diagnosis.

Relative to uninfected patients, those recovered from severe COVID-19 younger than 65 years had a 233% increased risk of dying in the next year. The increased risk was greater than that of survivors of severe COVID-19 who were 65 years or older.

The finding that most deaths were not due to COVID-19 complications suggests that the health of these patients had declined since their initial diagnosis, leaving them susceptible to different medical conditions.

COVID-19 infection rewires immunity "pathways" in pregnant mothers, affecting newborns' immune systems

New UCLA-led research finds that severe COVID-19 illness during pregnancy triggers an inflammatory "cascade" that may lead to damage associated with the disease — and which, in turn, may alter the infants' own immune system, according to a news release.

The findings, published in *Cell Reports Medicine*, may explain why COVID-19 during pregnancy can cause severe, damaging disease, said Karin Nielsen, Professor of Pediatrics in the Division of Infectious Diseases at the UCLA David Geffen School of Medicine. These findings also demonstrate the impact COVID-19 can have on infants born to women who had the infection during pregnancy, even when the infants are not infected with the virus.

The researchers focused on cellular proteins called cytokines, which are important components of the immune system. Chemokines are a type of cytokine that guide white blood cells to the site in need of repair. Levels of these proteins are in balance in healthy bodies, but in the presence of COVID-19, some cytokines are triggered, and others silenced in a process called upregulation or downregulation.

The researchers tested 93 mothers infected with COVID-19 and 45 of their infants at day 1 that were exposed in the womb using next generation sequencing (NGS)-based Olink proteomic profiling. This NGS technology enabled screening of nearly 1,500 cytokines, thus identifying alterations in immune system proteins and pathways.

They found that severe COVID-19 appears to rewire the immune systems in mothers and their newborns. In mothers with severe disease, COVID-19 upregulated and downregulated specific cytokines during pregnancy and delivery. These inflammatory "pathways," as they are called, are associated with liver and heart disease. In addition, infants born to mothers with severe COVID-19 had inflammatory profiles, identified by the presence of specific cytokines and chemokines, which were different from those normally found in newborns.

These altered cytokine levels are usually present in infants with respiratory problems and, in some cases, poor neurodevelopment. The researchers did not find these immune alterations in infants born to mothers who were infected but asymptomatic or who had mild to moderate COVID-19 disease.

Study shows COVID-19-related brain complications

A multi-institutional international study on brain complications of COVID-19 has found that approximately one in 100 patients hospitalized with COVID-19 will likely develop complications of the central nervous system. These can include stroke, hemorrhage, and other potentially fatal complications, according to a news release from the Radiological Society of North America (RSNA).

"Much has been written about the overall pulmonary problems related to COVID-19, but we do not often talk about the other organs that can be affected," said Scott H. Faro, MD, FASFNR, Professor of Radiology and

Neurology and Director of the Division of Neuroradiology/Head & Neck Imaging at Thomas Jefferson University in Philadelphia.

To derive a more complete picture, Faro and his colleagues analyzed nearly 40,000 cases of hospitalized COVID-19 positive patients from seven U.S. and four western European university hospitals. The patients had been admitted between September 2019 and June 2020. Their average age was 66 years old, and there were twice as many men as women.

The most common cause of admission was confusion and altered mental status, followed by fever. Many of the patients had comorbidities like hypertension, cardiac disease, and diabetes.

There were 442 acute neuroimaging findings that were most likely associated with the viral infection. The overall incidence of central nervous system complications in this large patient group was 1.2%.

The most common complication was ischemic stroke, with an incidence of 6.2%, followed by intracranial hemorrhage (3.72%) and encephalitis (0.47%), an inflammation of the brain.

The researchers also discovered a small percentage of unusual findings, such as acute disseminating encephalomyelitis, an inflammation of the brain and spinal cord, and posterior reversible encephalopathy syndrome, a syndrome that mimics many of the symptoms of a stroke.

Diverse genome sequences provide a tool for studying heart disease risk

In a large-scale study of people from diverse ancestries, researchers narrowed down the number of genomic variants that are strongly associated with blood lipid levels and generated a polygenic risk score to predict elevated low-density lipoprotein cholesterol levels, a major risk factor for heart disease, according to a news release from the National Institutes of Health (NIH).

The study, published in the journal Nature, was led by the Global Lipids Genetics Consortium. The authors include researchers at the National Human Genome Research Institute (NHGRI), part of NIH.

Lipids are fat-like substances that can be found in blood and body tissues. They come in two major forms - cholesterol and triglycerides. Humans need a certain amount of lipids in the body for normal function, but elevated lipid levels may increase the risk of developing a heart condition. Polygenic risk scores provide an estimate of an individual's risk for specific diseases, based on their DNA changes related to those diseases.

"Finding the set of genomic variants that are important for this trait is key for us to understand the biology and identify new drug targets," said Cristen Willer, PhD, Professor of Human Genetics at the University of Michigan. "These genomic variants then inform how well polygenic risk scores work to determine risk for such diseases."

Since the field's inception, the genomics community has performed over 6,000 studies looking at the association of specific genomic variants and cardiovascular disease. However, the design of these studies overwhelmingly included individuals from European ancestral populations.

To address this issue, researchers accumulated data from 201 previous genome-wide association studies, including about 1.65 million individuals from five ancestral groups: African, East Asian, European, Hispanic, and South Asian. About 1.32 million of those studies were from European ancestry, and the remaining 350,000 were non-European. The studies contained data on blood levels of the different classes of cholesterol and triglycerides.

The research group calculated the polygenic risk scores using data from each of the different ancestral groups, either separately or all together. Then, they tested the risk scores in a diverse set of studies, including Africans enrolled from Ghana, Kenya, and Nigeria as part of the Africa America Diabetes Mellitus study.

The results showed a polygenic risk score that includes diverse genomic data is much more predictive of whether a person of any ancestry will have elevated low-density lipoprotein cholesterol levels than a score that only includes European genomic data.

Pathologists find evidence of pre-existing chronic lung disease in people with long COVID

Researchers at University of Michigan Health, part of Michigan Medicine, are examining lung biopsies from patients living with persistent respiratory symptoms, such as shortness of breath, to help better define the pattern of damage associated with COVID-19. Their work has led to a surprising finding: some patients' symptoms could be due to damage that existed before getting COVID-19, according to a news release from the academic medical center.

Jeffrey Myers, MD, Professor of Anatomic Pathology, along with Kristine E. Konopka, MD, Associate Professor of Thoracic Pathology, and their Department of Pathology team, examined lung biopsies from 18 living patients who had ongoing respiratory symptoms or abnormal CT scans after recovering from COVID-19. Five patients were reported to have lung disease prior to their COVID-19 diagnosis. Fourteen patients had what is known as ground glass opacities on radiological scans, areas of the lungs that appear as a cloudy gray color as opposed to the dark color of normal air-filled lungs, on a chest X-ray or CT scan.

The most common finding in this set of patients was a condition known to pathologists as usual interstitial pneumonia, or UIP, also known clinically as idiopathic pulmonary fibrosis, or IPF, a well-studied chronic lung disease. IPF is the most common type of pulmonary fibrosis and causes scarring and stiffening of the lungs.

UIP/IPF is a progressive disease that gets worse with time and an infection, such as with SARS-CoV-2, can lead to accelerated illness or even death, what is known as an acute exacerbation of IPF, explained Myers. "SARS-CoV-2 comes along and does to the lung, from a pathology perspective, exactly what happens with an acute exacerbation."

Biopsies from these patients show evidence of the underlying pre-existing lung scarring with, layered on top, evidence of diffuse alveolar damage, a pattern of lung tissue damage commonly seen in patients with acute respiratory distress syndrome of any cause.

Testing for *Clostridioides difficile* and its disease

By Jodie Y. Lee, MS, MBA, and David M. Lyerly, PhD

C. difficile is an opportunistic anaerobic bacterial pathogen that causes diarrhea and colitis especially in hospital settings. There are other causes of hospital-acquired diarrheas (e.g., norovirus outbreaks, medications, laxatives, etc.), but *C. difficile* is a major cause, accounting for 5% to 15% of hospital-acquired diarrheas. Outside of *C. difficile* infections (CDI), the cause of many hospital-acquired diarrheas remains undiagnosed.

Variants of *C. difficil*e continue to cause problems in our healthcare systems. The most recognized variant is ribotype 027, which appeared in the early 2000s. This ribotype caused numerous outbreaks in Europe and North America because of its resistance to flouroquinolone antibiotics. However, it now is on the decline. Treatment does not differ between infections caused by the 027 ribotype and other ribotypes. As new hypervirulent strains continue to emerge, surveillance testing is important to help identify and proactively counteract these new threats.

CDI develops in persons who have a compromised intestinal microbiota. Hospitalized elderly patients receiving antibiotics are a prime target for the disease. Antibiotics kill the microbiota, allowing *C. difficile* spores to germinate, grow in the intestine, and produce two very potent tissue-damaging and inflammatory toxins designated A and B that cause CDI. The spores, which persist in the patient, cause recurrent disease in about 25% of CDI cases and are difficult to eradicate from hospital environments.

A closer examination of data over the past decade suggests a shift in the epidemiology in hospital-acquired versus community-acquired CDI.[1] Hospital-acquired CDI is defined as those patients who develop CDI while in a healthcare facility. Community-acquired CDI is defined as those patients who develop CDI prior to or shortly after admission to a hospital. The numbers most typically cited for CDI in the U.S. — 400,000 cases of CDI with up to 30,000 deaths — include both hospital-acquired and community-acquired cases. Lower rates of hospital-acquired CDI may be due to fewer cases caused by the hypervirulent fluoroquinolone-resistant ribotype 027, along with reduced use of fluoroquinolone antibiotics and greater attention to preventive measures. Community-acquired CDI, on the other hand, appears to be increasing, and may account for almost half of the cases of CDI, as indicated in more recently cited numbers.[1]

Overdiagnosis continues to be a challenge because of the large numbers of hospitalized patients who are carriers of *C. difficile* but who do not have CDI. Guidelines stipulate that carriers should not be treated because inappropriate treatment predisposes the patient to CDI and is not a good practice of antibiotic stewardship, possibly leading to antibiotic-resistant strains. **(See Figure 1)**

Gold standard tests for CDI

The more than 50 laboratory tests cleared by the U.S. Food and Drug Administration (FDA) for *C. difficile* all have the same intended use and patient population. Importantly, results with all of these tests are to be used in conjunction with clinical history when diagnosing CDI. About half of the FDA-cleared tests are immunoassays that detect toxins A and B or glutamate dehydrogenase (GDH). Immunoassay formats include microwell-based and rapid membrane tests. NAAT (nucleic acid amplification test) assays, representing the other half, detect the toxin genes as single gene targets or as part of multiplex gastrointestinal panels.

The gold standard tests, developed about 40 years ago to help diagnose CDI, continue to be used today as comparator tests to establish performance of newer laboratory tests. The gold standard tests include:

- the cell cytotoxicity neutralization assay (CCNA) for the detection of toxin in fecal specimens
- toxigenic culture on selective media (typically cycloserine cefoxitin fructose agar [CCFA]) followed by CCNA.

CCNA detects the cell-rounding activity (i.e., cytopathic effect) of the toxins, with confirmation by neutralization with specific *C. difficile* antitoxin. For toxigenic culture, colonies are picked from CCFA and further tested by CCNA to confirm the isolate is toxigenic.

The gold standard tests are very accurate and sensitive. CCNA detects picograms of toxin, and CCFA can detect <100 colony-forming units per gram feces. However, both are tedious and time-consuming, requiring 2 days minimum for CCNA and 4 to 5 days for toxigenic culture. For these reasons, these tests are seldom used in today's clinical laboratory. Instead, clinical labs have moved toward more rapid and easier-to-use formats.[2]

Toxin Immunoassays

There are variant strains that produce only toxin A or only toxin B. This is why immunoassays that detect both toxins must be used since variant strains that produce only one toxin are capable of causing severe and life-threatening CDI. Toxin immunoassays exhibit higher specificity, in some cases >99%, than GDH and NAAT assays because they detect the toxins that directly damage the intestinal mucosa and trigger the inflammation that occurs in CDI. The higher specificity results in higher positive

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LEARNING OBJECTIVES

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

1. Discuss the role of *C. difficile* as an opportunistic pathogen and the challenges of diagnosing CDI versus carriers
2. Recognize the advantages and limitations of laboratory tests for CDI
3. Describe how a diagnostic approach based on an algorithm provides optimal test results
4. Discuss treatment options for CDI

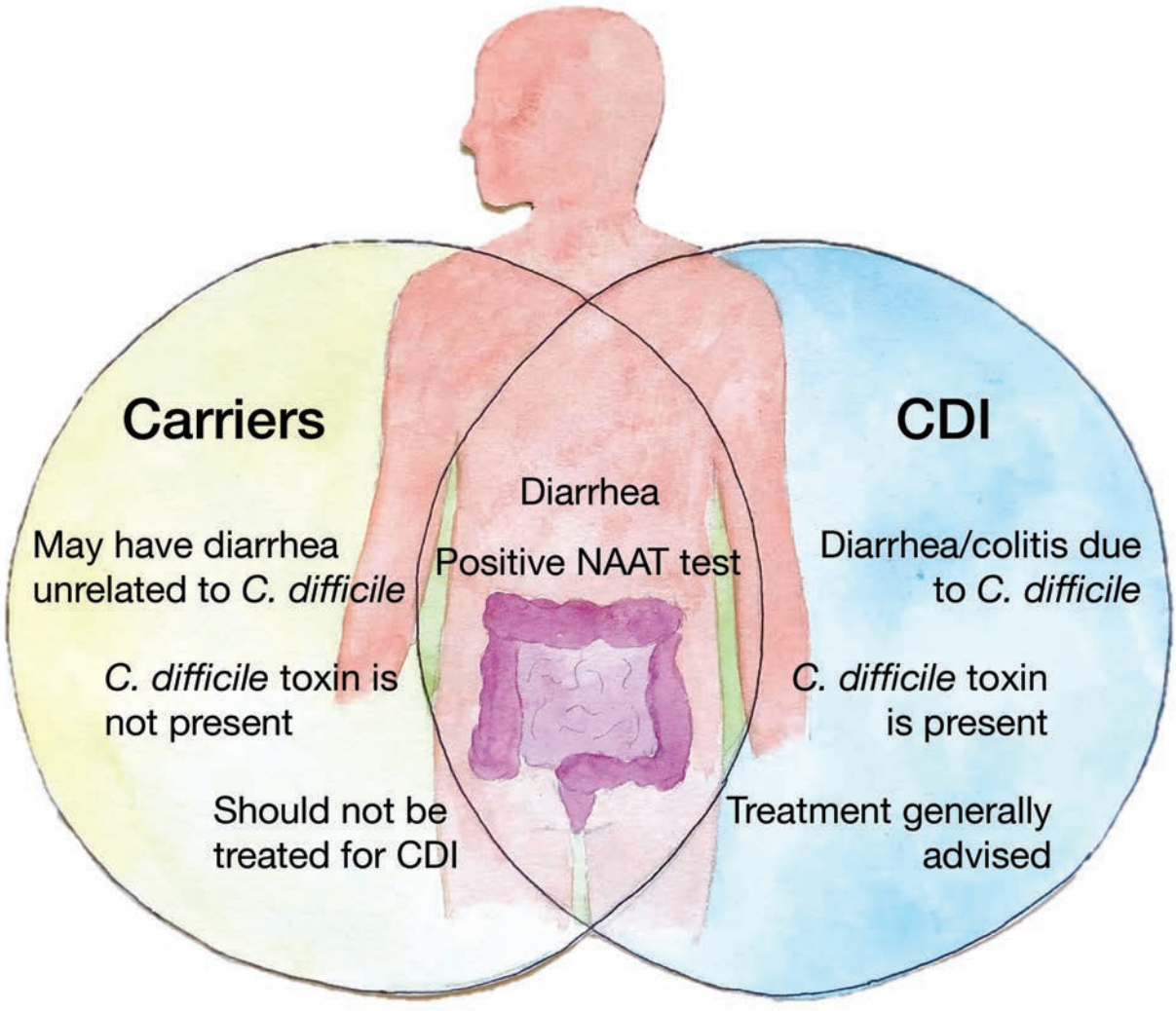

Figure 1. Distinguishing between carriers of *C. difficile* and patients with CDI can be challenging

predictive values (PPV) and fewer false positive results. PPVs are affected by the prevalence of the disease in the patient population being tested. The PPVs approaching >95% with some of the better performing toxin immunoassays have been reported from healthcare facilities with prevalence rates of 5% to 15%. The lower sensitivity of toxin immunoassays, compared to the picogram amounts detected by CCNA, has raised concerns about false negative results.[2] **(See Figure 2)**

GDH Immunoassays

GDH immunoassays detect glutamate dehydrogenase, a metabolic enzyme produced by *C. difficile*. GDH is an excellent biomarker for *C. difficile* because it is a stable enzyme produced when the organism is actively growing in the large intestine. GDH consists of six identical subunits, thus providing multiple repeating epitopes for increased binding of antibodies and better signal-to-noise ratios in immunoassays. As a result, GDH immunoassays are very sensitive with values comparable to those reported for NAAT assays for detecting the organism in fecal specimens. The high sensitivity results in high negative predictive values (NPV), and a negative GDH result accurately rules out CDI. GDH immunoassays do not differentiate between toxigenic and nontoxigenic strains. This is a limitation because nontoxigenic strains form spores and can spread in hospitals, although they are considerably less prevalent than toxigenic strains. Nontoxigenic strains do not carry the toxin genes and do not cause disease. In fact, they may be protective by outcompeting toxigenic strains in the intestine. A positive GDH result should be followed by a toxin assay when performing laboratory testing for CDI.

NAAT Assays

NAAT assays detect the toxins A and B genes (*tcd*A and *tcd*B), which are located on a large 19.6 kilobase pathogenicity locus called the PaLoc. The tests use PCR or isothermal amplification for detection. Most of the tests target *tcd*B although some target *tcd*A or both genes. A positive result confirms the presence of a toxigenic strain but does not confirm the presence of toxin. Many of the NAAT assays now available are very sensitive, detecting fewer than 50 cells per gram feces. The high sensitivity results in high NPV, and a negative NAAT result accurately rules out CDI. However, the exquisite sensitivity of the tests leads to overdiagnosis and overcalls patients who are carriers. NAAT assays will give positive results with spores and dead cells which do not cause active CDI.

Algorithm testing is recommended

C. difficile testing guidelines from the European Society of Clinical Microbiology and Infectious Disease (ESCMID), the Infectious Disease Society of America/Society for Healthcare Epidemiology of America (IDSA/SHEA), and the American Society of Microbiology (ASM) have been in place for several

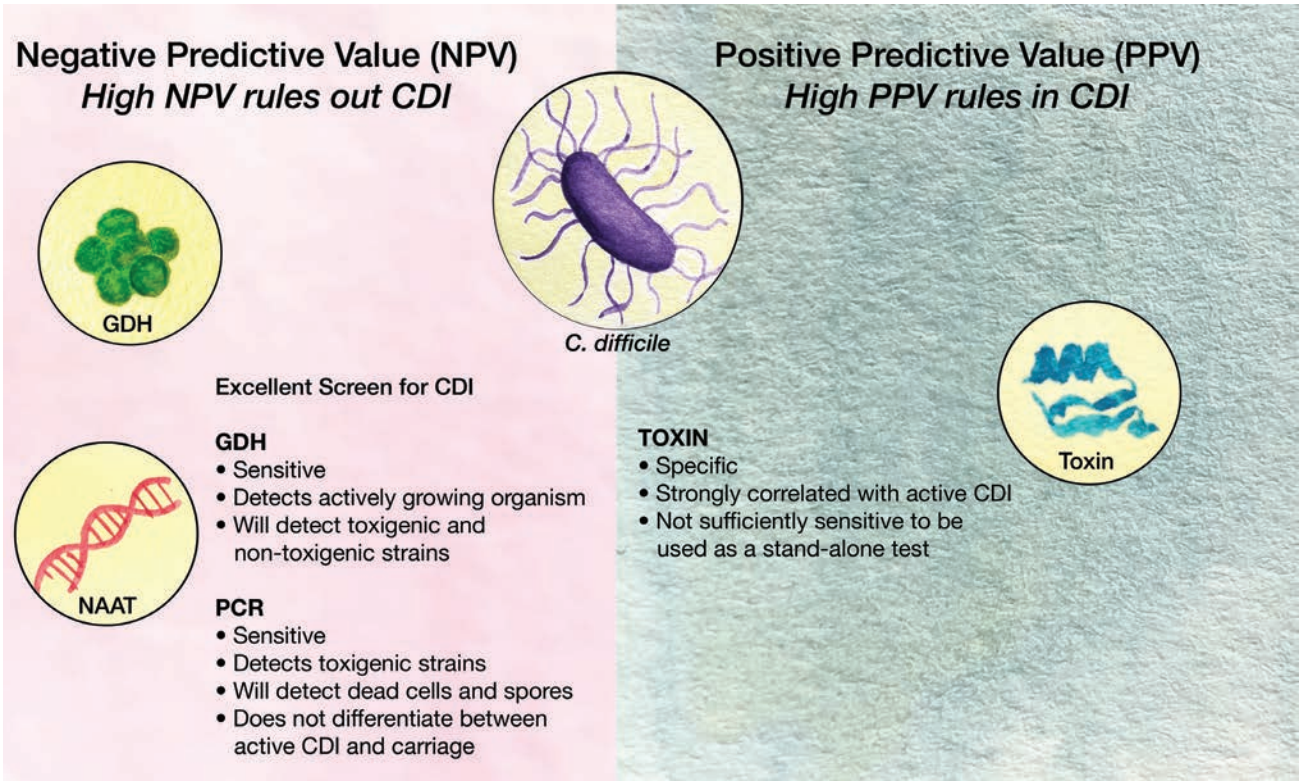

Figure 2. FDA-approved tests for *C. difficile* should be used with clinical history to diagnose CDI

years.[2] The most recent guideline is from the American College of Gastroenterologists (ACG).[3] The ACG recommendations follow those from ESCMID and IDSA/SHEA.

The ACG guidelines note that the case numbers of CDI increased significantly when many hospitals and healthcare facilities implemented NAAT assays, and the authors raised concerns that asymptomatic patients who were colonized with *C. difficile* tested positive, and there are other causes of diarrhea in patients colonized with toxigenic strains. The ACG guideline note further that complications are rare in patients who are NAAT-positive but negative for toxin. For these reasons, the ACG guidelines recommend an algorithm comprised of NAAT or GDH testing coupled with toxin testing.[3] These recommendations are directly in-line with those from ESCMID and IDSA/SHEA.

The IDSA/SHEA guidelines note that if specimen selection criteria are in place, the accuracy of a NAAT assay improves and recommend that a NAAT assay may be used as a stand-alone test under these conditions. Whether appropriate specimen selection procedures can be implemented in most hospital settings was a point of discussion at the recent 9th Annual International C. DIFF Conference and Health EXPO, which was held in November 2021.

Algorithm testing is considered optimal because it brings together the high NPV of GDH or NAAT assays with the high PPV of a toxin test, helping to limit false negative and false positive results, respectively. When used as the initial screen, GDH or NAAT assays accurately rule out patients who do not have CDI. This will be the majority of patients in most hospitals. Follow-up toxin testing with specimens that are GDH-positive or NAAT-positive provides the most accurate information to the physician tasked with diagnosing CDI. This approach provides confirmatory results for >90% of specimens submitted for testing. Of the remaining low number of specimens that are positive by GDH or NAAT but negative for toxin, the ESCMID guidelines define these patients as carriers, although some probably have true CDI.

Efforts have been made by several companies to produce ultrasensitive toxin tests.[4] These types of tests have the potential to simplify laboratory testing and the reporting structure for CDI, assuming they can provide adequate sensitivity. However, these tests also need to exhibit high specificity to achieve the PPVs now available with higher quality FDA-cleared toxin immunoassays.

Many patients will continue to test positive for weeks or months by NAAT even after symptoms have resolved because of the persistence of spores. Resolution of symptoms more accurately correlates with the disappearance of toxin. Even so, none of the guidelines recommends a "test of cure" for CDI. Repeat testing is not routinely recommended, although on occasion, it may be performed to confirm recurrent CDI. All of the guidelines stress that the diagnosis and decision to treat is a clinical decision, and that laboratory tests "aid" but do not diagnose CDI.

CDI is an inflammatory disease

Inflammation is a hallmark of CDI. The inflammatory process is triggered by the toxins and the tissue damage they cause to the gut mucosa. Both toxins are glucosyltransferases that bind to cellular receptors and kill cells by shutting down the cytoskeletal system. In addition, both toxins trigger inflammatory mediators that result in the diapedesis of white blood cells (WBCs) into the intestinal lumen. White blood cells become activated and stimulate the release of proinflammatory mediators. The multimeric inflammasomes that develop result in additional inflammation.

The severity of the disease can be monitored by circulating WBC counts, with >15,000 per mm^3 signaling severe disease. Fecal lactoferrin, a stable glycoprotein released from WBCs that migrate into the intestinal lumen, can be measured quantitatively in fecal specimens as a direct measurement of intestinal inflammation. Fecal lactoferrin and circulating WBC counts

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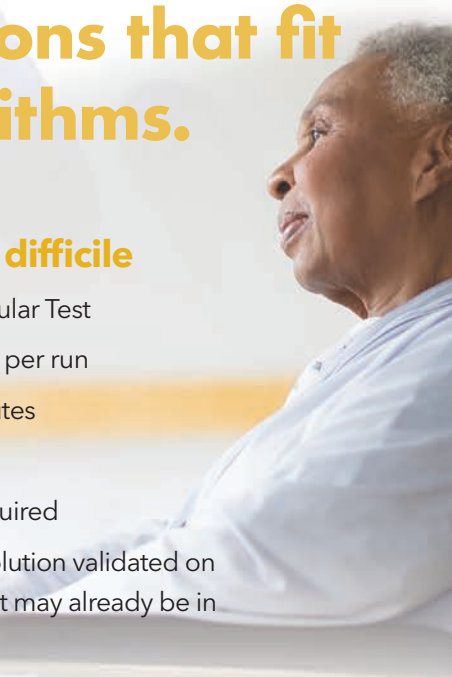

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both provide laboratory results that help physicians assess CDI severity and identify patients at high risk.

The onset of sporadic irritable bowel syndrome-like symptoms (IBS) may occur after CDI.[5-7] It is not uncommon for post-infectious IBS to develop after severe intestinal disease. This can happen, for example, following a norovirus infection. The triggering events in post IBS following CDI are unclear. Perhaps the persistence of spores is a complicating factor, although this has not been proven. In the absence of toxin-mediated mucosal damage, the ACG suggests that consideration of conditions "such as microscopic colitis or inflammatory bowel disease" be included as part of a clinical follow-up.[3] Another possibility in the onset of post IBS is the persistence of chronic low-grade inflammation. Low grade inflammation persists in patients with inflammatory bowel disease (IBD) who are in clinical remission, and is characterized by histological damage to the colon, which is a triggering event in clinical relapse in IBD patients. Perhaps a similar situation develops during severe inflammatory CDI.

Treating CDI

The disruption of the normal diverse intestinal microbiota predisposes a patient to CDI, especially in hospital settings. Antibiotics are very good at causing this disruption, and treatment with antibiotics continues to represent the primary triggering event for CDI. Other predisposing factors are conditions such as IBD in which the intestinal microbiota is less diverse or treatment with certain medications such as proton pump inhibitors that reduce the innate protective mechanism of stomach acidity.

Mild CDI may be treated simply by stopping the inciting antibiotic and allowing the intestinal microbiota to become reestablished, an approach recommended in the 2021 ACG guideline.[3] In more severe CDI, the disease typically is treated with metronidazole, vancomycin, or fidaxomicin, although vancomycin is now considered clinically superior to metronidazole. Fidaxomicin, which has a more narrow spectrum than vancomycin, is used increasingly because it has been associated with lower rates of recurrent disease.

Monoclonal antibody therapy with Bezlotoxumab (brand name Zinplava) was approved by the FDA in 2016 as a treatment for recurrent CDI in patients receiving antibiotics. The antibody, which is given as an intravenous infusion, binds to a specific epitope located in the combined repetitive oligopeptide (CROP) region of toxin B that constitutes the binding domain. Thus, protection is mediated by preventing the toxin from binding to its intestinal receptor.

Fecal transplants represent the latest efforts to treat CDI, especially in patients with recurrent CDI. The protection is based on the restoration of a more diverse intestinal microbiota capable of outcompeting *C. difficile*. Efforts are now underway to develop a more defined consortium of protective bacteria. The intestinal microbiota functions as a food pyramid with layers of diverse bacteria feeding off the byproducts produced by other bacteria, and it is challenging to identify specific consortia of bacteria that are protective. However, progress is being made in this area, and clinical trials are underway to establish efficacy in preventing recurrent CDI.

Patients who receive fecal transplants continued to carry *C. difficile* spores even though they become asymptomatic. This persistence of spores also occurs in patients following antibiotic treatment for CDI. Recommendations in Europe and the U.S. include antibiotic therapy for initial onset of CDI and consideration of fecal transplants in patients with recurrent disease.

CDI during COVID-19 pandemic

The Fall 2021 surge of the SARS-CoV-2 Delta variant overwhelmed hospitals, and resources such as laboratory instrumentation, reagents, and plastics have been limited. For these reasons, labs have looked for alternative ways to make testing more flexible and improve lab workflow. Modified approaches such as rapid testing for CDI have helped lower the burden.

During the early stages of the COVID-19 pandemic, antibiotics were heavily overused to treat COVID-19 patients due to the lack of alternative treatments and because of concerns about secondary bacterial pneumonias often seen with influenza illnesses. This overuse raised concerns about increased rates of CDI. By mid-2020, antibiotic overuse had been curtailed because secondary pneumonias were not being seen in COVID-19 patients at the levels seen, for example, following flu outbreaks. Although little data has been reported on rates of CDI in COVID-19 patients, it is no surprise that mitigation efforts against COVID helped reduce the incidence of CDI and other hospital-acquired infections.

Many COVID-19 patients, perhaps up to 25%, develop diarrhea.[8] The association of respiratory viruses with intestinal disease is not a new observation and has been reported, for example, with influenza virus. The SARS CoV-2 ACE2 receptor is present in high numbers in the intestine, so the binding of the virus to its intestinal receptor likely causes diarrhea due to cellular damage. SARS CoV-2 and other respiratory viruses appear to lower the diversity of the intestinal microbiota, raising the question of how this occurs and whether virus-induced dysbiosis is a predisposing factor for CDI.[9-11]

Conclusions

- The burden of CDI on healthcare continues to be a major problem, and elderly hospitalized patients treated with antibiotics continue to be a primary susceptible population. Rates of hospital-acquired CDI are decreasing, but community-acquired cases continue to increase due to spores in the environment, exposure in healthcare facilities, and variants.
- There are numerous tests available in immunoassay and NAAT formats. Each offers advantages and each has limitations. An algorithm approach brings together the advantages of these tests and helps physicians establish an accurate diagnosis when used in conjunction with patient history. This approach results in optimal patient healthcare, minimizes overdiagnosis, and represents good antibiotic stewardship.
- With the epidemic strain 027 that appeared in the early 2000s, fluoroquinolone resistance developed following a single mutation in the gyrase gene. Variants of C difficile, such as the toxin B-only variant causing outbreaks in Asia, continue to be problematic. Surveillance efforts need to continue to identify variants, determine their involvement in CDI, and ensure that they are accurately detected in laboratory tests.
- Inflammation is a primary reason why CDI becomes severe. Elevated white blood cell counts and elevated fecal lactoferrin, a marker of intestinal inflammation, indicate severe CDI. Elevated levels of either marker are associated with worse clinical outcome.
- SARS-CoV-2 replicates in the intestine and is present in fecal specimens from COVID-19 patients. This respiratory virus binds to ACE2 intestinal receptors, causes diarrhea, and has been associated with co-infections with *C. difficile*, leading to questions about the etiology of intestinal disease in these patients.

- Testing strategies that minimize overdiagnosis of CDI will result in appropriate treatment for the patient. Treating carriers can lead to patient harm and has been associated with an increased prevalence of antibiotic resistance, including VRE.[12] Minimizing overdiagnosis represents good antibiotic stewardship and helps to prevent the evolution of antibiotic-resistant strains.

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Testing for *Clostridioides difficile* and its disease

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TEST QUESTIONS

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1. ***C. difficile*** **is a _____ pathogen that causes _____ and _____.**
   - A. viral; diarrhea and colitis
   - B. viral; respiratory distress and pneumonia
   - C. bacterial; diarrhea and colitis
   - D. bacterial; sore throat and tonsilitis

2. **How much hospital-acquired diarrhea does *C. difficile* account for?**
   - A. 5-10%
   - B. 5-15%
   - C. 15/30%
   - D. 50-75%

3. **The inflammatory toxins associated with *C. difficile* are_____.**
   - A. A and B
   - B. C and D
   - C. A and C
   - D. C and F

4. **Hospital-acquired CDI is currently increasing over community-acquired *C. difficile* infections (CDI).**
   - A. True
   - B. False

5. **FDA-cleared tests that detect *C. difficile* are about _____% immunoassay and ____% NAAT.**
   - A. 25; 75
   - B. 85; 15
   - C. 30; 70
   - D. 50; 50

6. **The main limitation to the gold standard CCNA and CCFA is:**
   - A. the turnaround time of results
   - B. it is expensive to run
   - C. it needs highly trained techs
   - D. all of the above

7. **The use of toxin immunoassays has raised concern because of its_____.**
   - A. positive predictive value
   - B. high sensitivity
   - C. low sensitivity
   - D. low specificity

8. **GDH immunoassay is a great biomarker test because it is a stable enzyme produced when the organism is actively growing in the large intestine.**
   - A. True
   - B. False

9. **The main limitation of the GDH immunoassay:**
   - A. is time consuming
   - B. doesn't differentiate between toxigenic and nontoxigenic strains
   - C. has a low sensitivity
   - D. is very expensive

10. **Many NAAT assays to detect *C. difficile* can detect fewer than _____ cells per gram of feces.**
    - A. 5
    - B. 15
    - C. 25
    - D. 50

11. **The main limitation of NAAT assays:**
    - A. will give positive results with spores and dead cells, which do not cause active CDI
    - B. is low sensitivity
    - C. is long turnaround time
    - D. is very expensive

12. **Algorithm testing guideline to accurately diagnose CDI is recommended by:**
    - A. ESCMID
    - B. ASM
    - C. ACG
    - D. All of the above

13. **Algorithm testing is optimal because it brings together the _____ of GDH/NAAT assays and the _____ of toxin testing.**
    - A. low NPV; low PPV
    - B. high NPV; high PPV
    - C. high NPV; low PPV
    - D. low NPV; high PPV

14. **Algorithm testing provides confirmatory results for _____% of the specimens submitted for CDI diagnosis.**
    - A. 65
    - B. 75
    - C. 80
    - D. 90

15. **Even though many patients will continue to test positive for weeks or months after CDI, the resolution of symptoms more accurately correlates with the disappearance of the toxin.**
    - A. True
    - B. False

16. **The hallmark of CDI is:**
    - A. vomiting
    - B. headache
    - C. inflammation that triggers diarrhea
    - D. inflammation that triggers joint pain

17. **The severity of *C. difficile* disease can be monitored by:**
    - A. WBC count and fecal lactoferrin
    - B. CRP and ferritin
    - C. WBC count and CRP
    - D. IL-6 and fecal lactoferrin

18. **Post CDI can trigger:**
    - A. autoimmune disease
    - B. heart disease
    - C. chronic fatigue
    - D. IBS

19. **Treatments for CDI include:**
    - A. fecal transplants
    - B. monoclonal antibody therapy
    - C. antibiotics
    - D. all of the above

20. **Questions have recently been raised about _____ and _____ possibly predisposing patients to CDI.**
    - A. other respiratory viruses
    - B. SARS CoV-2
    - C. Both A and B
    - D. other bacterial infections

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Detecting new and emerging fentanyls with confidence using LC-MS

By Stephanie Samra, MS

The opioid crisis is a public health emergency in the United States and an emerging problem in other countries. In 2019, nearly 50,000 people in the United States died from opioid-involved overdoses.[1] To keep ahead of the manufacturing and trafficking of illicit substances, drug enforcement agencies rely on insights from forensic laboratories to track the rise of new forms of opioids in circulation. Yet emerging synthetic opioids, such as fentanyl and fentanyl analogs, are challenging to detect with conventional drug detection assays. To meet the changing demands of forensic drug analysis, new mass spectrometry (MS)-based methods that allow more sensitive, accurate and efficient detection of opioid analogs are coming to the forefront.

The challenge of analyzing fentanyls

Around 73% of opioid-involved deaths are due to synthetic opioids, such as fentanyl and fentanyl analogs.[2] Carfentanil, the most potent fentanyl analog detected in the U.S., is estimated to be 10,000 times more potent than morphine.[3] According to the National Forensics Laboratory Information System, the number of reported fentanyl-related overdose cases increased from 945 in 2013 to 71,341 in 2017.[4] More recent numbers from the Centers for Disease Control and Prevention (CDC) show that overdose deaths involving synthetic opioids, such as fentanyl and fentanyl analogs, were nearly 12 times higher in 2019 than in 2013.[5]

Forensic and clinical toxicology laboratories must be able to confidently identify fentanyls in clinical samples from an increasing caseload of overdoses, and rapidly provide results to health professionals and law enforcement agencies. Yet with more than 200 different fentanyl analogs in circulation and many more rapidly emerging, this presents a challenge both in terms of efficiency and accuracy.

The increased potency of fentanyl analogs relative to morphine means they are often present in the blood at much lower levels and can be below the sensitivity thresholds of conventional drug testing methods. Moreover, although forensic laboratories have established methods for known fentanyls and other opioids, the rapidly expanding list of new illicit fentanyls means standard techniques and reference reagents are not fit-for-purpose. The chemical similarity between different fentanyl analogs also pushes the capabilities of current forensic analytical chemistry tools to the limit in terms of their sensitivity and specificity.

Current analytical methods for fentanyls

Most forensic laboratories perform immunoassays to detect specific drugs. But these assays are limited by the specificities of the antibodies used and the availability of antibodies that are validated against different opioid analogs. As new drugs emerge, antibodies are not readily available to detect them, and developing and validating new antibodies for immunoassays can take up to 12 months. By this time, new opioid analogs may well be in circulation.

Most conventional immunoassays are relatively low throughput, involving multiple wash steps and only suitable for detecting a few analytes simultaneously. More advanced analytical methods, such as liquid chromatography (LC) coupled with high-resolution accurate mass (HRAM) MS (e.g., Orbitrap mass spectrometry), enable parallel analysis through their multichannel liquid chromatography capability. These alternative approaches are quickly becoming the new gold-standard method in many forensic laboratories.

However, there is still a challenge. LC-MS techniques require libraries of standards against which fentanyls can be correctly identified. But there are so many new analogs emerging with no existing standards that it is difficult for laboratory analysts to feel confident they understand the limits of detection for these new compounds, and that they have accurately and comprehensively detected every isomer in a sample.

More sensitive analytical solutions

To help forensic laboratories confirm the presence of new fentanyl analogs, the Centers for Disease Control and Prevention (CDC) released a Fentanyl Analog Screening Kit (FAS kit) and four Emergent Panels (FAS V1-4) in 2019 in response to the rapid emergence of further analogs. In total, these reference materials support the screening for 250 opioids, including over 210 fentanyl analogs.

To demonstrate the potential of LC-MS methods for identifying closely related synthetic opioids, reference panels were used to develop optimized workflows based on Orbitrap mass spectrometry for detecting new and emerging fentanyl analogs. By creating an MS spectral library using the 213 fentanyl analogs, it tested the potential of HRAM MS for its ability to detect closely related fentanyls from biological samples. It was found that HRAM MS can identify fentanyls in urine with high sensitivity. The limit of detection for a majority of the compounds tested was 0.5 ng/mL and ≥75% of the fentanyl standards had a limit of detection of 1.0 ng/mL or better in a synthetic biological matrix, surine.[6]

One key advantage of LC-MS over immunoassays is that LC-MS is more specific and can identify individual compounds within a compound class. The addition of LC to high-resolution MS means fentanyl isomers with identical mass spectra can still be differentiated because they are well separated by LC. Similarly, because some fentanyl isomers co-elute chromatographically, but have different MS2 spectra and unique product ions from other isomers, these can accurately be distinguished from each other. Although there are some scenarios where analogs have similar retention times and spectra, and cannot be distinguished, these high-resolution MS protocols and the spectral library make it possible to narrow down the analytes to one of two isomers.

More efficient workflows

A further advantage of using high-resolution MS for fentanyl analysis is the potential for more efficient analysis to help meet the demands of increasing caseloads in forensic laboratories. Although it might seem that optimizing samples for analysis in an LC-MS workflow might require more time-consuming and laborious sample preparation steps than traditional immunoassays, sample preparation with HRAM MS is surprisingly straightforward. Diluted urine samples can be directly injected into

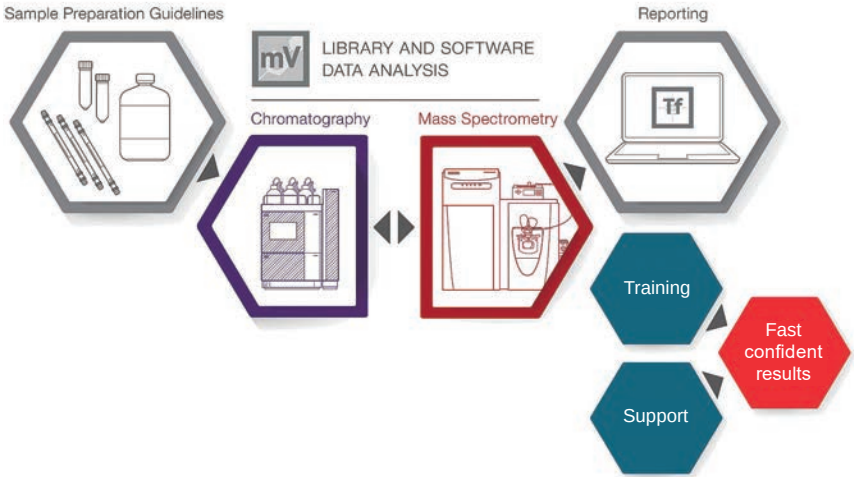

Figure 1: LC-MS screening workflow utilizing an extensive compound database and library

an ultra-high-performance LC system coupled with HRAM MS to screen and semi-quantify fentanyl and its analogs in a single analytical run. When you compare this to immunoassay workflows, which require a series of incubation and wash steps, the use of HRAM MS can make testing much more efficient and by reducing manual tasks, frees up analysts to perform other tasks **(see Figure 1).**

An alternative emerging workflow for targeted fentanyl analysis is the use of paper spray tandem MS. In this workflow, the sample is deposited as a fluid (urine) on a paper sampling substrate containing previously loaded labeled standards. This paper substrate is then dried and analyzed quantitatively with tandem MS. No other sample treatment is needed between the spotting of the sample on the paper cartridge and analysis. This method allows for rapid analysis without an LC step and offers a significantly simplified process for biological sample collection. This workflow is being tested in real-time at substance use disorder clinics and has already shown comparable quantitative results to LC-MS for identifying fentanyl analogs at low levels.[7]

Detecting the unknowns

Importantly, high-resolution MS has potential for untargeted analysis, which gives you information about everything in the sample, not just known compounds of interest. In an experiment to identify novel unknown fentanyl opioids, an MS^2 spectra library generated for the CDC FAS panel of 213 fentanyl analogs was used to establish a set of class- and structural similarity-based rules to identify unknown fentanyls from urine samples.[8]

The workflow for this experiment comprised three key steps.

First, it used multiple search tools to find class-based common fragments among the reference spectra. Eight common fragments of fentanyl compounds were identified through this search. Second, it conducted a similarity search using spectral data found in reference libraries, and an internal in-house spectral library of 213 fentanyls generated using the FAS Kit v1-3 was searched for similarity patterns. In the third and final step, the workflow investigated molecular networks to identify compounds that differed by transformations, such as methylation, hydration, dealkylation and so on. These rules were used to correctly identify a set of 'unknown' fentanyl standards from the CDC Emergent Panels spiked into urine.

This ability to perform untargeted analysis is arguably one of the greatest advantages of high-resolution MS for fentanyl screening. Its potential for providing unbiased information on all constituents of the sample, and in turn identifying the presence of unknown fentanyl analogs, provides a rapid view on the emerging and changing illicit drug landscape. It can also allow the retrospective analysis of previous samples to see if newly identified fentanyls were present in past overdose cases.

Conclusion

Forensic laboratories face an increasing workload trying to analyze the growing number of illicit opioid analogs in circulation. The higher potencies of fentanyls and the large number of structurally similar fentanyl isomers found in illicit drugs present a unique analytical challenge that requires highly sensitive and accurate techniques that work quickly on biological samples.

HRAM MS coupled with LC can accurately distinguish between structurally similar isomers of fentanyls at low concentrations. Moreover, it is possible to develop rules from the MS spectra of existing fentanyl standards that allow the identification of unknown fentanyl analogs in urine, helping laboratory scientists obtain information on all the constituents of a sample – including retrospectively.

The advent of real-time, MS-based approaches, installed at harm-checking facilities alongside high-resolution clinical laboratory tools, will be powerful weapons in our arsenal in the war against drugs, helping to turn the tide against the rise in opioid-related overdoses and deaths.

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Laboratory safety management roles and responsibilities

Joanne P. Christopher, MA, ELS

Keeping laboratory workers safe is a top priority and challenge for laboratory management. Implementation of a laboratory safety program, efficient laboratory design, and effective security measures are some considerations for laboratory worker safety. The laboratory director, safety officer, hazardous chemical waste disposal coordinator, and radiation safety officer, as well as laboratory employees themselves, all play roles in ensuring safety, with the laboratory director bearing the ultimate responsibility.

This article presents a brief overview of the components of a laboratory safety program, important laboratory security concerns, as well as descriptions of the various roles and responsibilities of laboratory employees responsible for the safe operation of the laboratory.

Throughout this article, the phrase "the laboratory needs to" explains an action directly related to fulfilling requirements of international, national, and accreditation organizations. The phrase "the laboratory should" describes a recommendation provided in laboratory literature, a statement of good laboratory practice, or a suggestion for how to meet a requirement.

A comprehensive safety program encompasses all aspects of daily laboratory operations, including chemical hygiene, blood-borne pathogens, respiratory protection, radiation safety, fire prevention, emergency management, occupational health, safety education, and recordkeeping. [1]

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A comprehensive safety program encompasses all aspects of daily laboratory operations

A general safety program includes considerations for the following:

- Engineering controls
- Personal protective equipment
- Work practice controls
- Emergency aids
- Personnel responsibility
- Waste disposal
- Ergonomics

The National Institute for Occupational Safety and Health (NIOSH) has identified the following broad types of laboratory hazards:[1]

- Allergic sensitization
- Carcinogens
- Equipment
- General chemical hazards
- Infection
- Mutagens and teratogens
- Physical stress

Lab-acquired infections

The laboratory-associated hazards of working with microorganisms have been well documented.[2] Accidental or unrecognized exposure to specimens or cultures of highly transmissible microorganisms — such as *Brucella* species, *Mycobacterium tuberculosis, Neisseria meningitidis, Salmonella, Shigella,* and Shiga toxin–producing *Escherichia coli* — has resulted in either life-threatening infection or death of clinical laboratory workers. For some of these organisms, laboratory workers are at greater risk of acquiring such infections than the general population.[3]

Laboratory-acquired infections may occur through inhalation; ingestion; direct contact of the eye, nose, mouth, or skin; or parenteral inoculation. Laboratory workers, who are routinely exposed to potentially infectious materials, are a high-risk group for occupationally related infections. Implementing practices that decrease the worker's exposure to potentially infectious materials can minimize the risk of infection.[4]

Laboratories should develop guidelines that address security issues. Only authorized personnel should have access to the laboratory. For laboratories using biological agents or toxins capable of causing serious or fatal illness, additional security measures may be required, such as locking all storage cabinets, refrigerators, incubators, and doors to sensitive areas.[5-7] The threat of theft and tampering of biological agents, specimens, drugs, chemicals, and confidential information should be assessed. The biological, chemical, and radiation levels of the laboratory should be considered when establishing a security policy. The current version of the *BMBL*[5] outlines additional security recommendations for laboratories.

Laboratory director responsibilities

The laboratory director is responsible for laboratory safety. This includes providing a safety manual for laboratory employees that details all aspects of the laboratory safety program (e.g., chemical hygiene plan, blood-borne pathogens plan, respiratory protection plan, safety committee, and safety training).[8] The safety manual should be readily available in work areas and specific to the laboratory's needs. A safety officer may provide guidance to the laboratory director, but the ultimate responsibility rests with the laboratory director.[1]

The laboratory director is responsible for ensuring the following items and tasks:[1]

- Adequate policies, procedures, and practices are in place to ensure the safety of laboratory employees, patients, and visitors
- All policies and procedures are reviewed and approved before use

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- Appropriate engineering and work practice controls are in place in the laboratory to minimize the hazards inherent in the laboratory
- All necessary personal protective equipment (PPE) is provided to laboratory staff
- All laboratory employees are provided with the necessary vaccinations and health monitoring
- The employees are informed of significant risks associated with tasks being performed
- Immediate medical attention can be arranged, on any shift, for incidents involving exposure to blood-borne pathogens or a chemical or biological agent

Laboratory safety officer

The lab manager should appoint a laboratory safety officer who has experience and credentials in the field of laboratory safety. For small laboratories, one individual in the facility should take appropriate courses to become knowledgeable in all laboratory safety requirements. The safety officer's main function is to provide guidance to management officials and supervisors who are responsible for providing a safe workplace for all employees. The safety officer is expected to make recommendations and should be authorized to stop activities that are clearly unsafe, but the laboratory director remains ultimately responsible for safety in the laboratory.[1]

The safety officer may be either the safety director of the healthcare facility in which the laboratory is located, or a specifically designated laboratory safety officer. In those laboratories that have a laboratory safety committee, the laboratory safety officer should be at least an *ex officio* member of this committee. If the healthcare facility has a single safety committee, the laboratory should be represented, and specific laboratory issues should be addressed. Laboratories should review local, regional, and national regulations concerning the composition of a laboratory safety committee and meeting frequency.[1]

The laboratory safety officer or the laboratory director is responsible for providing guidance and direction to all sites that submit specimens to the laboratory. All specimens are to be transported to the laboratory in a manner that follows government regulations and prevents contamination of workers, the public, and the environment.[1]

The laboratory safety officer is responsible for periodically reviewing (at least annually) and updating the laboratory's hazard identification system to ensure its relevance to known hazards.[1]

Fire hazard controls for use within the laboratory or laboratory units should be properly identified, and all doors leading into these areas should be marked, identifying specific hazards within. A recognized emblem system, such as the National Fire Protection Association (NFPA)[9] or Globally Harmonized System of Classification and Labelling of Chemicals,[10] should be used for this purpose.

Hazardous chemical waste disposal coordinator

One person in the laboratory needs to be designated to coordinate and be responsible for the laboratory's hazardous chemical waste disposal. The coordinator should evaluate hazardous chemicals based on their hazardous properties, according to national and/or regional regulations. The evaluations should be documented and available for retrieval. Disposal procedures for hazardous waste should be communicated to employees using language that ensures their understanding.[1]

Radiation safety officer

Any laboratory that performs tests using radioactive materials needs to meet the requirements of the U.S. Nuclear Regulatory Commission (NRC), including a radiation control plan. The laboratory director needs to appoint a radiation safety officer (RSO) who is responsible for administering the radiation control plan and ensuring the laboratory complies with NRC requirements.[1]

Responsibilities of the laboratory employee

Laboratory employees are responsible for the following tasks:[1]

- Complying with safety policies
- Reporting unsafe working conditions
- Reporting any accident or incident involving hazards
- Using PPE appropriately and whenever required

Conclusion

Implementing a laboratory safety program is an effective way of protecting laboratory workers from hazards such as laboratory-acquired infections, radiation, and other hazardous materials. Although there are several employee roles responsible for ensuring safety in the laboratory, the laboratory director is ultimately responsible for the safety of the laboratory under his or her guidance.

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Planning the future of diagnostic testing systems and cervical cancer screening

By Brooke Story

The global pandemic has impacted women's healthcare in ways that must be addressed immediately. It is imperative that every woman has access to cervical cancer screening. According to the Center for Disease Control and Prevention, an estimated 41% of U.S. adults have delayed or avoided medical care because of the pandemic[1], leading to a substantial decline in cancer screening[2].

In addition to the impact of the pandemic making screenings harder to get (or patients avoiding them based on lock-down constraints and fear of infection), changing patient management guidelines can make it difficult for patients and clinicians to keep up on new testing recommendations. Furthermore, income and education disparities continue to impact when and how women get screened and treated for health issues.

In the U.S., it is estimated that about 12,000 new cases of HPV-associated cervical cancer are diagnosed each year[3]. Often, Black, and Hispanic women have a higher likelihood of getting diagnosed with these types of cancers[4], possibly because of having less access to screening tests or follow-up treatment.

As more women in the U.S. get vaccinated against two highly dangerous strains of HPV (HPV 16 and 18) and those strains decrease in prevalence, it is increasingly crucial to identify the other high-risk HPV genotypes (an individual's collection of genes)[5,6]. Only an HPV assay with extended genotyping can identify and track high-risk HPV genotypes beyond HPV 16 and 18, including HPV 31 compared to other FDA-approved HPV tests[7-12].

The BD Onclarity™ HPV assay is the only FDA-approved assay available today that provides extended genotyping, including HPV 31[11-16]. Screening is evolving into using an HPV test with extended genotyping to ensure patients receive the most precise risk estimate for developing cervical pre-cancer and cancer per American Cancer Society guidelines.

Run on the BD COR™ PX/GX System, which integrates robotics and sample management software algorithms to automate the laboratory workflow from sample to diagnostic test result, the BD Onclarity™ HPV Assay with extended genotyping enables high-throughput labs to both enhance and standardize the quality of results, improving the patient experience.

The system itself is modular, scalable and designed to address multiple needs within laboratories for expanding molecular testing and increasing test volumes. Moreover, its onboard capacity for reagents and samples offers six to eight hours of unhindered system processing, eliminating multiple technologist interactions, therefore lessening the chance of human error. BD is actively working on further expanding the BD COR™ menu to help address women's health issues.

No other assay on the market today provides extended genotyping, which could be significant to patient care by unmasking other high-risk HPV strains[11-16]. Being able to identify extended genotypes specifically, as well as track those genotypes in a patient over time, allows for improved patient care and reduction of unnecessary additional testing. With extended genotyping, clinicians can recommend follow-ups rather than an immediate colposcopy that is not necessarily needed for the detected HPV strain. It is time to provide more advanced insight on women's health and more informed treatment options for clinicians in the U.S. It is time for this comprehensive diagnostic approach to be made the norm.

Brooke Story is the Worldwide President at BD Integrated Diagnostic Solutions. Visit go.bd.com/onclarity to learn more about BD Onclarity™ HPV Assay and BD COR™ System.

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Automating body fluid analysis

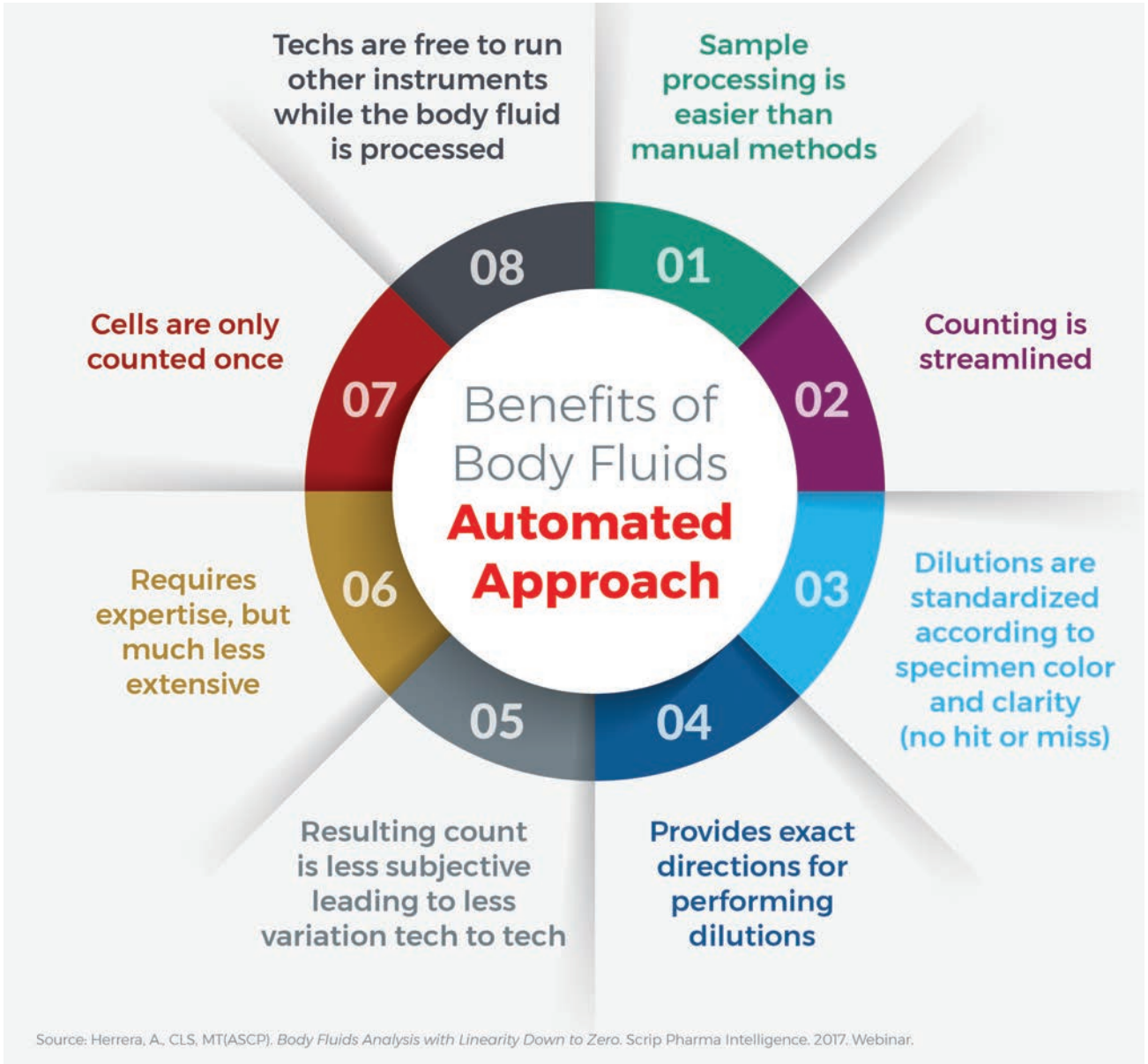
By Matt Rhyner, PhD, MBA

Body fluid cellular analysis is used by physicians to get substantial information regarding a patient's medical conditions, including inflammatory diseases, infection, hemorrhage and malignancy.[1] Traditionally, body fluid analysis is performed manually with an optical microscopy, which is considered the gold-standard method for cell counting.[2] However, manual counting is time-consuming, labor intensive, and subject to high inter-observer variability and potential poor reproducibility.[3] The burden of body fluids analysis is only increased with the high pressure, low resource environment we live in today.

The challenge for laboratories

Laboratories and healthcare systems across the world have been placed under sustained pressure during the COVID-19 pandemic.[4] This is exacerbated by the shortage of labor. In fact, according to Medical Laboratory Observer's 2021 annual salary survey of laboratory professionals, 80% of respondents noted shortages having a "moderate to large impact" on operational efficiency.[5] The challenge of labor shortages cannot be overcome easily as between 2007 and 2017, the number of active pathologists in the U.S. decreased by approximately 17.5%, a trend that has continued.[6] This dramatic lack of supply and demand equilibrium demands laboratories to do more with less, and this is where automation has a role to play — to help to reconcile this in equilibrium and deliver high-quality results.

Despite wage growth, the labor force participation rate remains near its lowest level since the 1970s. In the face of this shortage, hospitals are turning to automation.[7] Automated methods for body fluid cell counts have been rapidly replacing manual hemacytometer methods, while technological advances



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in hardware and software engineering have developed instruments with expanded analytical capabilities that enable processing multiple specimen types including urine, cerebrospinal fluid (CSF), peritoneal fluid, pleural fluid, synovial fluid, and lavages on a single analyzer.[8]

High quality body fluid analysis

Although electronic cell counters are available, they are usually ineffective for CSF and other body fluids owing in part to elevated background counts and other interference that can falsely elevate low cell counts.[9,10]

The introduction of automated methods of analysis has reduced interoperator variability and improved turnaround time and precision,[11] and many automatic analyzers have emerged in recent years, offering improved accuracy, efficiency, and standardization,[12] as well as rapid, reliable, and accurate counting of red blood cells (RBCs) and WBCs (differential) in cerebrospinal and other body fluids.[13] Beyond precision and reliability, automation can also provide insight for probable detection of multiple parameters, even in scant amounts, that could have been missed on manual analysis, like presence of yeast like cells, crystals, small round cells and others.[14]

Lower costs and labor for urinalysis

A clinical study that was done with an automated urinalysis analyzer in Spain that included a body fluid module showed that the comparison of annual costs for current practice versus the automated examination of urine samples found average cost savings of €340,003 per year. Assessment of body fluids using the automated analysis system would provide average annual savings of €1063. The use of automation would save 1,615 hours annually for laboratory personnel.[15]

Faster TAT for body fluid analysis

Manual screening of a urine specimen takes up to six minutes per sample while an automated system generates the result in around one minute.[14] This dramatically impacts turnaround time. Salem Hospital Regional Laboratory conducted a study to compare the number of tests that are resulted at 30, 45, and 60 minutes. The hospital found an improvement of 30% at 30 minutes, 9% at 45 minutes, and 3.2% at 60 minutes. The urinalysis staff also handled hematology duties, and the post-implementation improvement for CBCs was 44% at 30 minutes, 22% at 45 minutes, and 8% at 60 minutes.[16] In another hospital, automation helped to improve turnaround time with 92% of urines reported in 30 minutes from the time the laboratory received them.[17]

How to automate

There are several ways to automate body fluid analysis:[18]

- Method 1. Enhanced impedance counting is available on some hematology platforms and relies on the displacement of electrical field to provide information on particle size and volume. This aids in classifying particles.
- Method 2. Simple flow cytometry measures cell granularity, size, and light scatter. This can be performed on some hematology platforms. The laser detects size, volume and shape, which aides in classifying particles.
- Method 3. Particle and image recognition is a method in which the cells are counted by digital flow morphology, allowing the system to capture actual images of particles and classify them by size, shape, contrast, and texture. A visual inspection by a technician can be done after an analysis is complete if they need to identify real cells from artifacts.

Regardless of the methodology used, the benefits of automation are clear, and for laboratories, the most important one is that of lightening the load. And while modern urine analyzers have reduced some of the burden associated with manual body-fluid analysis, new innovations in body fluid analysis deliver faster speed to results and significant improvement in laboratory efficiencies.

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Artificial intelligence pushes lab automation forward

by Kara Nadeau

In a healthcare environment where clinicians and patients demand accurate diagnosis faster, laboratory professionals have turned to automated analyzer technology to replace time and labor-intensive manual analysis.

The latest advancements in this area include the application of artificial intelligence (AI) and machine learning (ML) to analyzer software. Today, most of these efforts are aimed at boosting efficiency in the face of ongoing shortages in skilled laboratory professionals. Labs are leveraging AL/ML to automate workflows, triage and prioritize samples, differentiate between conditions, verify results, and perform quality checks.

"Machine learning in microbiology represents a much-needed advance," said Glen Hansen, PhD, Medical Director Microbiology & Molecular Diagnostics Laboratory at Hennepin County Medical Center. "It's the distinction between robotics and software that can aid in decision making processes in the lab and this is a big distinction."

Glen Hansen, PhD

Looking forward, experts anticipate the use of AL/ML in automated analyzers will broaden in scope to encompass the entire testing continuum from research and development (R&D) through to proactive patient diagnosis and treatment.

In this article, manufacturers and users of automated analyzer technology share their insights on the current and future state of AL/ML, offering examples of where these advances provide benefit to laboratory professionals and patients.

Streamline workflows

As Brooke Story, Worldwide President, BD Integrated Diagnostic Solutions, explains, the flow of specimens through labs has typically required extensive handling by lab personnel both before and after a test. She describes how robotics and sample management software algorithms are now being applied to sophisticated molecular diagnostic instruments so that they can integrate and automate the complete molecular laboratory workflow from sample to result.

"BD has watched how specimens make their way through labs and we've seen how much handling was needed both before and after the test," said Story. "We also heard firsthand from customers that laboratory instruments have begun to manage them and their time instead of helping them improve efficiency of the lab. Systems that help address these needs, using AI and automation, give control back to medical laboratory scientists in high-throughput and large-scale laboratories."

Brooke Story

Story says cervical cancer screening with HPV extended genotyping has been a key focus area for complete molecular laboratory workflow automation given the hundreds of thousands of women who are tested each year.

"When robotics facilitates the processing of samples directly from liquid-based cytology vials, the creation of molecular aliquot tubes and assay testing — you cut out a lot of labor-intensive and error-prone manual processes," she explains. "Plus, being able to rely on onboard capacity for reagents and samples to provide hours of unimpeded system processing eliminates multiple technologist interactions currently required per shift."

Triage and organize specimens

According to Hansen, automation in plate reading and antimicrobial susceptibility testing are the most visible applications of AI/ML in laboratories today. He states that "AI/ML is currently being applied to microbiology plate reading, which has shown advances in accuracy and workflow efficiencies. Automated blood culture cabinets, which remove negative cases from the

workflow and reduce time to reporting, have also recently been widely accepted."

"Labs benefit from providing better 'tools' to the workforce and software and machine learning opportunities represent a chance to focus the work we do on cases that are of critical importance while targeting the work that we do in the lab to those aspects of reporting that require our attention," Hansen added.

Alanna Woodward, MLS(ASCP)CMSMCM, is Clinical Laboratory Microbiology Manager for UMC Health System, Lubbock, Texas, which has served as a primary testing site for an AI-enabled laboratory automation solution. She speaks to her experience with the technology in triaging plates with growth and without growth, saying, "AI software is much more sensitive than the human eye and can detect colonies on a plate that may be missed by some medical laboratory scientists (MLS), especially when the MLS is tired or distracted. AI isn't subject to the same constraints as humans, such as tired, distracted, aging eyesight, etc. AI has potential to be a great tool for the MLS if/when implemented well into the microbiology workflow."


Alanna Woodward, MLS(ASCP)CMSMCM

"We are using it (the solution) to triage plates with growth and without growth, so that the MLS can spend more time focusing on the cultures that need their expertise to interpret," Woodward added. "It doesn't make sense, especially with this technology, to utilize our highly trained MLS personnel to read/interpret no growth plates."

Urine cultures, which make up a significant part of many labs' daily workload, is one prime area for automated sample triage because the majority of samples either have no growth or non-significant growth and, therefore, don't require additional work-up, Story explains:

"Batch review and release of large volumes of plates with non-significant growth can help medical laboratorians focus their time and expertise on more clinically relevant tasks and complex specimens," Story said.

Achieve fast and accurate diagnosis

Hansen points to the "speed and accuracy with which molecular testing for COVID-19 entered the clinical space" as an example of how AI/ML can be leveraged to bring needed diagnostics to the lab in a faster, more accurate and less expensive manner, compared to previous development routes.

"Within the molecular space, AI was used to screen genetic material of COVID-19, providing a blueprint of the virus without ever having access to live virus. The application of AI for molecular test development allowed COVID-19 PCR test kits to be developed in weeks versus months," he states.

Differentiate between conditions

Experts say advanced automation of laboratory technology enabled by AI and ML can help labs differentiate between conditions, providing patients with more accurate results in a shorter time period.

While AI/ML is fairly new for analyzers used in routine diagnostics, Dominic Andrada, Sr., MS, Manager, Global Market Development, qPCR Molecular Diagnostics, Genetic Science Division, Thermo Fisher Scientific, says he has seen increased interest in the use of these technologies in future software versions to analyze data from multiple markers in a test panel or screening test.

"I have only seen a few conference posters or examples where AI/ML could help," Andrada explained. "It could potentially be used to help with pattern recognition of immunofluorescence assay (IFA) results for Antinuclear Antibodies (ANA) testing. This is a common test ordered to help with autoimmune diagnosis. This sounds intriguing to me, especially since IFA is a bit subjective and requires significant training to interpret the many different IFA patterns a sample could have."


Dominic Andrada, Sr., MS,

Bruno Larida, MS, MBA, Vice President, Marketing, Seegene Technologies, notes how AI/ML can be used in the design of analyzers that can handle multiplex tests that interrogate multiple disease biomarkers in a single sample, stating:

"Recent reports show that the flu and COVID-19, for instance, can occur simultaneously in a patient. Flu or other respiratory viruses, as well as COVID-19 symptoms, overlap, making a non-tested diagnosis, challenging. By using a multiplex test and analyzer, designed using AI, practitioners would be able to detect and differentiate between multiple targets, eliminating the need for multiple tests. Additionally, these diseases can be detected early and, therefore, treated quickly. Using AI/ ML would also reduce the time taken to design and manufacture assays, from months to a few weeks."


Bruno Larida, MS, MBA

Verify results

According to Eric Carlsgaard, MS, Senior Product Manager Informatics/Cloud at Beckman Coulter Diagnostics, in a world where there is a shortage of labor and an increased demand for testing, AI is poised to help by reducing manual intervention. He points specifically to AI automation in test result verification.

"A routine urinalysis increases workload when technicians must confirm the analyzer's findings or identify unique particle types (manual microscopic reviews are time-consuming and can take up to six times longer per sample than with an automated system[1]),"Carlsgaard explains. "Digital flow morphology technology with Auto-Particle Recognition Software can enable laboratories to deliver standardized results. This technology isolates, identifies, and characterizes urine particles to provide immediate, accurate, and reproducible results verified directly on the screen."


Eric Carlsgaard, MS

Automate quality assurance checks

Noting how clinical laboratories have taken significant steps in the use of rule-based functions to improve efficiencies, Carlsgaard describes how AI/ML can be used to streamline quality control (QC) checks."For example, if QC is being run on an instrument every eight hours, it's a standardized check. With auto QC, patient results are reviewed continually to determine if they are trending toward normal or extended ranges — so that the laboratorian can be alerted if some thresholds are crossed, which we know, based on rules and past data, may lead to problems with QC. Because if we wait for the regular QC, which is conducted every eight hours, we may find out what's wrong after it's too late and must invalidate and re-run a significant number of tests," he said.

Enhance staff training

Another area where Woodward and her team at UMC Health System are leveraging AI and ML is in the training of new lab employees. Because the solution they are using captures images of culture growth, they can save and store rare organisms for training purposes.

"There are situations that only arise every 5+ years in the microbiology laboratory, and now we can use the software to give our new people a leg-up in recognizing these situations," Woodward explained. "Prior to the AI/ML, we depended heavily on having a long-term experienced MLS available to recognize and help the newest MLS. This meant reading cultures for multiple days and scheduling multiple people to read behind each other to not miss these rare situations. Now, with the AI/ML, we can give the newest MLS employees that knowledge earlier in their career before they've potentially missed a critical result for a patient."

When asked where they see AI/ML being used in the future when it comes to lab analyzers, our experts pointed to everything from research and development (R&D) efforts to identifying organisms in patient samples.

Rapid R&D

"Machine learning-based diagnostic analyzers will be the key to simplifying and automating the R&D process, allowing scientists to respond rapidly to widespread testing needs," said Larida, pointing to the use of ML and AI in the battle against SARS-CoV-2. He notes how scientists have already leveraged these advanced technologies in diagnosis, treatment and vaccine development efforts throughout the COVID-19 pandemic.

Colony identification

Woodward believes the "sky's the limit" when it comes to the application of AI/ML in the lab environment. She hopes to see AI/ML becoming more robust in recognizing colors and growth patterns to determine how many colony types are growing in any given culture.

"AI/ML is able to offer identification suggestions, based on the color that grows on that media type. However, I'd like to see this expanded out to other routine media types so that we can utilize the AI/ML in more culture sources such as tissue, wound, respiratory, etc.," Woodward explains. "I'm not sure I'm totally comfortable with AI/ML being able to identify organisms from the routine agar yet, but being able to differentiate multiple colony types and give the MLS an idea of how many organisms they may be looking for would be very helpful in interpreting cultures on the first read."

Meaningful worklists

Story sees AI and ML being leveraged to "automatically organize specimens into meaningful worklists driven by user-defined expert rules for critical criteria such as 'high risk patients' or 'complex specimens.'" She explains how this would help labs professionals work more efficiently by prioritizing their efforts on the most critical and complex specimens, stating, "Reading plates is one of the laboratory tasks that requires the most skill and, given the decrease of skilled technicians entering the workforce and the strain on resources created by the COVID-19 pandemic, it's that much more critical for labs to be able to be more efficient by taking advantage of automation."

Disease differentiation

As Andrada noted, AI/ML holds tremendous potential for running tests for autoimmune diagnosis. He said, "I expect to see AI/ML used in future algorithmic analysis of data to help or increase the confidence of diagnosing a specific autoimmune disease from several potential disease states."

Patient feedback loops

Carlsgaard believes the benefits of AI-enabled technologies will go beyond operational efficiency to transform patient care delivery. "With AI, we can detect patterns hidden in large amounts of data and predict the future, which is the backbone of both automation and decision support. AI-enabled technologies, in the future, will come together to create data feedback loops to help improve not only efficiencies but transform patient care from 'sick care' to 'well care.' The journey has already begun," he said.

Considerable change

Hansen comments on how the application of AL/ML in clinical microbiology could contribute to the considerable change we are already seeing in the field, stating, "The reality of the global Sars2-covid-19 pandemic has forever shaped the field of clinical microbiology and placed emphasis on accuracy, efficiency, and support of a critical underappreciated workforce in need of new diagnostics support tools. However, it's also the craziest of times when one realizes the greatest opportunities for change and adaptation."

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Multi-system inflammatory syndrome is the storm that follows COVID-19 waves

By Bruno Larida, MS, MBA

While vaccines and continued public health measures are helping to control the COVID-19 pandemic and move us into a new normal, a troubling new syndrome has emerged. In the spring of 2020, doctors first noted a constellation of symptoms that suggested hyperinflammation and affected multiple organs at once in children, following exposure to or infection with SARS-CoV-2. The symptoms are not linked to any other microbial cause.

This syndrome was termed multi-system inflammatory syndrome in children MIS-C.[1] As similar symptoms began to rise in adults,[2] it prompted nomenclature that would include adults (MIS-A). At the population level, MIS cases appear to peak approximately one month after waves of elevated COVID-19 cases. While MIS is rare, it can be severe and require admission to the ICU. The underlying cause for MIS remains unclear, however, minimizing COVID-19 transmission using well-documented public health measures is a logical path towards better MIS management. In this article, we provide an overview of MIS and how communities can work to curb its prevalence.

Multi-system inflammatory syndrome (MIS) is a serious condition associated with COVID-19 in which different organs become inflamed. The syndrome can occur in children and adults.

MIS in the United States

As of November 1, 2021, the Centers for Disease Control and Prevention (CDC) reported approximately 5,000 cases of MIS-C out of approximately six million COVID-19 cases in children.[3] So far, four MIS-C case peaks have occurred within the United States. Each MIS peak has followed a corresponding COVID-19 peak by approximately four weeks. Prior to the most recent COVID-19 wave in the summer of 2021, a relatively consistent relationship existed between the magnitude of COVID-19 and MIS-C spikes. But during the summer of 2021, MIS-C cases dropped to their lowest levels compared to COVID-19 cases. Researchers are still unsure why the pattern changed.

Sixty-one percent of the CDC's reported MIS-C cases occur in non-Hispanic Black, Hispanic, or Latinx children. Additionally, similar to how COVID-19 has spread, most reported MIS-C cases are in male patients. Compared to adults and unlike the incidence of COVID-19, the inflammatory syndrome occurs more often in younger patients. The median age of reported cases is 9 years old, and half of the reported cases fall between 5 and 13 years old.

As we noted above, MIS has been more challenging to track in adults since the syndrome can be difficult to discern from other occurrences of COVID-19-related hyperinflammation. In spite of this, it is clear that MIS-A is rare, with only a few hundred reported cases[2] as of this writing. Similar to MIS-C, MIS-A appears to cluster in younger patients. The median age of MIS-A patients[2] is 21 years old and 50% of cases fall between 19 and 34 years old.[4] Most MIS-A cases occur in male, non-white patients.[4]

MIS around the world

Apart from the United States, MIS also has been reported in Europe.[5] As of this writing, experts at the World Health Organization (WHO) are unable to determine why cases appear to be concentrated in North America and Europe. More data will need to become available to fully understand the geographical occurrence of MIS.

How severe is MIS?

While MIS-C is rarely fatal, most patients experience severe symptoms and require aggressive treatment including ICU care and mechanical ventilation.[6] Out of the 5,526 MIS-C cases reported as of November 1, 2021, there have been 48 deaths.[3]

Though severity estimates may be distorted by the difficulties in finding instances in adults, especially if the adult has pre-existing health issues, MIS-A looks to be more lethal than MIS-C. Researchers reported that 57% of MIS-A cases were admitted to intensive care, with 47% requiring respiratory support and 7% of patients losing their life.[2]

Signs and symptoms of MIS

Patients with MIS commonly exhibit the following symptoms.

- Fever (for four or more days).
- Gastrointestinal symptoms including nausea, vomiting, and diarrhea. In a recent study, a whopping 71% of MIS-C patients reported gastrointestinal problems
- Skin rash
- Sores in the mouth
- Conjunctivitis
- Low blood pressure, which may lead to shock
- Heart symptoms including inflammation in the tissue lining the heart and the heart tissue itself.

In more severe cases, heart symptoms associated with MIS may lead to heart and kidney failure. Heart failure accounts for a large portion of the overall mortality in MIS.

What triggers MIS?

The cause of MIS is still unclear. As mentioned above, nearly every case of MIS has been associated with prior SARS-CoV-2 infection or exposure and is usually not the result of some other microbe. In fact, patients consistently show a time course in which MIS symptoms follow several weeks after they themselves tested positive, had COVID-19 symptoms or were exposed to someone who did. MIS cases tend to arise about four weeks after the onset of COVID-19 symptoms.

One study looking at what might cause MIS suggests that an unknown superantigen associated with SARS-CoV-2 triggers an excessive and abnormal immune

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response. Researchers have shown that the immune systems of patients with severe MIS tend to have a specific phenotype.[7] That phenotype is marked by increased and persistent immunoglobulin G (IgG) antibodies, a reduced white blood cell count, reduced neutrophil count, reduced total T cell count, and increased CD8+ T cell activation. CD8+ T cells are activated as part of the body's antiviral response and are responsible for "killing" the viruses. The specific superantigen[8] that triggers this heightened immune response is still unknown. Some researchers have suggested that components of the infamous SARS-CoV-2 spike protein might trigger the response, while others suspect unknown secondary microbes or opportunistic microbes (ones that typically might not cause disease but become pathogenic secondary to another infection), might be the culprit.

Another theory traces MIS symptoms to SARS-CoV-2 related antigens leaking from the intestines and into the bloodstream. A recent study found that MIS-C patients exhibit detectable virus within their stool several weeks after initial infection. Researchers suspect that the release of a protein called zonulin makes the gut "leaky" and, thus, allows the antigens to escape the intestines and into the bloodstream.[9]

While these studies offer some insight into why MIS may happen following COVID-19 infection or exposure, there is still a lot to learn.

How is MIS diagnosed?

Because the symptoms are non-specific, diagnostic criteria for MIS still varies from one institution to the next. Nonetheless, an American College of Rheumatology guideline[10] states that if an individual has had a recent SARS-CoV-2 infection or exposure and reports an extended fever, skin rash, gastrointestinal symptoms, and conjunctivitis, it is likely they're experiencing MIS.

Physicians evaluate these symptoms while considering other possible infections and other inflammatory diseases. Diagnostic studies might also include imaging and blood tests to study antibodies and blood cell counts. An echocardiogram might be used to study and diagnose heart dysfunction.

How is MIS treated?

Currently, there is little evidence that one optimal treatment works for treating MIS.

Nonetheless, treatment of MIS can be divided into two broad strategies:

- multi-system supportive care
- anti-inflammatory therapies.

MIS patients can present or develop complications in various systems. Thus, a multidisciplinary team of healthcare providers might be managing a case. Physicians may manage MIS patients using immune-modulatory therapy such as intravenous immune globulin (IVIG) and glucocorticoids.[11] So far, these two treatments have been widely used to treat MIS with success.

Curbing MIS

Like COVID-19, there is still so much to discover about MIS in adults and children alike. Yes, the condition is rare, but there is very little to predict who might develop the syndrome beyond a prior or current positive COVID-19 test. And because it affects multiple organs with irregularity, it is more important than ever to work at slowing or preventing the spread of the virus.

During the thick of the COVID-19 pandemic and as the world reopened, widespread routine testing was critical for slowing the spread of the virus. The prevalence of free testing in many communities and accessible at-home testing measures can still help individuals self-quarantine or get COVID-19 treatment early. At a community level, detection of COVID-19 waves, through testing, can concurrently warn of an impending increase in MIS cases. At the individual-case level, knowledge of your COVID-19 status can help doctors determine if they should be looking for symptoms of MIS so it can be treated early.

Community surveillance using wastewater testing is helping cities catch large outbreaks early. All these tools have helped communities and countries to blunt the severity of the pandemic. In doing this, we can also curb the incidence of MIS. Collectively, the tools listed above have also helped to identify MIS and its relationship to COVID-19 and illuminated parallel cyclic patterns in COVID-19 and MIS cases.

In conclusion, though vaccination has dramatically improved the course of the pandemic, continued vigilance is important. MIS can be a severe, even life-threatening, condition, but like the broader pandemic, this syndrome can be prevented with broad testing and informed policy decisions that minimize virus spread.

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Establishing expiry date for clinical diagnostic reagents

Alireza Ebrahim, PhD, Karl DeVore, BA, and Tim Fischer, MS

Product shelf life is an essential product performance requirement that, along with other design requirements, are used to determine the safety and efficacy of a clinical diagnostic reagent, whether they are made by a laboratory or commercially produced. Product shelf life can be determined following various domestic and international guidance documents.

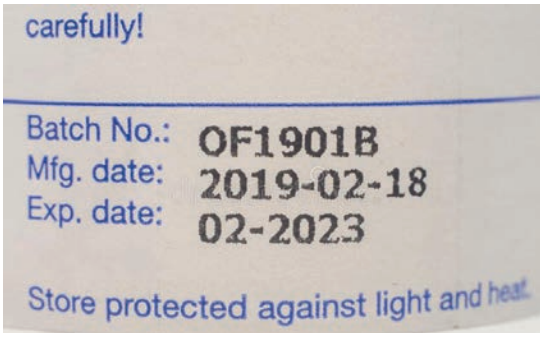
In this article, we provide a high-level overview of how product shelf life is determined and how accelerated stability studies can be used to estimate product shelf life. We also discuss some of the limitations and key considerations lab should consider before using an accelerated stability model to estimate shelf life for lab-developed reagents.

The discussion also will help laboratory employees understand how their suppliers determine the shelf life of products, which has the potential to impact test results.

Product expiration date

Establishing the expiry date (expiration or shelf life) for in vitro diagnostic products — such as reagent kits, calibrators, and quality controls — is a key quality and regulatory requirement for these products and is required by national and international regulatory agencies.

Since laboratory developed tests (LDTs) may be subject to these design control requirements, laboratories also need to demonstrate that specified design requirements, such as shelf life and stability, are also met during the design verification process. (LDTs are assays that are intended for clinical use and are designed, manufactured, and used within a single laboratory.[1])



There are two methods for determining product expiration dates: real time stability studies and accelerated stability studies

The expiration date shown on the product label, as well as the instructions for use (IFU), indicate that the product will perform as designed and was developed to meet product design requirements and user needs within the time period — from the manufacturing date to the expiration date (last day of use).

There are several domestic and European standard and guidance documents providing recommendations and direction on how to design and execute stability experiments to generate the necessary stability data, which could be used to establish shelf life at the recommended storage conditions for these products. For example, the Clinical and Laboratory Standards Institute (CLSI) documents for EP25-A2 and EN ISO 236403, provide guidance on the establishment and verification of shelf life stability claims for quantitative and qualitative in vitro diagnostic products.

There are typically three questions that need to be answered for defining stability:

1) Which product characteristics/metrics are considered key performance indicators?

2) What are the acceptance criteria or changes for each characteristic/metric that can be tolerated?

3) What is the statistical confidence level required for analyzing stability results?

Since each of these characteristics is different for various diagnostic reagents, it is not practical to provide a single protocol that is appropriate for all diagnostic products. For example, the stability acceptance criterion for a calibrator, quality control and a wash buffer of a reagent kit are very different and may vary from ±1% to ±20% of the initial value for the key measurement of these products. The appropriate sample size and statistical confidence level may also vary, depending on the intended use of the reagent.

Appropriate statistical methodology (for example, detection of outliers, determination of replicate runs, etc.) should be used to ensure that quality results are generated for shelf life stability determinations. Therefore, manufacturers of diagnostic reagents, as well as laboratory professionals involved in development of reagents for LDTs, should establish standard operating procedures for performing stability studies consistent with guidance and standardization documents, such as CLSI EP25-A and EN ISO 2346, and select and monitor physical, chemical, biological, or microbiological properties of the product in which their changes (for example, potency, activity, or concentration) impact the product shelf life and ultimately the safety and efficacy of the product.

Stability studies for shelf life determination

Expiry dates are assigned to in vitro diagnostic reagents, calibrators, quality controls, and other components by the manufacturer based on experimentally determined stability properties. Typically, two approaches can be used to generate the stability data necessary to establish an expiration date for a diagnostic reagent.

Real time stability studies can be done by storing the reagent at its recommended storage temperature and regularly sampling and testing the reagent at defined timepoints and periods of time, typically 20% longer than the desired expiration date. For example, for a reagent with a desired shelf life of 1 year, the reagent may be tested weekly or monthly for 14 months (2 months past the desired shelf life). On the other hand, accelerated stability studies can be designed and performed at elevated temperatures compared to normal storage conditions for products, or the storage temperature on the product label, to observe changes in product performance, mainly stability, more rapidly than what would be seen under normal storage conditions.

For example, a product with a storage temperature of 2-8°C, can be exposed to temperatures such as 35°C or 45°C to accelerate the degradation process. Since many diagnostic reagents have long shelf lives, in the order of 1 to 3 years, the shelf lives for these products are first estimated with accelerated stability studies during the product development process, which usually take several weeks, and are later supported with real-time stability studies that require several years.

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Accelerated stability studies are based on the Arrhenius Equation, which describes the mathematical relationship between the rate constant of a chemical reaction, the reaction temperature, and the activation energy for the molecule under study.[4] Generally, for every 10-degree rise in temperature (°C), the reaction rate doubles. Therefore, by increasing the temperature at which the product is stored, you can increase the reaction rate and degradation rate of a molecule or a product. For example, assuming an activation energy of 20 kcal/mole/degree, storing a product at 35 °C for 30 days is approximately equivalent to storing the same product at 5 °C for 3 years, in terms of degradation rate.

Key considerations when designing accelerated stability studies

In a recent article, some of the limitations of accelerated stability studies were highlighted.[5] Exposure of reagents to raised temperatures during an accelerated stability study may create conditions and chemical environments that produce degradation in the product. However, this degration may not be observed during a real time stability study when the product is stored under normal and unstressed storage conditions. This is especially true when the normal storage temperature of the product is at -10°C or lower, which means the product is frozen and is in a solid state, and accelerated stability studies are performed at higher temperatures (for example, 35°C), which means the product is now in the liquid state. For this reason, the prediction from the accelerated stability model may not be accurate and may result in overestimation or underestimation of shelf life.

Some other factors that may result in poor agreement between real time and accelerated stability studies are increased solubility and evaporation due to exposure of a reagent to higher temperatures, susceptibility of certain measurands to photodegradation more than thermal degradation, changes in residual moisture in lyophilized reagents, increased or decreased proteolytic and enzymatic activities of liquid reagents due to exposure to raised temperatures, and changes in pH of the reagent due to escaping of dissolved gases and organic volatiles such as carbon dioxide, oxygen, and alcohols.

Conclusion

Generally, accelerated stability studies predict stability and shelf life accurately, and there is reasonable and acceptable agreement between the actual shelf life from real time stability studies, which are performed over several years, and the estimated shelf life from accelerated stability studies, which are performed in a shorter period of time, typically over a few weeks. However, exposure of reagents to elevated temperatures during the accelerated stability study may create conditions and chemical environments that produce degradation in products that may not be typically observed during real time stability studies when the product is stored under normal and unstressed storage conditions.

Product developers and labs should assess the applicability and suitability of the accelerated stability studies for their products by performing a few simple and fast experiments and consider the shortcomings of the accelerated stability studies prior to fully utilizing the model to estimate or establish product shelf life. For example, designing and executing pre-accelerated stability experiments, by storing key components of a reagent such as the liquid buffer system at the

recommended storage temperature of the product and also at its equivalent raised temperature (for example, 9 days at 5°C which is approximately equivalent to 6 hours at 35°C) and comparing a key chemical parameter such as pH for the two conditions may provide insight into if the raised temperature used in the accelerated stability studies creates a change in pH that is not normally observed during real time stability studies. The results of the rapid study may suggest that the accelerated model may not be appropriate for estimating shelf life for a reagent and, consequently, will avoid an accelerated stability study with poor predictive value.

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FDA guidance on using expired lab products

By MLO Staff

The U.S. Food and Drug Administration (FDA) offered guidance on the use of expired products in October 2021[1] due to shortages during the COVID-19 pandemic. Many critical supplies weren't available due to supply chain shortages, shipping delays and manufacturing problems, but testing needed to continue.

Throughout the COVID-19 response, laboratories have faced limited reagent, kit and material inventories, with some reagents approaching or passing expiration dates. The FDA offered guidance, saying that critical supply shortages may necessitate the use of expired reagents to maintain testing.

According to the FDA, a medical product is typically labeled by the manufacturer with an expiration date. This reflects the time period during which the product is expected to remain stable, or retain its identity, strength, quality, and purity, when it is properly stored according to its labeled storage conditions.

In some cases, testing has shown that certain properly stored medical products can be used beyond their labeled expiration date if they retain their stability. The FDA is engaged, when appropriate, in various expiration dating extension activities.

During the COVID-19 public health emergency, the Centers for Disease Control and Prevention (CMS) will allow laboratories to use expired COVID-19 test kits, reagents and swabs, as long as the appropriate testing and documentation is maintained and the manufacturer's instructions do not prohibit use of the expired material (see CMS FAQ #27 [12-17-2020]) (CMS, 2020).[2] This guidance stated, "When in-date reagents are unavailable, it may become necessary to frame written policies for their temporary use beyond their expiration dates until non-expired supplies become available. Under no circumstances, however, should a laboratory adopt policies that would allow for the regular use of expired reagents." Thus, laboratories may use expired supplies until non-expired supplies become available provided that they put policies and procedures in place to ensure the reagents are performing as expected (e.g., ensuring that any expired supplies pass quality control tests with each assay run).

Guidance offered by the FDA

The FDA says laboratories should establish written policies and procedures regarding use of expired reagents, and consider the following key points

- When possible, request that the manufacturer provide a letter of expiry extension for the reagent or test kit, or document in writing that the manufacturer would vouch for the accuracy and reliability of the reagents while in use past the expiration date.
- Place these letters in the laboratory's reagent QC binders. It is important to note that regardless of CMS enforcement discretion, laboratories remain responsible for ensuring the accuracy of their test results.
- Setting expiration dates is historically the manufacturer's responsibility. Expiration date establishment requires considerable effort, particularly for a testing laboratory during a pandemic.

Expiration date practices are outlined in: CLSI EP25-A Evaluation of Stability of in vitro Diagnostic Reagents and Approved Guideline and ISO EN 13640, Stability Testing of in vitro Diagnostic Reagents.

If using expired reagents in a laboratory, QC must be closely monitored and consider doing additional QC. Shortages of supplies and reagents may necessitate additional verifications or validations to add new specimen types, media types or other testing components.

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Assays are key tools to diagnose diseases

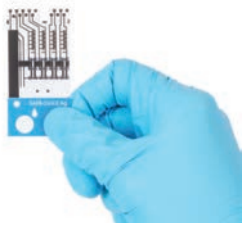
Assays are at the core of any clinical laboratory operation. Indeed, more than 7 billion lab tests are performed in the United States each year, according to the American Clinical Laboratory Association.[1]

In 2019, Medicare alone spent $7.68 billion on lab tests, ranging from HbA1c assays to genetic tests associated with various types of cancer. When measured by total spending, the most common tests among Medicare beneficiaries are blood tests, including those measuring a comprehensive group of blood chemicals; lipids, thyroid stimulating hormone, and a complete blood cell count.[2]

But a conversation about lab testing is not complete without mentioning SARS-CoV-2. Since the beginning of the COVID-19 pandemic in 2020, U.S. clinical labs have performed more than 673.5 million tests to detect the virus, the Centers for Disease Control and Prevention (CDC) reported.[3]

Medical Laboratory Observer features a small sampling of assays in this issue.

SARS-CoV-2 or flu assays



Microfluidic immunofluorescence assay

The LumiraDx SARS-CoV-2 Ag Test is a rapid microfluidic immunofluorescence assay for use with the LumiraDx Platform intended for the qualitative detection of the nucleocapsid protein antigen to SARS-CoV-2 directly from anterior nasal swab and nasopharyngeal swab samples collected from individuals suspected of COVID-19 by their healthcare provider within the first twelve days of symptom onset or from individuals without symptoms or other epidemiological reasons to suspect COVID-19. **LumiraDx**

Rapid flu test

The Immunocard STAT! Flu A&B is a rapid qualitative test for the differentiated detection of influenza A and B directly from nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/washes specimens from patients with signs and symptoms of respiratory infection. Results in 10-15 minutes for positives and 15 minutes for negative samples. It is a CLIA-waived test. **Meridian Bioscience**

Lateral flow antigen test

The Sofia 2 SARS Antigen FIA is a lateral-flow test, read by Quidel's Sofia 2 Fluorescent Immunoassay Analyzer, for rapid detection of SARS-CoV-2 from anterior nares specimens. It delivers accurate, objective, automated results in 15 minutes. Workflows include a walk-away mode for individual tests and read-now mode for batch processing. **Quidel**

SARS-CoV-2 variant detection

The NEXTFLEX Variant-Seq SARS-CoV-2 Kit v2 detects all mutations associated with SARS-CoV-2 variants in a PCR-positive sample. With the incorporation of normalization beads, this kit reduces turn-around time by three hours. 1,536 UDIs incorporated in the kit enable the sequencing of 6,000 SARS-CoV-2 libraries in one flow cell. **PerkinElmer**

PCR assays

Seegene's high multiplex real-time PCR assays include research-use-only tests designed to detect SARS-CoV-2 variants (including Delta and Omicron). The tests are compatible with Seegene STARlet, which is a liquid-handling workstation designed to manage primary sample tubes to nucleic acid (NA) extraction and PCR setup. **Seegene**

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84	26.15	0.897	3.4	29.94	0.950	3.2	56.09	1.313	2.3
84	8.57	0.391	4.6	102.94	3.428	3.3	111.51	3.474	3.1
84	104.54	3.313	3.2	10.07	0.561	5.6	114.61	3.455	3.0

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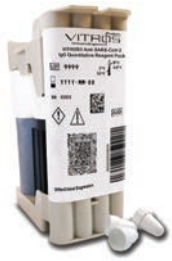

IgG antibody test

Ortho Clinical Diagnostics' COVID-19 IgG Quantitative Test is an antibody test calibrated to the International Standard for anti-SARS-CoV-2 IgG antibodies from the World Health Organization (WHO). It is a standard tool to measure antibody response and intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2. **Ortho Clinical Diagnostics**

Respiratory panel

The QIAstat-Dx Respiratory SARS-CoV-2 Panel, which runs on the QIAstat-Dx Analyzer, uses real-time PCR to detect 21 respiratory pathogens in about an hour. The QIAstat-Dx Analyzer and cartridges are designed as a closed system that contains all necessary reagents, ensuring a fast and easy workflow. **Qiagen**

SARS-CoV-2 and flu combo test

The Aptima SARS-CoV-2/Flu Assay is available for simultaneous detection and differentiation of three respiratory viruses - SARS-CoV-2, influenza A and influenza B. Hologic's multiplex test runs on Hologic's fully automated Panther system, which provides initial results in approximately three hours and can process more than 1,000 tests in 24 hours. **Hologic**

Point-of-care test

The CLIA-waived Acucy Influenza A&B Test on the Acucy System is a rapid (result in 15 minutes) chromatographic immunoassay that provides clinicians with flexibility in the workflow, with read-now or walk-away mode, easy transfer of results to the electronic health record (EHR), and standardized results for improved patient care. **Sekisui Diagnostics**

Miscellaneous assays

HPV assay

The BD Onclarity HPV Assay with extended genotyping can individually identify and track persistence for high-risk HPV genotypes beyond 16 and 18, including HPV 31, which poses a similar risk for cervical pre-cancer and cancer, as compared to HPV 18. **BD**

Alzheimer's disease assay

The Randox apolipoprotein E4 (ApoE4) assay saves laboratories time and money by eliminating the requirement for genetic ApoE4 testing. The direct determination of ApoE4 status from a plasma sample can be applied to determine Alzheimer's disease in basic clinical research, as well as in personalized medicine. The test runs on the Evidence Investigator analyzer. **Randox**

Body fluid assays

Beckman Coulter's body fluids module, with linearity down to zero, provides a fully automated method for the analysis of RBC count and nucleated cell count in cerebrospinal, synovial and serous fluids. Digital flow morphology technology isolates particles in body fluids to provide immediate, accurate, and reproducible results that can be verified on screen on the DxU Microscopy Series. **Beckman Coulter**

Bacterial immunoassay

Hardy Diagnostics is the United States' authorized distributor of the NG-Test CARBA 5. CARBA 5 is a rapid (15-minute) multiplex immunoassay for the detection and differentiation of the five most common carbapenemases from bacterial cultures of Enterobacterales and Pseudomonas aeruginosa. Thet test is manufactured by NG Biotech. **Hardy Diagnostics.**

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LABORATORY INNOVATOR

Innovations in transfusion medicine

By Linda Wilson

Scott Bush, MS, has been the CEO of **SunCoast Blood Centers** since September 2011. Before that, he held numerous other positions at both SunCoast and the American Red Cross. Bush holds a master's degree in nonprofit management from the University of Central Florida.

How did you become involved in the work of SunCoast Blood Centers?

Sixteen years ago, I joined the SunCoast Blood Centers as director of donor services and recruitment, and three years later I was appointed chief operating officer. In 2011, I became CEO. I'm honored to lead a team of 150-plus employees. I'm also a regular donor, having donated over 120 times — or more than 15 gallons of blood products.

Will you describe how SunCoast's activities have changed and broadened as the field has evolved from blood banking to transfusion medicine?

The SunCoast Blood Centers are known for being a leader within the blood donation industry. SCBC was the first blood center in the country to adopt and implement pathogen reduced technology for our platelet products. Pathogen reduction closes the window period on viruses (when an infection in a donor cannot be detected through testing), such as HIV, and destroys or inactivates bacteria, making these the safest platelet products in the world. As a community-oriented blood center, we wanted to provide the best and safest blood products to the hospitals that we serve. In 2020-2021, we were recognized as a national surge center because we manufactured and distributed over 15,000 therapeutic doses of convalescent plasma locally and to any COVID-19 hot spot in the country. All of this was provided free to the patient and to the hospital. We have expanded our patient testing platforms to include genetic compatibility testing for complex patient workups, while improving turnaround time to the hospitals. We also created the nation's only 1:1 Donate-at-Home donor program in which we send a phlebotomist directly to a donor's residence. This program is growing. There is no waiting in lines, and it is a perfect solution for those individuals who have reservations about heading into a public setting to donate blood. Lastly, each blood donor receives a complimentary COVID-19 antibody screening, which will notify our donors about the presence of antibodies and allow them to monitor their antibody status as they continue to donate with SunCoast Blood Centers.

How do you think the work of blood centers will evolve over the next 3-to-5 years?

As new technology and innovations emerge, many blood centers have already been evolving more into transfusion medicine and cellular therapies. Blood centers are far more complex and have a wider array of product and service offerings to hospitals, clinicians, and research firms than ever before. Though the core of any blood center is blood donation — as well as the manufacturing and distribution of those biological products to the hospitals that depend on them — blood centers have figured out that they can fill unique niches within transfusion medicine and cellular therapy. Blood centers keep meticulous records and manage highly regulated manufacturing practices, which uniquely positions them to step into the transfusion and cell therapy space. Therefore, over the next several years you will see blood centers take a much deeper role in supporting and participating in various clinical trials and expanding to new product lines and therapies such as CAR-T cell products for various cancer studies.

SunCoast recently joined the Blood Emergency Readiness Corps (BERC). Will you explain the BERC's mission?

On December 1st, we announced that the SunCoast Blood Centers have been tapped to be Florida's partner of the Blood Emergency Readiness Corps (BERC), a cooperative of 16 local blood centers from 29 states that have committed to collecting extra blood units on a rotating "on call" schedule. The extra blood products will be held in reserve for any critical-need scenario, such as a mass shooting or natural disaster. Once BERC is activated, blood will be shipped immediately to the impacted area.

Why do you think BERC's work is important and why did SunCoast join now?

The nation's blood centers have faced widespread blood shortages in recent months, creating a severely strained national safety net for mass traumas and other high casualty disasters. As the Suncoast's exclusive, local blood supplier, we help BERC to be proactive in its emergency planning, rather than rely on an increasingly unstable backup supply plan.

What other steps has SunCoast taken to address shortages in blood donations during the COVID-19 pandemic?

Our exclusive, Donate-at-Home service was birthed out of necessity during the pandemic. The service provides donors with a safe, socially distant, and a one-to-one personalized donation experience from the convenience of the donor's driveway. The pandemic shut down businesses and schools — about 60% of our collections were done on our mobile buses our mobile units; high schools accounted for 20% of our collection. The day everything shut down, we lost about 95% of our blood drives. Organizationally, we also pivoted by providing convalescent plasma and by offering free COVID antibody testing to all donors. As a result of these efforts, we were able to address the blood needs of patients and hospitals in the region. During this time, we also added more medical centers like the Moffitt Cancer Center, the new Sarasota Memorial/SMH-Venice Hospital and the new Sarasota Memorial/SMH-Jellison Cancer Institute.

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LumiraDx SARS-CoV-2 Ab Test has not been FDA cleared or approved but has been authorized for by FDA under an EUA for use by authorized laboratories.The LumiraDx SARS-CoV-2 Ab Test has been authorized for use only for detecting the presence of total antibodies to SARS-CoV-2, not for any other viruses or pathogens.This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
*Frederick National Laboratory for Cancer Research (FNLCR), sponsored by the National Cancer Institute (NCI) , see LumiraDx SARS-CoV-2 Ab Test Product Insert for additional study details.

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