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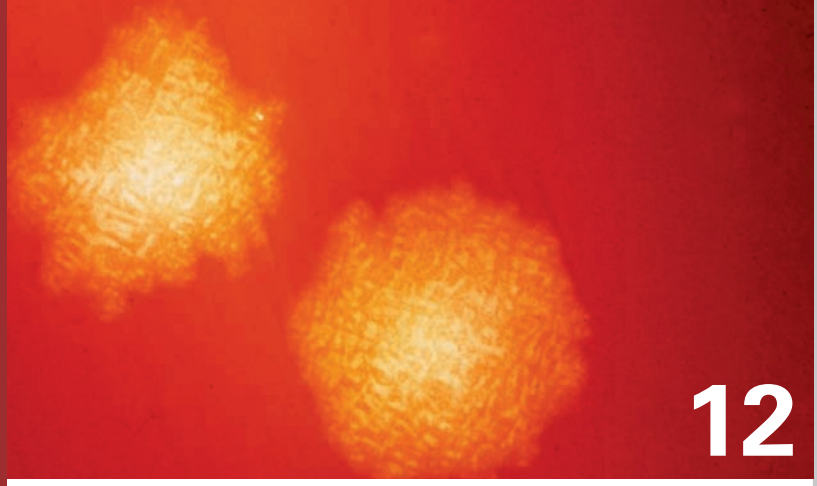

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Doing well by doing good

This issue's Management Matters article, "How healthcare CRM can drive profitability and efficiencies in toxicology labs," by Diane Janowiak, BS, MT(ASCP), argues convincingly that higher volume does not necessarily mean more profit in toxicology testing, and that healthcare-focused customer relationship management systems can help labs analyze their business effectively and enhance business decisions.

In one subsection, "Facing strong headwinds," the author summarizes some of the pressures that toxicology (and other) labs face these days, including, for example, reduced reimbursements. In addition, the article goes on to say:

> ...high-deductible healthcare plans are forcing consumers to foot a higher percentage of the bill for their testing. According to TransUnion Healthcare, patients paid 11 percent more in out-of-pocket costs in 2017 than the previous year. This is creating more savvy and discriminating healthcare consumers who are more likely to question the necessity of healthcare tests and procedures. (That's a good thing for everyone in the long run, but a challenge for labs nonetheless.)

May I draw your attention to that last sentence above, the one in parentheses. The author did not write that sentence. I did, because I didn't want to give the impression that the author, or MLO, thinks that educated consumers is a bad thing, or that we would prefer that patients accept prices without checking. MLO doesn't believe that, and I'm sure Diane Janowiak doesn't either, but I was afraid the paragraph would come off that way, so I added the sentence in question. (I sent the edited manuscript to the author for approval, of course.)

When I proofread the manuscript later, I thought about it again. Did I really need to add that sentence? Why did it seem necessary? Sometimes our most self-teachable moments occur when we look back at something we have done and use our critical thinking skills to analyze it.

The reason I added the sentence is that I felt defensive, because there may seem to be a grain of truth in the idea that the clinical lab industry can make more money if patients don't think too much—if they just follow the doctor's orders, submitting to all prescribed tests, some of them probably unnecessary or duplicative, and then labs collect their share of the money.

I believe most laboratorians don't think that way, because they know that an educated healthcare consumer is actually good for their business. Such a consumer will comply with necessary testing, be responsible about follow-up testing appointments, and be pro-active in a number of ways—with regard to cancer screenings, for example—that a less informed patient might not be inclined to embrace.

The lab industry, like most industries, is sometimes challenged by the question: Should we do well (make as much money as we can this quarter) or do good (the morally or ethically right thing, even if it means leaving a few dollars on the table this quarter)?

Fortunately, in the long run, and probably in the short run too, we do well by doing good. If labs do their part to educate and inform patients (doing good), they are also increasing their business (doing well). And, they are staying true to the reason why most laboratorians entered the field in the first place: to be part of a system that promotes good health.

Alan Lenhoff

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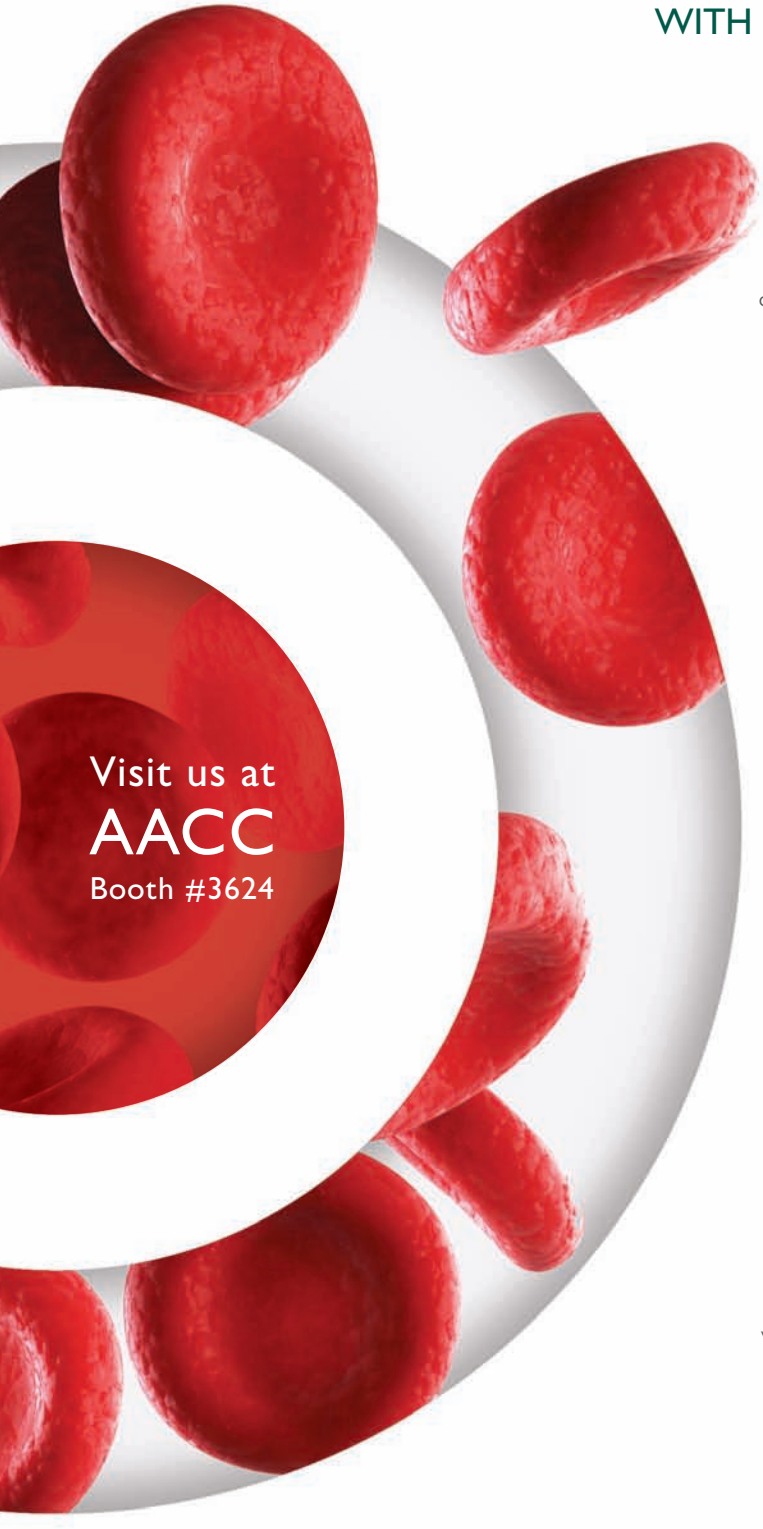

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Fast Facts Opioids in America

What do Americans say about opioid use in America, and their own experience with opioids? These statistics come from a study conducted by Lake Forest, Illinois-based Stericycle.

42 percent
of Americans have one to three bottles of unused prescriptions, including opioids, in their medicine cabinet.

9 percent
of Americans have four to six bottles.

2 percent
of Americans have seven to ten bottles.

15 percent
of Americans admit they've offered or given unused opioids to a friend or family member.

25 percent
of Americans admit they've been offered or given unused opioids by a friend or family member.

30 percent
of Americans admit they've kept leftover prescriptions for future use.

74 percent
of Americans believe that sharing and selling of unused prescriptions is contributing to the growth of the opioid epidemic.

25 percent
of Americans admit to flushing unused prescriptions.

83 percent
of Americans say they have never participated in a "drug takeback program."

27 percent
of Americans say they are concerned about the U.S. government solving the nation's opioid epidemic.

52 percent
of Americans believe that the opioid epidemic can be stopped.

• Source: The Opioid Epidemic and Unused Prescriptions Study. *https://www.stericycle.com/knowledge-center/ebook/takeback-study-info-stats*

Infectious Diseases

The immune system does not recover despite cured hepatitis C infection. Changes to the immune system remain many years after a hepatitis C infection is cured, a new study by researchers at Karolinska Institutet and Hannover Medical School shows. The findings, presented in *Nature Communications*, increase understanding about chronic infection and the way it regulates and impacts composition of the immune system.

Infection with hepatitis C virus (HCV) almost always turns chronic and poses a major health problem around the world. The infection can lead to cirrhosis and cancer of the liver when the immune system fails to fight the virus. Eventually the immune system becomes exhausted. However, most patients with HCV can now be cured in a matter of a few weeks with revolutionary new medications that became available in about 2015.

The current study included 40 patients with chronic HCV infection whom researchers followed before, during, and after treatment with these new medications to investigate impact on the composition and diversity of the immune system. Diversity is vital to the ability of the immune system to fight infections. Of particular importance are natural killer (NK) cells, a type of white blood cells. The researchers used flow cytometry and a new measurement method to derive the composition of the immune system, as well as the appearance of NK cells and their function in the blood.

The results showed that the overall composition of the immune system was affected by the chronic infection, with significantly reduced diversity among the NK cells. Many of the changes remained long after the virus had been eliminated by means of medication. Researchers have not yet determined the long-term implications but are currently exploring whether patients have a harder time fighting future infection.

A number of questions are outstanding. Researchers would like to investigate consequences for a good deal longer than a few years, as well as identify strategies for rejuvenating the immune system and increasing its diversity.

Genomics/Proteomics

Genome-editing tool could increase cancer risk. CRISPR-Cas9 is a molecular machine first discovered in bacteria that can be programmed to go to an exact place in the genome, where it cuts the DNA. These precise 'molecular scissors'" can be used to correct faulty pieces of DNA and are currently being used in clinical trials for cancer immunotherapy in the United States and China. New trials are expected to be launched soon to treat inherited blood disorders such as sickle cell anemia.

Two independent articles published in the journal *Nature Medicine* now report that therapeutic application of the genome-editing tool may, in fact, increase the risk of cancer. In one of the studies, scientists from Karolinska Institutet and the University of Helsinki report that use of CRISPR-Cas9 in human cells in a laboratory setting can activate a protein known as p53, which acts as a cell's "first aid kit" for DNA breaks. Once active, p53 reduces the efficiency of CRISPR-Cas9 gene editing. Thus, cells that do not have p53 or are unable to activate it show better gene editing. Unfortunately, however, lack of p53 is also known to contribute to making cells grow uncontrollably and become cancerous.

"By picking cells that have successfully repaired the damaged gene we intended to fix, we might inadvertently also pick cells without functional p53," says Dr. Emma Haapaniemi, who was co-first author of one of the studies. "If transplanted into a patient, as in gene therapy for inherited diseases, such cells could give rise to cancer, raising concerns about the safety of CRISPR-based gene therapies."

"CRISPR-Cas9 is a very powerful tool that has staggering therapeutic potential," adds Dr. Bernhard Schmierer, who served as co-supervisor of the study. "As is the case with all medical treatments, however, CRISPR-Cas9-based therapies might have side effects, which patients and caregivers need to be aware of. Our study suggests that future work on the mechanisms that trigger p53 in response to CRISPR-Cas9 will be critical in efforts to improve the safety of CRISPR-Cas9-based therapies."

Diagnostics

Finances are a major factor in patient avoidance of diagnostic testing. Patient preferences for diagnostic testing differ significantly across levels of risk, benefit, and cost of testing, but cost is the strongest and most consistent factor associated with decreased desire for testing. Those are the findings of a study published in the June 2018 issue of *Academic Emergency Medicine* (AEM), a journal of the Society for Academic Emergency Medicine (SAEM).

The lead author of the study is Jonathan D. Porath, University of Michigan Medical School. The emergency department-based study by Porath and colleagues utilized a copay to "penalize" for the test. The results suggest that a credit for foregoing the test (comparable to a safe driver discount) might be an interesting direction for future research. With patients having a growing personal contribution to healthcare, the findings support the need for further study to determine how best to implement financial considerations to alter testing behavior.

Erik P. Hess, MD MSc, professor and vice chair for research at the University of Alabama at Birmingham Department of Emergency Medicine, comments: "This study highlights the importance patients may place on the cost of low-value diagnostic testing. Implementing these findings requires careful consideration, as discussing cost in this context may have unintended effects on physician trust and runs the risk of disproportionately influencing decision-making in uninsured patients. Nonetheless, it is important for physicians to recognize the importance of cost as a driver of patient decision-making in low-value diagnostic testing."

Vitamin D

High vitamin D levels linked to lower cholesterol levels in children. There is a link between higher serum vitamin D levels and lower plasma cholesterol levels in primary school children, University of Eastern Finland research shows. Children whose serum 25-hydroxyvitamin D levels exceeded 80 nmol/l had lower plasma total and low-density lipoprotein (LDL) cholesterol levels than children whose serum 25-hydroxyvitamin D levels were below 50 nmol/l, which is often regarded as a threshold value for vitamin D sufficiency. 25-hydroxyvitamin D is the major circulating form of vitamin D. The findings were reported in the *Journal of Clinical Endocrinology and Metabolism*.

Vitamin D is known to be essential for bone metabolism, and low serum 25(OH)D levels increase the risk of rickets, osteomalacia, and osteopenia. Vitamin D may also improve plasma lipid levels and have beneficial impact on other risk factors of cardiovascular diseases.

Lifestyle factors, such as healthy diet, physical activity, and spending time outdoors leading to the production of vitamin D in the skin, may be linked to both higher serum vitamin D levels and lower plasma lipid levels. The researchers found that the link between higher serum vitamin D levels and lower plasma cholesterol levels was independent of body adiposity, dietary factors, physical activity, parental education, and day length prior to blood sampling. Moreover, hereditary factors that have been linked to serum vitamin D levels did not modify the observed association. More research is needed to uncover the reasons behind the inverse association of serum vitamin D with plasma lipid levels.

The new findings provide support for the importance of following recommendations for vitamin D intake, which vary from country to country. The most important dietary sources of vitamin D are vitamin D fortified products such as dairy products and spreads, and fish.

In addition to the dietary intake, vitamin D supplement use is also recommended for the general population in several countries. The recommended use of vitamin D supplements varies considerably among these countries (mostly 5-50 µg/d, corresponding to 200-2000 IU/d), depending on age group and other factors.

Vitamin D is synthetized endogenously in the skin in the presence of UV-radiation from the sun. However, in northern latitudes, exposure to sunlight alone is inadequate to maintain sufficient serum 25(OH)D levels, especially during winter.

Molecular Diagnostics

Mayo Clinic researchers take a step closer to developing a DNA test for liver cancer. A group of researchers from Mayo Clinic and Exact Sciences Corporation have completed a phase II study comparing a set of DNA markers to alpha fetoprotein as a method to test for liver cancer. The researchers presented their findings last month at the 2018 Digestive Disease Week conference in Washington, D.C.

"We currently test for liver cancer using ultrasound and a blood protein marker called alpha fetoprotein," says John Kisiel, MD, a gastroenterologist at Mayo Clinic. "Unfortunately, these tests are not very sensitive for curable stage liver cancers, and most patients who need this testing do not have it easily available or [are] not able to receive it often enough to be effective."

Dr. Kisiel and his colleagues developed a simple blood test using abnormal DNA markers that are known to exist in liver cancer tissues. They were able to confirm that the abnormal DNA markers were present in the overwhelming majority of blood samples that came from people with primary liver cancers. Simultaneously, these markers were absent in healthy individuals and individuals with cirrhosis of the liver, but no evidence of tumors on their clinical follow-up.

"We were most excited that our DNA markers were able to detect more than 90 percent of patients with curable stage tumors," says Dr. Kisiel. "This is the main reason why we think a DNA test will make a difference, compared to currently available tests." Dr. Kisiel says the next step will be to validate these markers in blood testing on much larger patient cohorts.

According to the National Cancer Institute, the number of new cases of liver and bile duct cancer in the U.S. was 8.8 per 100,000 men and women per year. Dr. Kisiel says primary liver cancer is a major cause of suffering and death for patients who have cirrhosis of the liver or patients with hepatitis B infections. Worldwide, liver cancer is the second most common cause of cancer death.

This month's CE is comprised of two articles and one test.

Multistep algorithm testing accurately identifies *C. diff* patients who need treatment

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

Clostridium difficile has leapfrogged over MRSA as the most common healthcare-acquired infection (HAI). Its ability to cause antibiotic-associated diarrhea (AAD) and colitis (AAC) is well-established. In the United States, there are about 500,000 cases each year, with up to 30,000 deaths attributed to the disease. Healthcare costs for *C. difficile* infection (CDI) now run more than $6 billion annually. The numbers in Europe probably are similar, although cases in the United Kingdom have been declining. The success in the U.K. is in part due to an extensive educational awareness program about the disease in that nation and its near-universal adherence to multistep-algorithmic diagnostic testing, which enables the healthcare system to appropriately find and treat patients with CDI and thereby limit its spread.

Emergence of CDI

Prior to the early 2000s, the incidence of CDI was rising, but it was not considered to be nearly as significant as healthcare-acquired MRSA infections. Recognition of *C. difficile* as an emerging pathogen quickly coalesced with the appearance of the hypervirulent strain 027, a fluoroquinolone-resistant strain of *C. difficile* that generally produces more toxin and grows to higher numbers in the intestine than other strains. Because of these increased virulence traits (primarily antibiotic resistance), 027 spread quickly among medical facilities, moving from Europe to Canada and then to the U.S. With the spread of 027, the overall incidence of *C. difficile* began to increase dramatically. Hospitals, particularly in Canada, noted rising mortality rates. In fact, some Canadian institutions observed a 20 percent mortality rate in patients infected with 027.

C. difficile disease occurs primarily in hospitalized elderly patients, but it has extended beyond this population into the community, infecting younger, healthier patients. As the hypervirulent 027 strain spread, *C. difficile* began to appear as a community-acquired pathogen. The community-acquired patient population skewed younger and more female than the hospital-acquired population. Some researchers also are categorizing particular *C. difficile* ribotypes as foodborne pathogens. A more thorough understanding of the emerging pattern of *C. difficile* is continuing to develop.

CDI sometimes does not cause symptoms, or the infection can be subclinical, or the infection can result in clinically apparent disease. That is one reason why diagnosing CDI is challenging. Consider the following:

- *C. difficile* can be found in healthy infants at levels that would cause pseudomembranous colitis (the severe stage of CDI) in elderly adult patients; we still do not understand the mechanism of this protection in infants.
- The organism often can be found in hospitalized patients who do not have diarrhea. In fact, a patient's chance of picking up *C. difficile* in the hospital and carrying it asymptomatically is greater than developing active CDI while in the hospital.
- *C. difficile* can be carried in persons who have diarrhea caused by norovirus or *Campylobacter* or any one of a number of other infections or conditions (for example, other intestinal pathogens, inflammatory bowel disease), but not be involved in causing the diarrhea.
- *C. difficile* can be a passive bystander in patients with diarrhea for a host of other reasons including laxative use, chemotherapy, liquid diets, stress, and pharmaceutical side effects. These *C. difficile* carriers should not be treated because the antibiotic regimen puts the patient at greater risk for a true active CDI.
- *C. difficile* can cause a mild self-limiting diarrhea that probably does not require antibiotic treatment, although the patient most likely will receive such treatment if *C. difficile* is detected.
- And, of course, *C. difficile* can cause severe diarrhea and colitis that, if left untreated, can progress to severe pseudomembranous colitis.

Therefore, due to the prevalence of carriers, accurately identifying patients who have true CDI and need treatment remains a significant challenge.

The diagnostic landscape

C. difficile and its ability to cause diarrhea and colitis in patients treated with antibiotics was first recognized more than 40 years ago. Decades later, we still are trying to determine the most accurate approach for diagnosing

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LEARNING OBJECTIVES

Upon completion of these articles, the reader will be able to:

1. Recognize healthcare statistics associated with *C. difficile* infection and barriers to accurate diagnosis.
2. Explain the diagnostic landscape of *C. difficile* identification and the advantages and disadvantages to available tests.
3. Identify the organizations that recommend advanced algorithms for *C. difficile* diagnosis and describe the algorithm used and the organizations' new guidelines for treatment.
4. Describe the efforts involved in decreasing the incidence of the spread of *C. difficile* smong hospital patients.

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this disease. Increased awareness has resulted in efforts to improve diagnostic tests for this pathogen. The *in vitro* diagnostic tests on the market today detect three types of analytes: 1) *tcdB* and/or *tcdA* (the genes for toxins A and B); 2) glutamate dehydrogenase (GDH), a metabolic enzyme that is produced in high amounts by actively growing (i.e., vegetative) *C. difficile* cells; and 3) toxins A and B, which cause the disease.

Nucleic acid amplification testing (NAAT) for *tcdB* and/or *tcdA* uses PCR or isothermal amplification and is very sensitive for detecting toxigenic *C. difficile* in fecal specimens. To produce toxin, strains of *C. difficile* must have toxin genes and must express them. NAAT tests do not detect toxin, nor do they demonstrate that toxin is being produced and is present in the specimen. Since roughly half of the patients colonized with *C. difficile* are not colonized with strains producing toxin, this high sensitivity for the toxin gene(s) results in the overdiagnosis of CDI in hospitals, leading to treatment, including antibiotic usage, of patients who do not need treatment.

Additionally, some of these tests detect extremely low numbers of *C. difficile* in the specimen, numbers that are not clinically relevant. Even if patients only have spores in their intestine, without actively growing cells and therefore without active disease, NAAT tests will give positive results. The ability of *C. difficile* to live transiently in the patient without causing problems and the fact that it forms very hardy spores that persist in host and hospital environments are reasons why NAAT testing as a standalone test leads to overdiagnosis and low positive predictive values.

GDH is an accurate indicator of actively growing cells and is produced by toxigenic and nontoxigenic cells. Nontoxigenic strains usually are present only at low incidence rates. In many hospitals, they are negligible. Studies have shown that nontoxigenic strains were not present in some institutions. In others, nontoxigenic strains represented less than 10 percent of GDH-positive fecal specimens. Some other organisms that live in the human intestine produce an immunologically related GDH, so optimal performance requires GDH testing performed with immunoassays that have highly specific antibodies for GDH from *C. difficile*. All ribotypes of *C. difficile* produce identical GDH molecules except for the GDH of one ribotype that has a single amino acid substitution. This substitution has no effect on detection in GDH assays.[1] The positive predictive value using GDH as the biomarker is comparable to that observed with NAAT testing and delivers this performance more cost-effectively.

Toxins A and B are virulence factors that cause disease. The detection of toxin indicates the presence of actively growing toxigenic cells. In symptomatic patients, the detection of toxin correlates closely with true CDI. Over the past five years, studies looking at assay performance have taken a critical look at test results in conjunction with patient history. The analyses have demonstrated strongly that patients who have the presence of toxin have more severe disease, longer bouts of diarrhea, longer hospital stays, higher mortality rates, and more inflammation than patients who were positive by NAAT testing but negative for toxin. In fact, there is no clinical difference between patients who are positive for *C. difficile* but negative for the presence of toxin and patients who are negative for *C. difficile* overall.

C. difficile disease often has a primary inflammatory component, noted by increased white cell counts and inflammatory biomarkers such as lactoferrin, and many experts believe it should be considered an inflammatory disease. The inflammation is triggered by the extensive tissue damage caused by the toxins and their chemotactic activity. Although guidelines have not discussed the value of inflammatory biomarkers for *C. difficile*, studies have shown that these markers help identify severity in CDI.[2,3]

Most toxin immunoassays detect both toxins. This is important because toxin B-only strains, although uncommon, can cause disease. No toxin A-only strains have been reported. The standard for toxin detection is the tissue culture assay, also referred to as the cell cytotoxicity neutralization assay (CCNA). CCNA offers exquisite sensitivity and detects picogram amounts of toxin due to the high activity of toxin B. However, the assay is tedious and time-consuming. A positive result, noted as cell rounding that is neutralized by specific *C. difficile* antitoxin, can be noted within eight to 12 hours in samples with medium to high levels of toxin. However, it takes 48 hours to call a specimen negative or to identify samples that contain only very low amounts of toxin. Toxin enzyme immunoassays offer the advantages of ease of use and rapid turnaround time (one hour or less), but they are less sensitive than the tissue culture assay. Trying to develop toxin immunoassays that approach the sensitivity of CCNA is challenging but critical, since toxin testing provides positive predictive values that are considerably higher than those observed with NAAT or GDH testing.

Recommended testing algorithms

For the reasons described above, multiple analyte algorithms rather than single analyte assays have been recommended by two societies well versed in *C. difficile* testing. The European Society of Clinical Microbiology and Infectious Disease (ESCMID) guidelines that became available two years ago recommended an algorithm approach for CDI.[4] Optimal results were obtained using a multistep algorithm. NAAT or GDH tests were recommended as a first step since their performance is comparable, followed by a high-performing toxin assay. This approach offers the most accurate diagnostic strategy for CDI, based on results obtained from large clinical studies in which test results were correlated with patient clinical findings.[5,6] Symptomatic patients who have diarrhea from another cause but who are positive by a NAAT or GDH test and negative for toxin are defined as carriers. Symptomatic patients who are positive by a NAAT or GDH test and positive for toxin are identified as patients with true CDI.

The recent guidelines from the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) similarly support an algorithm approach, although the guidelines include additional information on using a standalone molecular test when the institution had a specimen selection guide in place.[7] Although the performance of all testing methods improves from testing the correct samples, algorithm testing does not need to depend upon this prerequisite enhancement for accurate results. As with the ESCMID guidelines, the IDSA/SHEA guidelines state that an algorithm approach that incorporates toxin testing results in the highest predictive positive value of true CDI. In addition, both guidelines note that a negative NAAT or GDH test accurately rules out CDI.

continued on page 16

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There are a large number of NAAT and immunoassay tests available to the clinical lab. Not all of these tests perform equally well, a point repeatedly noted in the IDSA/SHEA guidelines. The quality of reagents that go into these tests varies. There are differences in purity, specificity, and affinity for *C. difficile* analytes. Some immunoassay tests use polyclonal antibodies, whereas others use monoclonal antibodies, each with their own advantages and disadvantages for testing fecal specimens.

In this context it is worth noting that fecal specimens are challenging because they are among the most complex clinical samples. Variation is due to bile content, bacterial/host degradative enzymes, diet, and a number of other host factors. Testing fecal specimens is therefore much more complex than testing serum samples, and the development of diagnostic reagents, diluents, and procedures optimized for fecal samples is imperative. Something as simple as mixing a specimen properly can be crucial because in feces, the analyte is not evenly distributed throughout the matrix. Specimens also often contain amplification inhibitors, affecting the performance of NAAT tests. NAAT tests or immunoassays that perform well with "neat" reagents can be totally ineffective with fecal specimens. Therefore, quality and demonstrated performance are critical factors when choosing a *C. difficile* test.

The multistep approach

Although there are a large number of tests and formats available, adherence to a multistep algorithm clearly seems to be the best approach. According to recent surveys, it appears that roughly half of the responding labs in the U.S. use NAAT testing as a standalone test or as part of a multistep algorithm. The other half use immunoassays, with more than half of that group using an algorithm approach consisting of GDH and toxin testing. Additional surveys are needed to determine more accurately the current prevalence and adoption of multistep algorithm testing in U.S. labs. An algorithm that incorporates a screening assay (NAAT or GDH) followed by a toxin test with demonstrated performance will provide a higher positive predictive value than standalone testing. The testing can be made more cost-effective by using GDH as the initial screen, since it is comparable in performance to NAAT testing. An algorithm approach is supported by the ESCMID and IDSA/SHEA guidelines and, when paired with the clinical history of the patient, provides the most accurate assessment of true *C. difficile* infection, resulting in optimal patient care.

Please visit mlo-online.com for references.

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New guidelines and studies suggest improved approaches to *C. difficile* testing

By Sherry Dunbar, PhD, MBA

Clostridium difficile represents a significant health threat around the world. In the United States, infections caused by *C. difficile* are now the most common type of healthcare-associated infection.[1] Nearly half a million infections occur in the U.S. annually, with an estimated 29,000 deaths within 30 days of the initial diagnosis.[2]

Consequently, much effort is ongoing toward the development of better testing and treatments for *C. difficile*. This year, new clinical guidelines were released that included significant changes to how healthcare teams respond to *C. difficile* infections. In addition, scientists and clinicians are conducting a number of studies and generating useful information that could guide new expectations or policies about testing and treatment.

For example, studies have shown that molecular tests targeting a marker specific to a single *C. difficile* strain are less useful now, as other strains of the pathogen have become more prevalent.[3-5] These findings could help clinical labs fine-tune their *C. difficile* testing procedures to ensure the most reliable results. Also, several recent studies have demonstrated that *C. difficile* infections occur more frequently in hospitals when asymptomatic carriers are mixed in with the general patient population.[6,7] Screening for *C. difficile* upon admission and isolating patients colonized with the organism are not yet standard practices, but these studies suggest that healthcare facilities could improve outcomes and lower costs by taking such steps.

Together, new approaches to infection control along with adherence to updated guidelines could make a real difference in patient care and help to check the spread of this pathogen.

Guideline details

Earlier this year, the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) released new clinical guidelines about diagnosing, treating, and preventing *C. difficile* infections.[8] This update, which replaces guidelines issued eight years ago, offers healthcare and clinical laboratory professionals important standards for handling this public health threat.

The new guidelines include substantial changes from earlier recommendations about how to treat patients with *C. difficile* infections. For example, based on evidence from clinical trials, advocacy of the previous first-line treatment, metronidazole, has been replaced with a strong preference for vancomycin or fidaxomicin, delivered for ten days for mild or moderate cases in adults. (Metronidazole is still considered a first-line option for children.) Patients with recurring infections—a common situation, as one in four people with *C. difficile* have the infection return—are now to be treated with either ten days of fidaxomicin or a tapered course of vancomycin spanning several weeks. Doctors may also consider probiotics for these cases, although the guidelines make clear that there is not yet sufficient evidence to determine whether these treatments are effective in preventing recurrence. Finally, the recommendations support the use of fecal transplants for patients whose *C. difficile* infections have recurred at least twice.

The IDSA/SHEA guidelines also reflect the diagnostic challenge presented by *C. difficile* infections: when the organism is detected, how can clinical labs discern whether those positive results represent the cause of infection or simply asymptomatic colonization? Molecular diagnostics are frequently used for *C. difficile* testing, but they cannot distinguish between those states. Because of this, diagnostic testing might overestimate the number of infections attributable to this pathogen.

To address this challenge, the guidelines offer clear recommendations about when to test—and when not to test—patients for *C. difficile*. According to the latest protocols, testing should be restricted to patients who have at least three bouts of diarrhea in a 24-hour period, and within those cases, testing is only recommended when diarrhea has started recently and cannot otherwise be explained. The guidelines also state that patients with a negative *C. difficile* test should not be retested for at least a week. Children less than a year old should not be tested at all, and children between one and two years old should be tested only after other potential causes of diarrhea have been ruled out.

According to the guidelines, clinical labs should test diarrhea samples and not formed stool. The particular type of test used is largely left to each institution to decide based on its broader diagnostic policies. Molecular tests are recommended in cases where facilities only use such diagnostics on patients who are likely to be suffering a *C. difficile* infection. When that is not possible, though, the guidelines urge clinical labs not to rely on molecular tests alone but rather to supplement them with advanced toxin tests to ensure that positive results can reliably be accepted as the source of infection.

Molecular testing

Molecular tests for *C. difficile* function by amplifying and identifying genes associated with toxins related to *C. diff*-triggered disease. They offer significant advantages over traditional typing methods, most notably in turnaround time; these tests can return clinically actionable results in hours rather than days.

Different tests cover different ribotypes of the organism; as new strains evolve, some of these ribotypes may become less important to detect. For example, an epidemic of the BI/NAP1/027 ribotype of *C. difficile* occurred in Europe and North America in the early 2000s, dramatically changing the epidemiological patterns of *C. difficile* infections. As a result, some molecular tests include this ribotype, even as its incidence decreases and other strains become more common. The identification of this ribotype can help with disease prognosis because it has been associated with more severe cases, but the strain is still typically addressed with the same treatments as other infection-causing strains of *C. difficile*.

Emerging strains that appear to be closely related to the BI/NAP1/027 ribotype have made it more difficult to accurately

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distinguish among ribotypes. Ribotype 244 is relatively new and is misidentified by some molecular tests as the BI/NAP1/027 ribotype.[9] This has resulted in higher false-positive rates for NAP1 and a lower specificity for these tests.[10]

Surveillance studies have shown that other ribotypes are quickly overtaking BI/NAP1/027 among the patient population. In the U.S. from 2010 to 2014, a study of 673 *C. difficile* isolates collected during a clinical trial representing six different geographic regions found that ribotype frequency shifted over time.[3] In the populations examined, ribotype BI/NAP1/027 went from a frequency of 43 percent at the beginning of the study to just 14 percent by the end. During that time, other ribotypes—notably 014/020, 106, and 002—became more prevalent.

In Canada, epidemiological analysis showed that NAP1 isolates decreased by 29 percent between 2008 and 2013.[4] In Europe, studies describe a similar shift. A 2012-2013 point prevalence study that spanned 1,196 isolates, 482 hospitals, and 19 countries found the most common ribotypes to be BI/NAP1/027 at 19 percent, 001/072 at 11 percent, and 014/020 at 10 percent.[5] Compared to an earlier study in 2008, only ribotype 014/020 was consistently among the three most frequent. Ribotype BI/NAP1/027, which had previously been more common in the UK and Ireland, was by 2013 more likely to be found in Germany and Eastern Europe.

Given these patterns, it seems unwise to focus *C. difficile* testing on the identification of NAP1, which is often used today as an indicator of disease severity. Patient history and symptoms should be used with the standard guidelines to ensure optimal treatment for each patient, regardless of the presence or absence of the NAP1 marker.

Testing for colonization

While IDSA/SHEA treatment guidelines recommend testing only for cases in which patients have symptoms consistent with *C. difficile* infection—with the important goal of avoiding misdiagnosing patients who are colonized by *C. difficile* but not suffering symptoms from it—there has been increased recognition in the community that *C. difficile* colonization is more common than previously understood. Studies have shown that nearly 30 percent of patients carry *C. difficile,* though it seems to have no impact on their health.[11]

However, asymptomatic patients represent a reservoir of *C. difficile*, and there are benefits to identifying them in hospital settings so they can be kept away from non-colonized patients who are at risk of acquiring the pathogen and suffering an infection from it.[12] Two recent studies found that screening for *C. difficile* carriers can support hospital infection control efforts.

In one study, investigators examined the prevalence of nosocomial infections in a 354-bed acute care tertiary facility in Canada.[6] The study looked at data before and after the facility began a policy of screening patients at admission and isolating asymptomatic carriers of *C. difficile*. Results showed that the incidence of hospital-associated *C. difficile* infections dropped from 6.9 per 10,000 patient-days before the isolation policy to 3.0 per 10,000 patient-days when carriers were isolated. The team estimated that the new approach prevented 62 percent of expected hospital-associated *C. difficile* infections, with potential savings of as much as $627,000.

The other study involved two university hospitals with 188 beds in Denmark and measured rates of *C. difficile* infection among patients exposed or not exposed to asymptomatic patients colonized with *C. difficile*.[7] Patients were screened for the pathogen at admission; anyone found positive, whether suffering infection or not, was put into isolation with strict contact precautions. Results revealed that patients who were not exposed to asymptomatic carriers developed *C. difficile* infections at a lower rate: 2.6 percent compared to 4.6 percent of patients who were exposed to carriers.

Together, these findings suggest that screening for *C. difficile* upon admission at healthcare facilities may provide actionable information that could be used to isolate asymptomatic carriers and reduce the incidence of hospital-associated *C. difficile* infections.

Looking ahead

The use of more comprehensive molecular testing, combined with the recently updated clinical guidelines for *C. difficile* testing and treatment, as well as consideration of more robust infection control policies, could dramatically reduce the transmission of this organism, improve patient outcomes, and reduce overall healthcare costs. Clinical labs are a key link in this chain. By ensuring optimal testing protocols and considering screening policies for all patients admitted to hospitals, laboratorians stand to make a real impact in the *C. difficile* infection arena.

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TEST QUESTIONS

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

1. ***C. difficile*** **has surpassed _____ and is now the most common healthcare-acquired infection.**
   - a. VRE
   - b. flu
   - c. MRSA
   - d. rotavirus

2. ***C. difficile*** **is known to cause**
   - a. colitis.
   - b. antibiotic-associated diarrhea.
   - c. both and a and b
   - d. neither a nor b

3. **What country has seen declining cases of *C. difficile* due to educational awareness and multistep algorithmic diagnostic testing methods?**
   - a. United Kingdom
   - b. Canada
   - c. Europe
   - d. United States

4. **Which strain of *C. difficile* has emerged across the world, quickly increasing the incidence of *C. diff*?**
   - a. 014
   - b. 02
   - c. 025
   - d. 027

5. ***C. difficile*** **infection (CDI) can be challenging to diagnose because it can be asymptomatic or subclinical in some patients.**
   - a. True
   - b. False

6. **Which in vitro test(s) is/are on the market today to detect *C. difficile's* types of analytes?**
   - a. *tcdB/tcdA* and GDH
   - b. GDH and toxins A and B
   - c. *tcdB/tcdA* and toxins A and B
   - d. *tcdB/tcdA*, toxins A and B, and GDH

7. **The use of which test as a standalone test has led to a low positive predictive value and overdiagnosis of *C. difficile*?**
   - a. GDH immunoassay culture
   - b. NAAT testing
   - c. rapid toxin A and B testing
   - d. both a and c

8. **Which analyte is produced by toxin and nontoxin-producing *C. difficile* and gives a good positive predictive value?**
   - a. GDH
   - b. NAAT
   - c. toxin A/B
   - d. none of the above

9. **The detection of toxins A/B demonstrates that the patient has less severe disease and symptoms.**
   - a. True
   - b. False

10. **What is the specimen of choice for the detection of toxins A/B?**
    - a. serum
    - b. fecal
    - c. tissue
    - d. gastric fluid

11. **What is/are the main limitation(s) of the highly sensitive CCNA assay?**
    - a. It is expensive.
    - b. It requires highly skilled lab scientists to perform and interpret the results.
    - c. It is tedious and time-consuming.
    - d. none of the above

12. **Which society(ies) recommend(s) algorithm approaches for the accurate diagnosis of *C. difficile*?**
    - a. ESCMID
    - b. IDSA
    - c. SHEA
    - d. all of the above

13. **The algorithm approach to the accurate diagnosis of *C. difficile* recommends using NAAT or GDH as a screening step, followed with a high-performing toxin A/B assay.**
    - a. True
    - b. False

14. **Evidence from clinical trials has changed the treatment preference for CDI. Instead of metronidazole, clinicians now tend to prefer _____ for adults.**
    - a. vancomycin/fidaxomicin
    - b. penicillin
    - c. erythromycin
    - d. clindamycin

15. **For patients with recurring *C. difficile* infections, the recommendation for treatment is**
    - a. antibiotics along with steroid administration.
    - b. increased dosing of antibiotics.
    - c. fecal transplants.
    - d. none of the above

16. **Molecular testing needs to continuously evolve in terms of detectable strain types because the strain patterns are continuously changing.**
    - a. True
    - b. False

17. **According to the article by Dunbar, what percentage of hospital patients are carriers of *C. difficile*?**
    - a. 10
    - b. 20
    - c. 30
    - d. 40

18. **In one study, what was done to help control the spread of nosocomial *C. difficile* to at-risk noncolonized patients?**
    - a. screening all admitted patients and isolating the asymptomatic carriers
    - b. treating all carriers with high doses of antibiotics at the time of admission
    - c. assigning all at-risk patients to isolation rooms
    - d. none of the above

19. **The investigators in the study described in question 18 say that their approach resulted in a decrease of _____ percent of expected hospital-acquired *C. diff* infections.**
    - a. 15
    - b. 28
    - c. 46
    - d. 62

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The importance of specificity in diagnostic tests for autoantibodies

By Nina Olschowski, PhD, and Lorraine Damico, MBA

Detection of autoantibodies in the blood of a patient helps to support the diagnosis of autoimmune disease. A challenging aspect is that autoantibodies often appear in a myriad of diseases, and it is important to consider this before making a diagnosis. For example, antibodies against the protein Sm could indicate that a patient may have lupus (systemic lupus erythematosus, SLE). However, only five percent to 30 percent of lupus patients have Sm antibodies[1]; thus, a negative result does not exclude the disease in question.[2] On the other hand, the marker is very specific for lupus; therefore, clinicians may erroneously presume that all patients with Sm antibodies have the disease.

Diagnostic ambiguity with RA

Another example could involve diagnostic markers for rheumatoid arthritis (RA). Early identification and a correct diagnosis of RA is critical. If left untreated, or undertreated, RA is associated with progressive and irreversible joint destruction leading to disability, reduction of quality of life, and increased mortality.[3] In the twentieth century, rheumatoid factor was the only autoantibody used as a diagnostic marker for RA. It is present in about 70 percent to 90 percent of RA patients. However, rheumatoid factor is also positive in other diseases and even in healthy individuals.[4]

In 2000, a team led by Walther Van Venrooij in the Netherlands introduced a new marker for RA, which created an unrivalled success story in autoimmune diagnostics: antibodies against CCP (cyclic citrullinated peptide).[5] Antibodies to CCP appear more than 10 years before the first symptoms.[6] In older patients, serum anti-CCP is detectable well before the development of clinical symptoms of RA, while in younger patients, the detection of anti-CCP occurs closer to the time of disease onset.[7]

The important difference between anti-CCP and rheumatoid factors is the high specificity of anti-CCP for RA.[5,8] This means test selection and timing are important. There is a large variability among different anti-CCP assays, and numerical test results are not interchangeable.[3] During the last 20 years, anti-CCP tests have evolved significantly,[7] with several generations of anti-CCP assays introduced. The anti-CCP assays using the CCP2 peptide mix have repeatedly demonstrated the highest sensitivity for RA when compared at a stratified 98.5 percent specificity.[9]

Alone, RF or anti-CCP tests are not sufficient for the diagnosis of RA, because some patients have only one or the other biomarker, or lack both. However, in combination with other clinical measures, the biomarkers together provide important diagnostic and prognostic information about different RA patient populations depending on which of the biomarkers (if any) are present in patient sera.[7] Combination testing with anti-CCP, RF, sIgM, and RFIgA is beneficial when excluding the diagnosis of RA, rather than testing for individual antibodies.[10] In fact, combining the presence of anti-CCP antibodies and the presence of any of the individual RF isotypes in samples predating symptom onset results in a higher specificity, with increases to 99 percent and 100 percent,[6] providing a greater sense of confidence in the diagnosis.

The use of enzyme immunoassays to detect autoantibodies is common. In enzyme immunoassays, the antigen (the target of the antibodies) is coated to the wall of a small cavity. The antibody in the patient's blood binds to this antigen. The quality of the assay is tied directly to the purity of the antigen used. When it is not purified thoroughly enough, contaminants may bind to the cavity, resulting in antibodies against those contaminants causing a false positive result. With that in mind, consider the assay attribute of specificity and its importance particularly in low prevalence disease.

Specificity and accuracy

A laboratory's decision about test selection plays a critical role in the accurate detection of antibodies. Using anti-CCP as an example, since there are assays available that use different antigens, the specificity for rheumatoid arthritis can differ among these tests.[8]

Small differences in assay specificity make a large difference in the accuracy of a result, mostly due to the very low prevalence of autoimmune diseases in general. The specificity of a test is the proportion of unaffected individuals with a negative test. The sensitivity of a test is the proportion of affected individuals with a positive test.[2] In a setting of relatively high clinical suspicion (high pre-test probability), a positive anti-CCP result means the patient has a strong likelihood of having or developing RA.[11] The pre-test probability in a large commercial lab is particularly low, because the sera are often sent in by non-specialists who want to exclude autoimmunity in patients with unclear clinical scenarios. Since the prevalence of autoimmune disease is low in the general population, for most autoimmune markers tested in immunological labs, most samples are probably not from patients with the diseases in question. In addition, most antibodies are only positive in a subset of the patients with the disease.[2]

	Anti-Sm positives	Anti-Sm negatives	Sum
Lupus patients	88	312	400
Non-lupus patients	192	9,408	9,600
Total	280	9,720	10,000

Table 1. A routine lab evaluates 10,000 sera for anti-Sm antibodies per year. The prevalence of lupus in this cohort is four percent. The lab's anti-Sm test has a sensitivity of 22 percent and a specificity of 98 percent. The positive predictive value is 31 percent.

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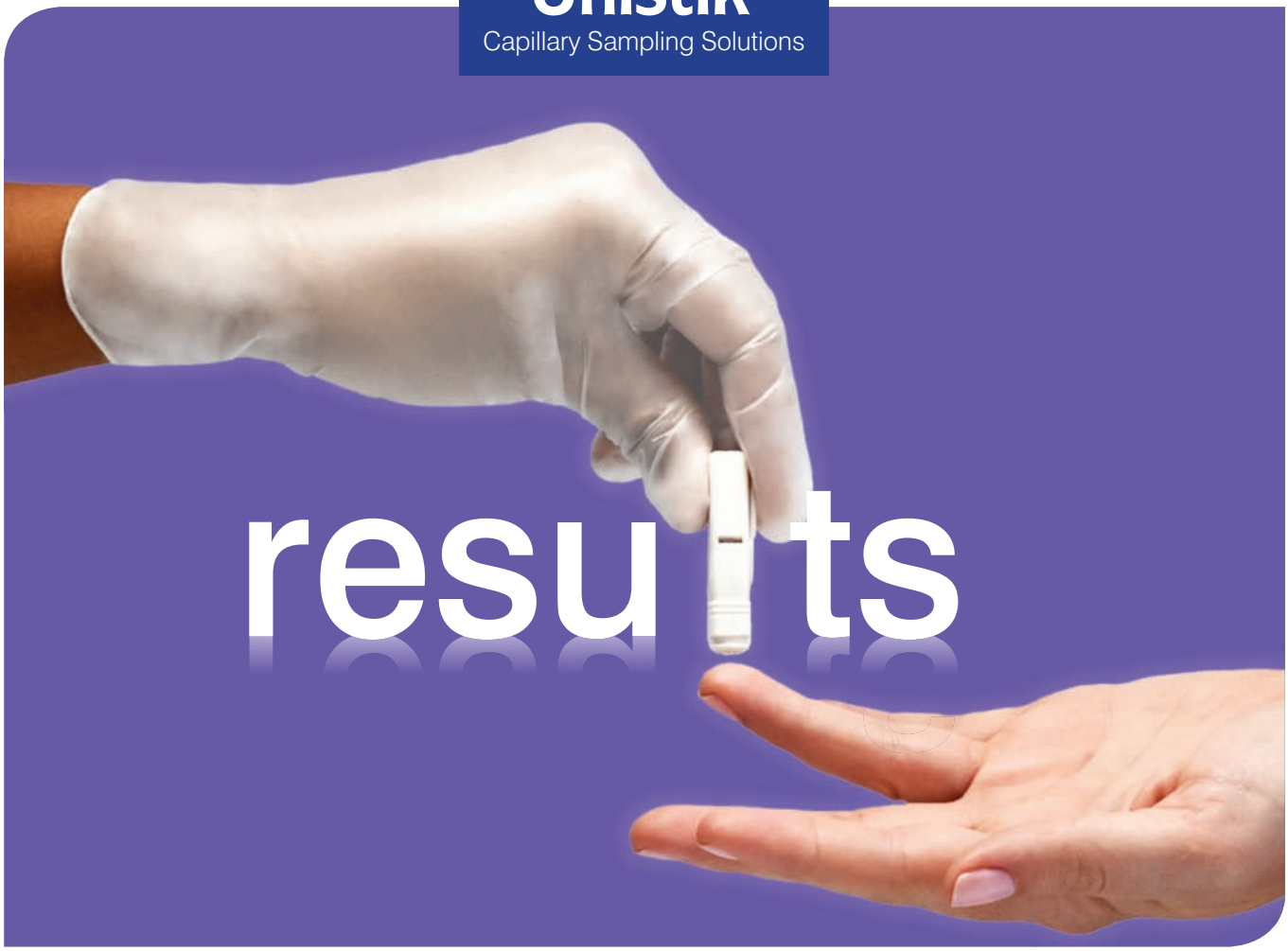

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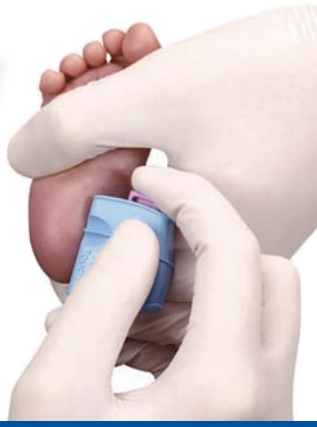

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	Anti-Sm positives	Anti-Sm negatives	Sum
Lupus patients	88	312	400
Non-lupus patients	96	9,504	9,600
Total	184	9,816	10,000

Table 2. The same lab uses another anti-Sm test, which has the same sensitivity (22 percent) but a higher specificity of 99 percent. The positive predictive value increases to 48 percent.

The earlier mentioned Sm example is also useful to elaborate on with regard to this topic. Natural Sm protein is complex and consists of six single proteins (Sm B, B', D1/D2/D3, E, F and G). Anti-Sm antibodies react with the double protein Sm-B'/B and SmD but rarely with E, F, and G.[1] However, only the presence of anti-D can be regarded as characteristic for an anti-Sm serum, as antibodies against Sm B'/B may occur in other diseases. Hence, a good, specific Sm-test should contain only the SmD protein. If the test contains the whole Sm complex, it will deliver false positive results by being positive in patients with other autoimmune diseases.[1]

Keep in mind that no assay is 100 percent sensitive and 100 percent specific. While the anti-Sm antibodies are known for being extremely specific to lupus, a diagnosis should not be made based solely on a single test result taken in isolation. Doing so could lead a clinician to form a presumptive diagnosis of lupus and prescribe a treatment for the patient for a disease which is not present, with drugs that may not help and often have devastating side effects.

Hypothetical illustrations

Let's look at two hypothetical examples that demonstrate this concept. If a lab evaluates 10,000 sera per year for the presence of Sm antibodies, there actually might be fewer than 400 sera from patients with lupus. A test with an average sensitivity of 22 percent and an average specificity of 98 percent will correctly find 88 lupus sera to be positive. Alternately, false positive results will occur in two percent of the 9,600 non-lupus-patients. Therefore, a total of 88 correct positives occur with 192 false positives, and less than a third of the positive results are really from lupus patients **(Table 1)**. If the test has the same sensitivity, but a 99 percent specificity (one percent more than in the first example), only 96 non-lupus patients will falsely be identified as positive. This means about half of the positive results are correctly identified as positive **(Table 2)** due to the improved specificity of the assay, instead of a third of the patients. On the other hand, an increase in sensitivity of one percent, from 22 percent to 23 percent, results in only an additional four sera being classified as positive (92 instead of 88),

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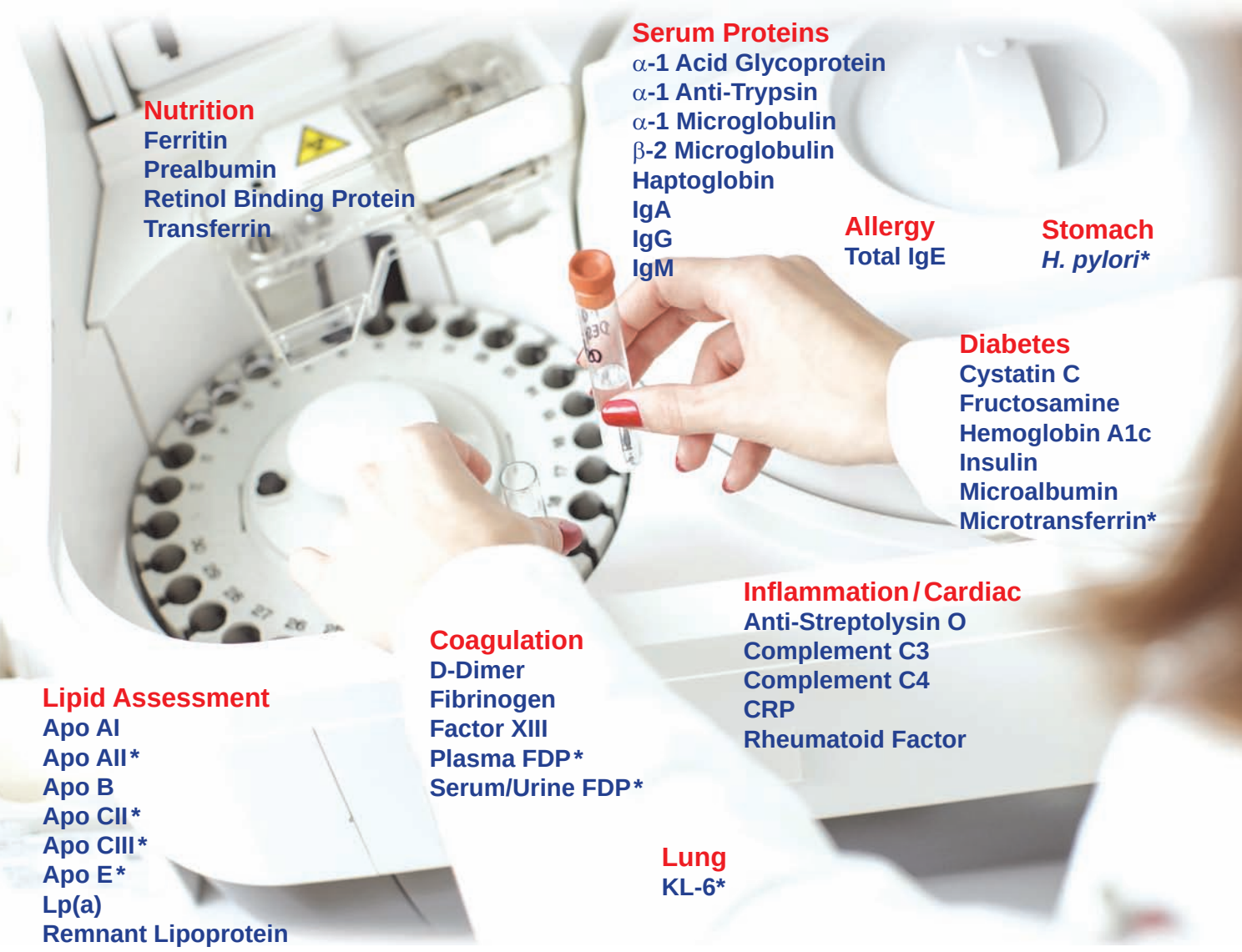

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yielding very little change in the number of correct versus false positive results. (**Table 3)**

Why does this matter? Due to low prevalence, the pretest probabilities of disease for almost all autoantibodies are very low. A difference in specificity of two percent, as demonstrated in a study by Van Hoofels et al, causes a marked impact on whether sera are classified correctly as positive versus false positive results.[3] This means that a slightly lower specificity can lead to a wrong diagnosis in many patients.

	Anti-Sm positives	Anti-Sm negatives	Sum
lupus patients	92	308	400
Non-lupus patients	192	9,408	9,600
Total	284	9,716	10,000

Table 3. While a one percent higher specificity has a large impact, a one percent higher sensitivity has almost no influence on the positive predictive value; in this example, 32 percent instead of 31 percent. Still, less than a third of the positive sera are from lupus patients.

Specificity is a critical metric in assays associated with autoimmune disease. Unfortunately, during a typical assay comparison performed in many laboratories, the specificity for each assay in review is not usually assessed. Without knowing the clinical characteristics and histories of the patients and samples, a laboratory can only determine whether the two tests in the evaluation deliver comparable results. In addition, in the rare cases where the clinical diagnosis is known for samples, often too few sera from controls are available to form a statistically valid conclusion about the specificity of each assay being evaluated.

Expanding validation testing

A consequence of the difficulty in determining specificity is the underestimation of its importance. This is mirrored and exemplified in the United States in proficiency testing (PT) programs where usually only the sensitivity of a test method is judged. Comparable to the evaluations in a clinical laboratory, the providers of sera for quality assessment programs often do not have any information about the diagnosis of the serum donors. This limitation may be the reason behind the need to rely upon a consensus, where the results reported by the dominant method used by participants (the majority) influence whether a sample is deemed negative or positive. The market leader and respectively the market-dominant method therefore essentially determine whether a test result is considered correct or not. The question to ponder is whether this inadvertently causes innovative tests, which are markedly different from the majority, to be underutilized, even when they have a higher diagnostic value as it relates to

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disease. Usage of clinically characterized samples in PT programs would be a positive step forward in how these programs are designed and allow for not only sensitivity to be assessed, but, importantly, specificity of assays as well.

In summary, the specificity in diagnostic tests for autoantibodies matters a great deal, and laboratories considering the adoption of an assay would benefit greatly from expanding the validation testing to include clinically characterized samples; the same can be said for PT programs.

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Standardization and harmonization in state-of-the-art anti-nuclear antibody testing

By Oliver Sendscheid, PhD

Anti-nuclear antibody (ANA) testing is a cornerstone of autoimmune diagnostics. ANAs occur in a variety of autoimmune diseases, including systemic autoimmune rheumatic diseases (SARD), systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome (SjS), systemic sclerosis (SSc), polymyositis (PM), dermatomyositis (DM) and primary biliary cirrhosis (PBC). Detection and classification of ANA is a major criterion for diagnosing and differentiating these diseases.

ANAs are detected by indirect immunofluorescence assay (IFA) followed by specific confirmatory tests. IFA on human epithelial (HEp-2) cells is the standard for detection of ANAs, as endorsed by the American College of Rheumatology.[1] Different types of ANAs give rise to characteristic staining patterns on the HEp-2 cells, depending on the cellular location and properties of the antigenic target. Analysis of the fluorescence pattern enables classification of the antibody or antibodies present in the patient sample, as the cell substrate contains the complete relevant antigen spectrum.

However, ANA testing strategies and result reporting vary greatly among laboratories. The International Consensus on ANA Patterns (ICAP) was released in 2015 with the goal of harmonizing the nomenclature and interpretation of ANA results.[2,3] Additionally, the use of automation solutions and computer-aided microscopy has significantly increased the level of standardization in laboratories. This article reviews these latest advances and highlights the benefits for diagnostic laboratories.

Overview of ICAP classification tree

The umbrella term ANA is, for historical reasons, used to refer to all cellular antibodies that are detected on HEp-2 cells. The ICAP classification identifies three major fluorescence patterns; nuclear (true ANA), cytoplasmic, and mitotic. Each category is subdivided into groups and subgroups of patterns, creating a classification tree. Each pattern and subpattern is assigned an anti-cell (AC) pattern code, from AC-1 to AC-28. The patterns are additionally designated as competent-level or expert-level reporting, depending on the clinical relevance and ease of recognition. The reporting level is a fluid system, which will be adapted in the future based on feedback from users.

Nuclear patterns (true ANA)

Nuclear patterns are defined as any staining of the HEp-2 interphase nuclei. The nomenclature for nuclear patterns is primarily based on the reactivity observed in the nucleoplasm (for example, homogeneous or speckled) and the nuclear subcomponents that are recognized (for example, centromere or nucleolar). The nuclear patterns are comprised of homogeneous, speckled, centromere, discrete nuclear dots, nucleolar, nuclear envelope, and pleomorphic patterns. Dense fine speckled 70 (DFS70) belongs to the speckled group. The centromere pattern belongs to the discrete nuclear dots, but is given its own grouping due to its characteristic pattern and frequent occurrence in the clinical setting.

The nuclear pattern groups are further divided into subgroups. The classification of the nuclear pattern provides information about the likely target antigens and thus possible disease indications **(Table 1)**. For example, the homogeneous pattern arises from reactions with chromatin components such as double-stranded DNA, histones, and/or nucleosomes. These autoantibodies are associated with SLE, drug-induced lupus, and juvenile idiopathic arthritis. Other nuclear reactions can help clinicians to delimit, for example, MCTD, SjS, SSc, PM, DM, PBC, or other autoimmune diseases. Mixed patterns can occur if more than one autoantibody/pattern is present in the patient sample; for example, autoantibodies to both centromere (nuclear) and mitochondria (cytoplasmic) frequently coexist in PBC.

Pattern (ICAP)	Code	Antigen association	Disease association
Homogeneous	**AC-1**	dsDNA, nucleosomes,histones	SLE, drug-induced lupus, juvenile idiopathic arthritis
Speckled	**AC-2,4,5**	hnRNP, U1RNP, Sm SS-A/Ro (Ro60), SS-B/La, RNA polymerase II, Mi-2, Ku	MCTD, SLE, SjS, DM, SSc/PM overlap
Dense fine speckled	AC-2	DFS70/LEDGF	Rare in SLE, SjS, SSc
Fine speckled	AC-4	SS-A/Ro (Ro60), SS-B/La, Mi-2, TIF1y, TIF1ß, Ku, RNA helicase A, replication protein A	SjS, SLE, DM, SSc/PM overlap
Large/coarse speckled	AC-5	hnRNP, U1RNP, Sm, RHA polymerase III	MCTD, SLE, SSc
Centromere	**AC-3**	CENP-A/B (C)	Limited cutaneous SSc, PBC
Discrete nuclear dots	**AC-6,7**		
Multiple nuclear dots	AC-6	Sp100, PML proteins, MJ/NXP-2	PBC, SARD, PM/DM
Few nuclear dots	AC-7	p80-colin, SMN	SjS, SLE, SSc, PM, asymptomatic individuals
Nucleolar	**AC-8,9,10**		
Nucleolar homogeneous	AC-8	PM/Scl-75, PM/Scl-100, Th/To, B23/ nucleophosmin, nucleolin, No55/SC65	SSc, SSC/PM overlap
Nucleolar clumpy	AC-9	U3-snoRNP/fibrillarin	SSc
Nuclear envelope	**AC-11,12**		
Smooth nuclear envelope	AC-11	Lamins A,B,C, or lamin-associated proteins	SLE, SjS, seronegative arthritis
Punctuate nuclear envelope	AC-12	Nuclear pore complex proteins (i.e., gp210)	PBC
Pleomorphic	**AC-13,14**		
PCNA-like	AC-13	PCNA	SLE, other conditions
CENP-F-like	AC-14	CENP-F	Cancer, other conditions

Table 1. Targeted antigens and associated diseases for nuclear patterns

Cytoplasmic patterns

Cytoplasmic patterns are defined as any staining of the HEp-2 cytoplasm. The nomenclature is primarily based on the reactivity observed

Pattern (ICAP)	Code	Antigen association	Disease association
Fibrillar	AC-15, 16,17		
Linear/actin	AC-15	Actin, non-muscle myosin MCTD	MCTD, chronic active hepatitis, liver cirrhosis, myasthenia graviss, Crohn's disease, PBC, long-term hemodialysis, rare in SARD other than MCTD
Filamentous/ microtubules	AC-16	Vimentin, cytokeratins	Infectious or inflammatory conditions, long-term hemodialysis, alcoholic liver disease, SARD, psoriasis, healthy controls
Segmental	AC-17	Alpha-actin, vinculin, tropomyosin	Myasthenia gravis, Crohn's disease, ulcerative colitis
Speckled	AC-18, 19,20		
Discrete dots	AC-18	SGW182, Su/Ago2, Ge-1	PBC, SARD, neurological and autoimmune conditions
Dense fine speckled	AC-19	PL-7, PL-12, ribosomal P proteins	"anti-suynthetase syndrome," PM/DM, SLE, juvenile SLE, neuropsychiatric SLE
Fine speckled	AC-20	Jo-1/histidyl-tRNA synthetase	Anti-synthetase syndrome, PM/DM, limited SSc, idiopathic pleural effusion
Reticular/AMA	AC-21	PDC-E2/M2, BCOADC-E2, OGDC-E2, E1a subunit of PDC, E3BP/proteinX	Common in PBC, SSc, rare in other SARD
Polar/Golgi-like	AC-22	Giantin/macrogolgin, golgin-95/GM130, golgin-160, golgin-97, golgin-245	Rare in SjS, SLE, RA, MCTD, GPA, idiopathic cerebellar ataxia, paraneoplastic cerebellar degeneration, viral infections
Rods and rings	AC-23	IMPDH2, others	HCV patients post-IFN/ribavirin therapy, rare in SLE, Hashimoto's and healthy controls

Table 2. Targeted antigens and associated diseases for cytoplasmic patterns

in the cytoplasm (for example, fibrillar or speckled) and the cytoplasmic structure that is recognized (for example, rods and rings). The five main cytoplasmic patterns are fibrillar, speckled, reticular/mitochondrion-like (AMA), polar/Golgi-like, and rods and rings. Cytoplasmic autoantibodies of different specificities are found in a range of diseases **(Table 2)**, including MCTD, PM, DM, SLE, SSc, PBC, Crohn's disease, ulcerative colitis, and myasthenia gravis. As per the ICAP statement, cytoplasmic patterns should now be reported in patient results and no longer considered ANA negative.

Mitotic patterns

Mitotic patterns are defined as patterns that address cell domains strongly related to mitosis. These patterns include centrosomes, spindle fibers with subpattern nuclear mitotic apparatus (NuMA), intercellular bridge, and mitotic chromosome coat. Some mitotic patterns (for example, centrosomes) are not exclusively associated with mitosis, but exhibit very distinctive features in mitotic cells. Mitotic antibodies rarely occur in diseases such as SSc, SLE, SjS, and Raynaud's phenomenon **(Table 3)**.

Pattern (ICAP)	Code	Antigen association	Disease association
Centrosome	AC-24	Pericentrin, ninein, Cep250, Cep110, enolase	Rare in SSc, Raynaud's phenomenon, infections (viral and mycoplasma)
Spindle fibers	AC-25	HsEg5	Rare in SjS, SLE, other SARD
NuMA-like	AC-26	Centrophilin	SjS, SLE, other
Intracellular bridge	AC-27	Aurora kinase B, CENP-E, MSA-2, KIF-14, MKLP-1	Rare in SSc, Raynaud's phenomenon, malignancy
Mitotic chromosome coat	AC-28	Modified histone H3, MCA-1	Rare in discoid lupus erythematosus, chronic lymphocytic leukemia, SjS, and polymyalgia rheumatica

Table 3. Targeted antigens and associated diseases for mitotic patterns

Recent innovations aid standardization

Automation of IFA evaluation increases the consistency among different readers and laboratories and boosts the speed and efficiency of the evaluation procedure. The need for standardization and automation in IFA is tremendous in all fields of autoimmune diagnostics. Manual evaluation of results is both time-consuming and subjective. Automation platforms with harmonized software and hardware components have in recent years contributed enormously to the standardization and simplification of the evaluation process, especially for ANA, but also antineutrophil cytoplasmic antibodies (ANCA) and *Crithidia luciliae* immunofluorescence test (CLIFT).

Advanced software provides positive/negative classification, pattern recognition, and titer designation. Today's systems offer fully automated identification of immunofluorescence patterns on HEp-2 cells, including nuclear, cytoplasmic, mitotic, and, importantly, mixed patterns. The recording of tissue substrates, such as liver, kidney, stomach, esophagus, small intestine, heart, and neuronal tissue, is also feasible with a cutting-edge system. It is anticipated that the continued development of automated evaluation systems will lead to even greater standardization of IFA and further improvements in workflow for diagnostic laboratories.

The ICAP initiative has laid the foundation for standardized nomenclature and reporting of ANA test results. It is an ongoing process, which could potentially mature into a global standard, incorporating input from laboratories and clinicians worldwide. A further important contribution to ANA standardization is the increasing use of computer-assisted microscopy by labs. These systems offer fully automated identification of immunofluorescence patterns on HEp-2 cells.

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Oliver Sendscheid, PhD, serves as Scientific Affairs Director for the U.S. subsidiary of **EUROIMMUN, Inc**.

Understanding key performance indicators and benchmarks for anatomic pathology labs

By Diana Brooks

Reporting and benchmarking are critical components in managing any pathology practice or clinical laboratory. Gone are the days of waiting to review a month-end reporting packet. Real-time feedback on operational and financial performance is becoming increasingly necessary as the healthcare industry demands more work for less reimbursement and laboratories are required to adapt leaner, more efficient processes.

While benchmarking is a somewhat rudimentary method for managing the financial performance of a pathology practice, the information that core indicators provide helps to identify whether the organization is performing on par with, better than, or not as well as similar pathology practices within the market. In addition, monitoring benchmarks can provide early indications of process or staff training issues that should be remediated.

Using a large pathology and clinical laboratory claims database, current claims data and trends were analyzed and key performance indicators (KPIs) developed to help pathology labs benchmark operational and financial performance and better understand the quality of current revenue cycle management (RCM) processes. Top KPIs identified for anatomic pathology include:

- net collections (%)
- bad debt (%)
- days in Accounts Receivable (AR) (#)
- denials (%).

Net collections rate

The net collections rate is calculated by dividing net charges (gross charges – contractual adjustments) by net collections (gross payments – refunds). The national rate average for net collections is 88 percent to 92 percent, based on payor mix and patient demographics. A below-average performance on net collections rate may indicate that claims aren't being followed-up on in a timely manner. An above-average performance on net collections rate may mean RCM processes are being managed and collected exceptionally well, or it could also indicate that the billing department is taking what should be bad debt adjustments as contractual adjustments, which erroneously inflates net collections statistics.

Bad debt rate

The bad debt rate is calculated by dividing the total amount written off as bad debt by the total amount that was eligible to collect. In this explanation, the specific reference is to bad debt associated with patient and third-party transactions.

Bad debt rate can be challenging to benchmark due to differences in how labs and billing service providers choose to classify contractual adjustments and denials. For example, timely filing is considered a bad debt by some practices, but others consider it a contractual adjustment. The basic rule is that anything that isn't a formal contractual adjustment (the difference between what is billed and the contracted reimbursement rate or total allowable for that service) should be written off as bad debt. However, there may be exceptions.

When bad debt write-offs are classified accurately, a bad debt benchmark of 10 percent to 12 percent is considered average or good for most pathology practices. While bad debt write-off benchmarks were lower in previous years, the increase in patient responsibility due to growing high deductible health plans has resulted in most pathology groups trending slightly higher bad debt percentages than historical averages.

Key Performance Indicators

- NET COLLECTIONS
- BAD DEBT
- DAYS IN ACCOUNT RECEIVABLE
- DENIALS

In instances where bad debt performance is better than average (less than 10 percent), it's likely that patient demographics (for example, propensity to pay) and follow-up policies are resulting in higher collections and, subsequently, fewer bad debt write-offs. That said, it could also indicate that non-contractual denials are being written off as contractual adjustments instead of bad debt. In addition to auditing write-off policies, it is also worth reviewing days in AR to ensure that there isn't a large volume of claims older than 90 days sitting in receivables. There may be balances that have not yet been adjusted or accounted for as a potential bad debt accrual.

Below average performance (more than 12 percent) on bad debt may indicate claims are not followed-up on in a timely or appropriate fashion, and/or claims are being written off too quickly, resulting in missed opportunities to appeal denials, missed filing deadlines, or writing off patient balances without proper follow-up or engagement in order to increase likelihood of payment.

Days in Accounts Receivable

To calculate days in AR, compute average daily charges (for example, total charges for six months divided by the number of days in the last six months) and divide the total accounts receivable by the average daily charges.

Although days in AR seems to be a straightforward calculation, the benchmark can vary based on the type of work performed by the laboratory and the timing

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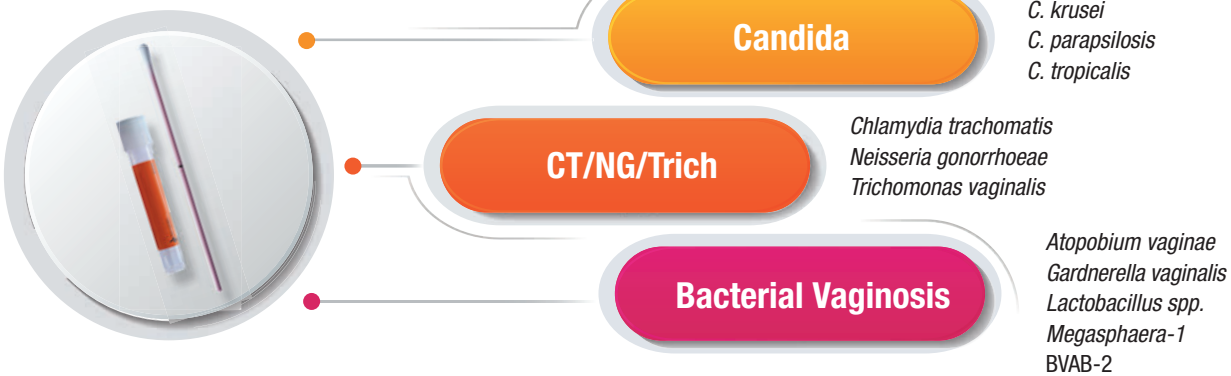

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of adjustments. Overall, a benchmark of 35 to 50 days is fairly standard. If days in AR exceed 50 days, that warrants additional investigation.

Below-average performance on days in AR (less than 35 days) may be the result of a terrific payor mix that pays quickly. On the other hand, it may also mean that adjustments are being made or claims are being written off too quickly, resulting in missed opportunities to collect on cases that are payable. It is a good practice to review bad debt write-offs by category to determine if there are any unusual outliers (such as how quickly patients are rolled over to collections).

Above-average performance on days in AR (above 50 days) may be related to the type of work a laboratory performs. Groups that work with capitated plans and IPAs, bill out-of-network plans, or perform molecular testing that may require appeals will have above-average days in AR without there being an issue. A good way to analyze AR is to benchmark the top 10 payors (which likely make up close to 80 percent to 90 percent of revenue) and determine if there are any clear outliers. If the laboratory performs complex testing with a high likelihood of denials, also review days in AR by CPT code to see which tests have the longest turnaround (and success/failure rates) on payment.

Denial rate

Denial rate is calculated by dividing the total dollars billed for denied claims by the total dollars billed on all submitted claims for each processed remittance. The goal is to maintain a denial percentage of less than 10 percent.

Depending on the revenue cycle management system in use, and the granularity of data provided by the billing software or service provider on denial types and gross and net revenue, a pathology practice may or may not have insight into its denial percentage. Yet a practice's denial rate is one of the most critical performance measures of AR, as it is an important indicator of how well RCM processes are working, and reflects payor-specific trends that can impact the practice and its patients.

Pathology labs can only improve what they measure. A sound technology infrastructure that supports optimized business processes and delivers the right financial and operational benchmarks and KPIs is essential to success.

Diana Brooks serves as Director of Program Development, Anatomic Pathology, for San Diego-based **XIFIN, Inc.**, developers of a technology platform used to streamline revenue cycle management and business decision-making.

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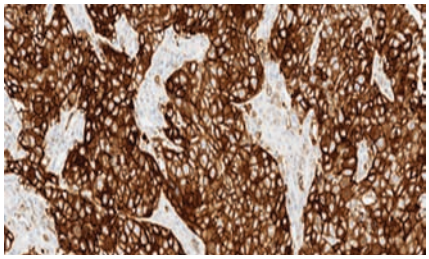

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An examination of two critical IHC biomarkers

By Jonathan Weinreich, BS

The detection of cervical intraepithelial neoplasia (CIN) is of increasing importance to cervical cancer screening, and has been fundamental to the overall decrease in the rate of cervical cancer incidence in the United States and around the world. The most common method for investigating CIN levels is immunohistochemistry (IHC). As has been shown in the literature, p16 and Ki-67 have been the leading biomarkers for high-risk HPV infections, which can lead to cancers of the cervix. Infections of HPV genotypes 16, 18, 31, 33, and 51 have been shown to be the leading causes of cervical cancer, and it has further been shown that there is a significant difference in diagnostic result when scoring between a CIN1 sample and a known negative. In this article, we will show how histologists and pathologists can help detect and identify CIN with these two critical biomarkers.

In a clinical histology lab, tissue blocks are received from biopsy specimens, mainly as part of the accessioning and grossing process. Briefly, the patient is entered into the clinical system and is assigned a unique case number. The tissue received is fixed in formalin and set in paraffin; the paraffin blocks are then sectioned into slides. The slides can then be subjected to an immunohistochemical stain.

In IHC, the tissue slide is incubated with a series of antibodies, with the ultimate goal of selectively staining a specific antigen that is expressed by the patient's cells. The principle of the test involves using antibodies specific to the antigen of interest, followed by an antibody conjugated to an enzyme (for example, horseradish peroxidase, HRP) that can react with a substrate to create an insoluble colored product for visualization of the biomarker. Immunohistochemistry is widely used in the clinical lab because the test is done directly on a biopsy of the tissue in question, can be completed in under three hours, and is relatively inexpensive, and because a qualified pathologist can interpret the results as a diagnostic and/or prognostic result for the patient.

Since 1993, when p16 was first identified as a cyclin-dependent kinase inhibitor, this tumor suppressor protein has gained near-universal recognition among pathologists and oncologists alike. p16 was first implicated in human cancer cell lines through genomic analysis; researchers observed that p16 was frequently mutated, which suggested an important role in cancer development. Liggett and Sidrasky[1] showed that in tumors, p16 can be inactivated by homozygous mutation, methylation of the promoter, or a point mutation. Furthermore, it has been suggested that the deletion of p16 is an early event in the progression of cancer, because the deletion of one copy of the gene is found in pre-cancerous cells. However, the expression of p16 is increased under abnormal situations, yet this increase doesn't result in the suppression of cancer because p16 protein is inactive. Interestingly, under pre-cancerous conditions (CIN1-CIN3), p16 is upregulated and used as a biomarker for the detection of cervical cancer.

While p16 can suppress tumor progression, tumor growth and proliferation are dependent on another key transcription factor, Ki-67. During cellular interphase, Ki-67 is exclusively found in the nucleus, which gives the advantage of making it easy to "spot" in an IHC stain. Ki-67 is present during active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells. During tumorigenesis and cell growth, Ki67 is unregulated, therefore mediating the uncontrolled cell growth. These facts make Ki-67 an antigen of choice when evaluating the growth fraction of a patient's tissue section. The approximate fraction of Ki-67 positive cells has been shown to correlate with the clinical diagnostic result. A higher percentage of proliferating cells indicates a fast-growing tumor, while a low number of Ki-67 positive cells usually indicates a pre-cancerous lesion or an early-stage cancer.[2]

In a routine clinical lab, p16 and Ki-67 IHC tests are often run together, side-by-side, to allow pathologists to have a full understanding of the extent of a patient's diagnostic and prognostic outcomes. While p16 scoring can commonly be used to identify the grade or progression of the cancerous tissue, Ki-67 is used to measure the rate of cell proliferation. Combining the two offers a superior diagnostic result when compared with p16 alone or Ki-67 alone. This is reflected very well in the literature, as Zhong et al found that p16 and Ki-67 expression significantly increased with disease progression (p16, $P < 0.001$; Ki-67, $P < 0.001$).[3] Clearly, p16 and Ki-67 are useful in the evaluation of progression of cervical dysplasia. Together, they are a powerful tool for evaluating cancer progression.

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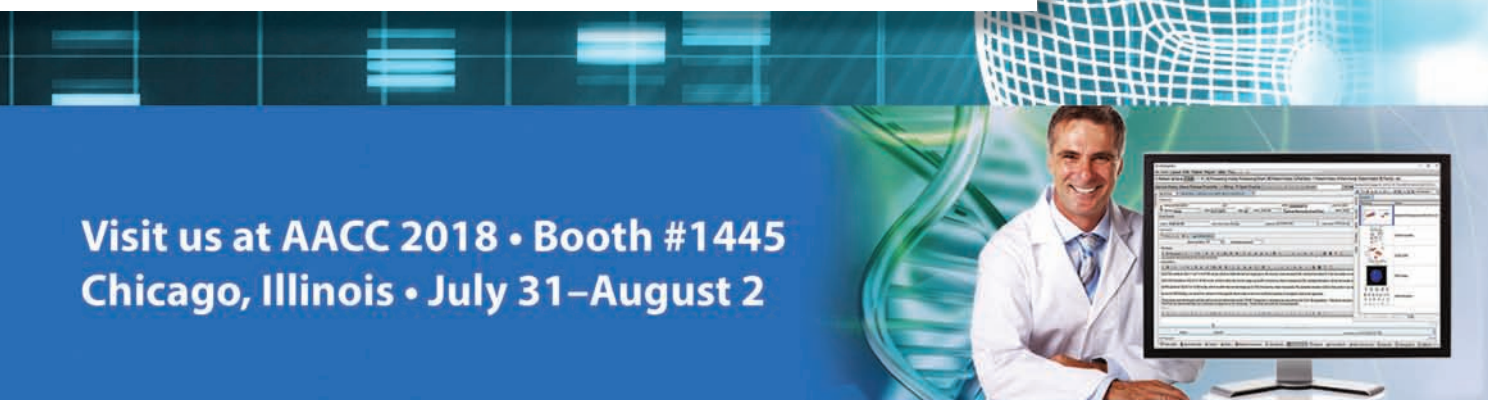

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Measuring adiponectin levels to assess type 2 diabetes risk

By Carolyn Humphrys, BSc (Hons)

Approximately seven percent of the United States population is affected by diabetes, and millions are at risk of developing the condition. One in three adults has prediabetes, and 90 percent of them don't know they have it.[1,2] Fifty percent of people with diabetes die of cardiovascular disease (CVD,) and diabetes is the leading cause of newly diagnosed adult blindness for people between the ages of 20 and 74.[3,4] Early risk assessment is extremely important, as type 2 diabetes can have detrimental long-term consequences, including damage to kidneys, nerves, eyes, and heart, and places an ever-increasing burden on healthcare systems.[4]

Adiponectin and diabetes

Adiponectin is a protein hormone with anti-inflammatory and insulin-sensitizing properties that plays an important role in a number of metabolic processes such as glucose regulation and fatty acid oxidation. Adiponectin levels are inversely correlated with abdominal visceral fat (AVF),[5] which is the fat that develops around the abdominal cavity, close to many vital organs including the liver, intestines, and pancreas. This visceral fat plays a potentially dangerous role, affecting how hormones function, with high levels having been shown to be a strong indicator of type 2 diabetes, metabolic syndrome, and CVD. Research has found that this type of fat also secretes retinol-binding protein 4 (RBP4), which has been found to increase insulin resistance.

A study published in *JAMA*,[5] involving meta-analysis of 13 prospective studies with a total of more than 14,500 participants and 2,623 cases of type 2 diabetes, demonstrated a correlation between increasing adiponectin levels and a declining risk of the disease, across diverse populations. This study highlighted the potential of adiponectin as a risk biomarker.

Traditional methods of measuring risk

Non-biochemical ways in which a patient's risk of developing type 2 diabetes is evaluated include the consideration of various factors such as gender, age, family history, body mass index (BMI), waist size, and blood pressure. In particular, BMI (the measurement of weight kg/height m^2) is commonly used to determine whether a patient is overweight or obese. However, this has proved to be unreliable as a diabetes marker, and it has its limitations when other factors are taken into account, such as age, sex and race. There is a significant need for a more accurate method of early risk assessment.

With regard to clinical testing, routine tests used to assess risk of type 2 diabetes include measuring fasting plasma glucose (FPG), but this has been shown to have poor specificity; many individuals are identified as having impaired fasting glucose, but their absolute risk of conversion to diabetes is only five percent to 10 percent per year.[6-8] Another common test is the oral glucose intolerance test (OGTT), which is more accurate but is rarely used in practice due to the length of time it takes to perform and because drinking the "Glucola" is unpleasant enough for many patients that it negatively affects compliance. Additionally, oral glucose intolerance can be detected only when the underlying disease has been progressing for many years, which makes the test inadequate for early risk assessment and prevention.[9]

Automated adiponectin assay vs. ELISA

In those contexts it becomes increasingly clear that measuring adiponectin levels is the most accurate way to assess prediabetes. But to become a routine test, it must become one that can be easily used by clinical laboratories. Traditionally, adiponectin has only been available in an enzyme linked immunosorbent assay (ELISA) format, but new research has contributed to the development of adiponectin in an automated format. This means that adiponectin can help to improve efficiencies and facilitate expansion of laboratories and their testing capabilities. ELISAs for clinical testing are notably time- and personnel-consuming, but moving from ELISA to an automated biochemistry method can help to avoid this, as well as increase confidence in clinical results by reducing the risk of error and contamination. Adding automated adiponectin to a routine testing panel can ensure accurate and early diagnosis of prediabetes for a larger population of patients.

> "Adding automated adiponectin to a routine testing panel can ensure accurate and early diagnosis of prediabetes for a larger population of patients."

Other applications for adiponectin testing

In addition to measuring risk of type 2 diabetes, the measurement of adiponectin levels can also be used to identify women at risk of developing gestational diabetes (GD) in early pregnancy. Interestingly, a study found that women who had a lower adiponectin concentration measured on average six years before pregnancy were associated with a five-fold increased risk

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of developing GD.[6,10] BMI is often used to assess the risk of developing GD, and women with a BMI of over 30 are urged to maintain a healthy weight before they become pregnant. However, some women develop GD regardless of their BMI, which emphasizes the need for adiponectin testing to ensure a safe and healthy pregnancy for both mother and baby.

To conclude, the need for measuring adiponectin levels has never been greater given the prevalence of the disease and the limitations of traditional means of risk assessment. Early risk assessment is not only vital because of the health implications of diabetes and its complications, but also because, economically, diabetes and its complications bring substantial costs not only for people with diabetes and their families, but for health systems and national economies, both through direct medical expenses and loss of work and wages. It is clear that an improved method for assessing risk, along with a convenient format for routine clinical use, would enable physicians to accurately evaluate more individuals, and as a consequence, lower the percentage of the global population with the disease in the future through lifestyle changes. Such adiponectin assays are for research use only and not for use in diagnostic procedures in the U.S. at present; stay tuned.

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3. StatStrip market share of 57% and Inform II market share of 30% from 2017 market share survey. Data available on request.

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Emerging diabetes biomarkers offer new insights

By Doug Borses, BS, MSc

Diabetes and its complications impose severe economic burden on individuals, families and the U.S. healthcare delivery system. The total costs of diagnosed diabetes due to both direct medical costs and lost productivity in the United States in 2017 was $327 billion dollars. After adjusting for population age and sex differences, the average medical expenditures among people with diagnosed diabetes were 2.3 times higher than non-diabetics.[1]

Currently, blood glucose and hemoglobin A1c are the standard measures for the diagnosis and monitoring of diabetes. There has recently been increasing interest in nontraditional diabetes biomarkers, including fructosamine, glycated serum protein (GSP), glycated albumin (GA), and 1,5 anhydorglucitol (1,5 AG). Recent studies suggest that expanded use of these tests has the potential to improve diabetes care, as these assays overcome the limitations of HbA1c in some patients, while providing additional insight into shorter-term glycemic control and improving risk stratification for diabetes and its complications.[2]

Glycation of hemoglobin and serum proteins

Blood glucose nonenzymatically and irreversibly attaches to intracellular and blood proteins via a slow Maillard reaction between glucose and amino acid residues of proteins to form glycated proteins **(Figure 1)**. HbA1c is produced in this manner by the reaction between glucose and hemoglobin and is considered the standard for monitoring long-term glycemic control in patients with diabetes. Because red blood cells are replaced every 90 days, HbA1c provides a glycemic picture of blood glucose over the previous two to three months. HbA1c has been shown to predict complications of diabetes such as cardiovascular disease, neuropathy, and nephropathy clinically through randomized clinical trials, including the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS).[3,4]

There are, however, some well-understood shortcomings of HbA1c testing for certain groups of patients, which result in lower diagnostic and prognostic performance. These patients include pregnant women and the elderly. There also exists the risk of decreased reliability of HbA1c in patients with decreased red cell lifespan, including patients on hemodialysis, as well as those with hemolytic anemia, end-stage renal disease, and heavy alcohol consumption. Additionally, there are potential analytical difficulties, depending on the HbA1c method used, with variant hemoglobins, persistent fetal hemoglobin, and chemically modified derivatives such as carbamylated and acetylated hemoglobins,[5] which may reduce the reliability of the assay.

Glycated serum protein (glycated albumin)

Blood glucose also reacts with serum proteins via a slow, non-revisable Maillard reaction to form glycated serum protein (GSP). Serum albumin composes 60 percent to 70 percent of serum protein and represents > 90 percent of total serum glycated proteins.[6] GSP is used for monitoring average blood glucose levels over the past two to three weeks, making it more useful for showing problems with control in the short term than HbA1c. By combining GSP measurements with HbA1c, it is possible to see a more complete picture of a patient's long term and intermediate glycemic control **(Figure 2)**. Although HbA1c continues to be considered the standard, a number of clinical studies have found that GSP is at least as predictive of future complications as HbA1c.[7] Armed with a better understanding of the patient's glycemic profile, the physician may be able to recommend a more informed treatment plan.

Fructosamine

In the literature, glycated serum protein (GSP) is also known as fructosamine. Fructosamine is traditionally measured by a non-specific chemical method using nitroblue tetrazolium (NBT) that is interfered with by various reducing substances in patient samples **(Figure 3)**. Although rapid, inexpensive, and available for automation, the method remains poorly standardized. Moreover, due to the technical nature of the assay, all molecules with reducing activity, such as bilirubin and vitamins, may interfere in the measurement, thus biasing the test results, especially when present in large concentrations. Recently, a more specific fructosamine

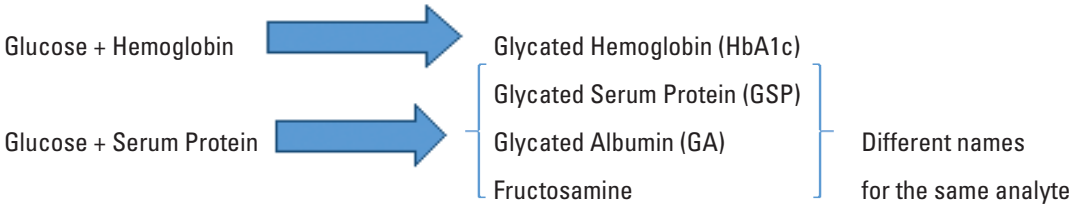

Figure 1. Enzymatic glycated serum protein methods measure primarily glycated albumin. Most current fructosamine assays are colorimetric and are less specific for glycated serum proteins than the enzymatic methods.

	Traditional Biomarkers		Non-Traditional Alternative Glycemic Biomarkers			
	Transit	Long-Term	Intermediate			Short-Term
	Fasting Glucose Electrochemical	**HbA1c HPLC/Immunoassay/ enzymatic**	**Fructosamine Colorimetric NBT Nitro blue tetrazolium [13]**	**Glycated Albumin/ Albumin ratio Enzymatic**	**Glycated Serum Protein/Albumin Enzymatic [14]**	**1,5 AG Enzymatic**
Time Frame	Snapshot of current blood glucose	Biomarker of average blood glucose over the past 2-3 months	Biomarker of average blood glucose over the past 2-3 weeks	Biomarker of average blood glucose over the past 2-3 weeks	Biomarker of average blood glucose over the past 2-3 weeks	One-to-two-week measure related to the average daily maximum blood glucose
Strength	Assessment of current glycemic status Inexpensive Standardized Reference materials	Non-Fasting Gold standard long-term marker of glycemic control. Standardized Reference materials	Non-Fasting Serum/Plasma Less expensive	Non-Fasting Less interferences from endogenous reducing substances Shown to improve screening for pre-diabetes in some populations	Non-Fasting Less interferences from endogenous reducing substances Shown to predict future complications of diabetic patients as effective as HbA1c US FDA 510k cleared	Non-Fasting Shown to correlate with post meal hyperglycemia and glucose variability over a period of 1-2 weeks
Weakness Assay Method	Requires fasting specimen, and only indicates the transit level of glucose	Requires whole blood – Needs blood lysing step. Cannot be used for patients who are having the following conditions: • Rapid changes in blood glucose • Larger blood glucose excursions • Shortened RBC lifespan • Various anemias • Variant hemoglobins • Advanced renal disease (including dialysis patients)	Limited specificity for the exact measurement of glycated proteins in serum. Non-specific, 50% of signal is not from glycated albumin. Significantly interfered by endogenous reducing substances	Not widely available in the USA. The product requires two separate measurements and ratio calculations.	Measures all glycated serum proteins including about 10% non-albumin proteins	Results dependent on renal threshold. No standard reference materials.

Table 1. Brief comparison of glycemic biomarkers

assay has been developed, a new enzymatic GSP assay for clinical laboratory determination of GSP or GA that is formulated with ready-to-use liquid stable reagents. Another GA assay has been developed in Japan, which measures the percentage of glycated albumin to total albumin; this assay is not yet widely available in the U.S.

1,5 anhydroglucitol

The Diabetes Control and Complications Trial (DCCT) effectively showed that HbA1c concentrations are associated with microvascular complications, but it also showed that HbA1c alone fails to explain all the observed risk.[8] This has led to the hypothesis[9] that microvascular complications may result from both chronic hyperglycemia and from glycemic excursion, also known as glycemic variability. 1,5 anhydroglucitol (AG) correlates with post-meal hyperglycemia and reflects trends over a period of one to two weeks. Unlike the glycated hemoglobin and glycated serum proteins, 1,5 AG does not reflect an average blood glucose but rather reflects hyperglycemia and glycemic variability.

1,5 AG is a monosaccharide that is freely filtered in the renal glomerulus and competes with glucose for active transport back to the bloodstream by the renal tubular cells. As the level of glucose increases, renal tubular cells preferentially reabsorb glucose, and 1,5 AG passes into the urine and is cleared from the blood. As blood glucose rises, 1,5 AG concentrations decrease. Recent studies have shown that 1,5 AG is strongly and independently associated with long-term risk of complications including microvascular outcomes,[10] retinopathy, albuminuria, and cardiovascular disease.[11] Most important, when adjusted for HbA1c and fasting glucose, 1,5 AG was associated with a fivefold increase of retinopathy and twofold increased risk of chronic kidney disease.

The future of non-traditional markers

Nontraditional glycemic biomarkers are not replacements

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for the HbA1c assay, but rather are complementary assays to HbA1c that can improve quality in diagnosing diabetes and monitoring glycemic control, especially for those patients whose HbA1c levels do not truly reflect the mean blood glucose levels or those with glycemic variability. GSP (GA) provides a picture of past two-week average blood glucose levels, and is a short- to medium-term index for glycemic control. GSP is especially useful in the management of various diabetic conditions such as diabetic pregnancy, dialysis of diabetic patients, and diet or medication adjustment. GSP bridges the gap between blood glucose testing (a transient index) and HbA1c testing (a long-term index), and provides a complementary and unique system to the existing methods for glycemic control. A small improvement in glycemic control may lead to a significant improvement in the quality of life and a huge reduction in the economic burdens currently imposed on the families of people with diabetes and the healthcare systems on which they rely.[12]

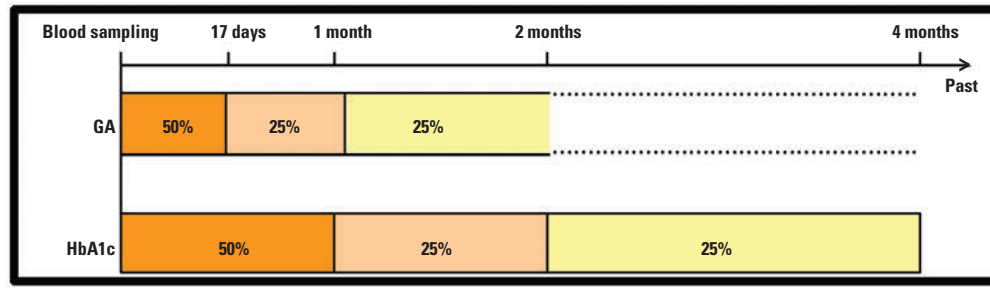

Figure 2. Measurement of glycated albumin can confirm changes in blood glucose status one to two weeks after the commencement of treatment, whereas HbA1c presents a longer term (two to three months) window. Blood glucose status may be more accurately assessed in a monthly interval with the measurement of glycated serum protein.

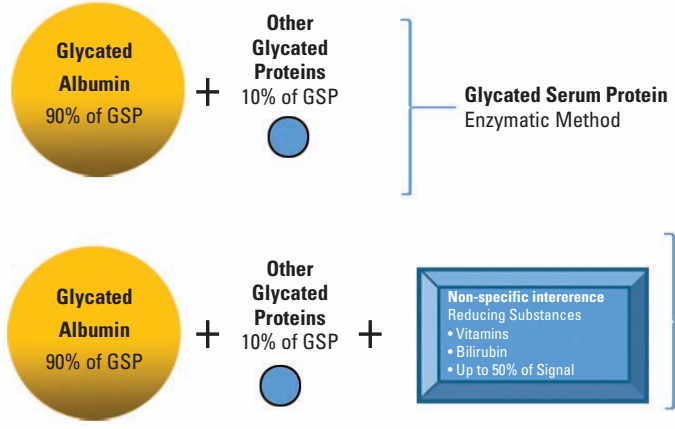

Figure 3. The difference between fructasomine and glycated serum protein.

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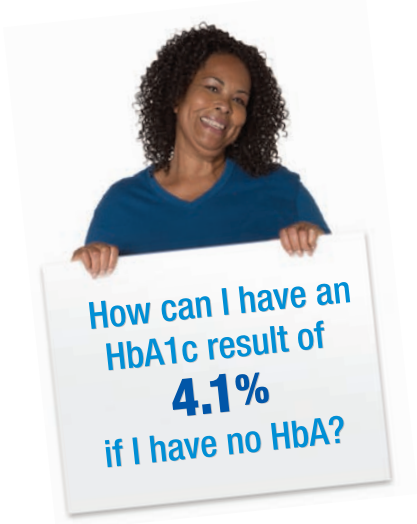

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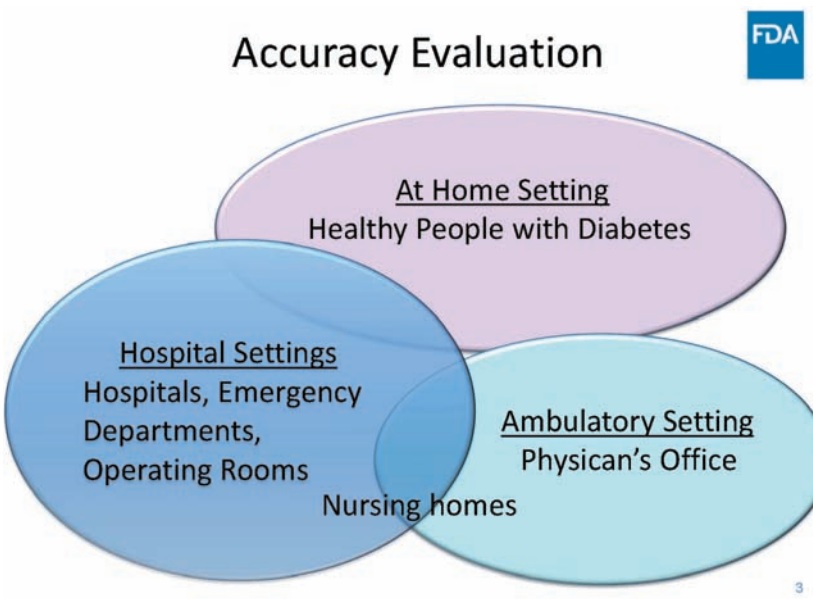
The FDA reviews guidelines for capillary glucose testing in critically ill patients

By Jeffrey A. DuBois, PhD, BCLD/CC (ABB), FACB

Capillary whole blood testing with point-of-care (POC) glucose meters in hospitalized patients and, particularly, in critically ill patients, remains a topic of interest in the medical and regulatory communities. However, determining the requirements for effective clinical use has proved challenging.

An FDA panel convenes

This past March, the U.S. Food and Drug Administration (FDA) convened its Clinical Chemistry and Clinical Toxicology Devices Advisory Panel, seeking guidance and recommendations on the acceptability of capillary specimens in critically ill patients based on benefits and risks, and whether capillary specimen testing in this patient population meets the criteria for waived status under the Clinical Laboratory Improvements Amendments (CLIA) regulations.[1]



The FDA began by summarizing the history of POC glucose testing for the panel and emphasized the need for manufacturers to submit data supporting their glucose meters' acceptability for use with critically ill patients. The FDA reviewed the data submitted for a glucose meter cleared for use with these patients using arterial and venous specimens,[2] and related that no manufacturer had submitted data for capillary whole blood.

The panel was then presented with data from three large studies that compared capillary whole blood glucose to arterial and venous glucose in critically ill patients. Conducted by unidentified manufacturers, the studies used two different glucose meters that were compared to central laboratory results. The cleared glucose meter's critical care claim data for arterial and venous testing was used as the benchmark for comparison.

The panel considers

Capillary performance was shown to be accurate, though less so than the other specimen types. The decrease in accuracy of capillary results surprised a number of panelists, including those specializing in critical care, who said that education was needed for clinicians and nurses regarding the difference in specimen performance.

The panel discussed at length both the analytical performance needs and the clinical factors that should be considered when determining whether capillary testing with a glucose meter is appropriate for critically ill patients, acknowledging that certain patient conditions may be more likely to produce questionable capillary results.

The inaccuracy of capillary glucose compared to arterial or venous glucose in certain conditions is due to inherent differences in the specimen types based on the physiology of capillary vessels and microcirculation. Capillary vessel networks diffuse glucose into surrounding fluid and tissue. The rate of diffusion is dependent on the rate of blood flow. Conditions such as hypoxia, hypotension, and hypoperfusion can cause capillary restriction, which reduces blood flow. When this happens, glucose diffuses more quickly and, as a result, capillary whole blood tested with glucose meters can produce elevated glucose readings compared to arterial or venous specimens.

The panel noted that in certain conditions, an unreliable capillary glucose reading could lead to misdiagnosing a patient and result in harm or death. However, a clear advantage of capillary testing is that the clinician receives immediate results. If the result does not agree with the patient's clinical presentation, an arterial or venous specimen can be drawn and measured at the bedside, provided the POC meter is FDA-cleared for use with critically ill patients.[2] This avoids the need for an alternative testing method yet ensures rapid results for faster clinical decision making and treatment.

The panel concludes

Ultimately, the panel decided that the use of capillary whole blood can be safe and effective for use with critically ill patients, despite the risk of inaccurate results associated with certain patient conditions or treatments. However, the panel did not reach consensus on how to determine which patients are eligible for capillary testing, due in large part to the lack of data and subjectivity regarding clinicians' comfort levels in making this decision.

On the topic of CLIA waivers, the panel also had trouble reaching consensus. Several panelists argued for user-proficiency testing as a consideration in granting CLIA-waived

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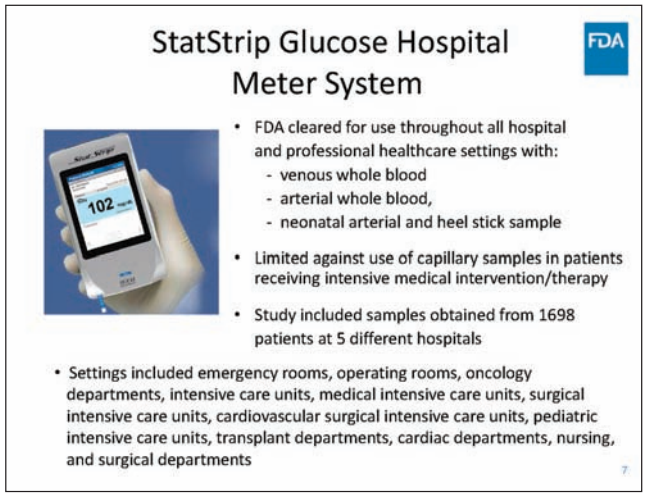

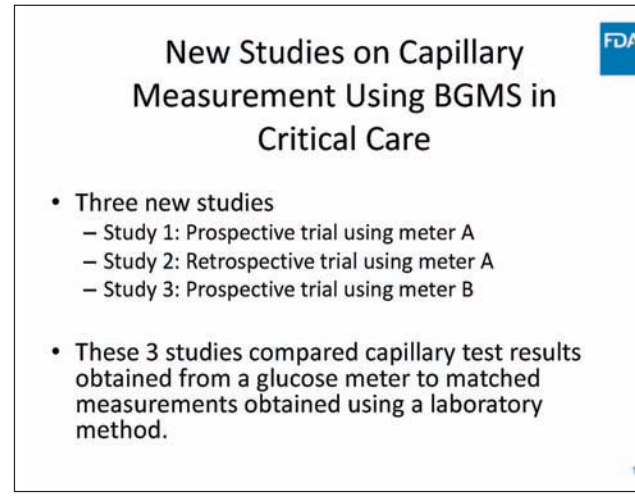

status for capillary testing with glucose meters, which would prove testing to be simple and have "an insignificant risk of an erroneous result" for untrained users. Other panelists felt that special controls were needed to issue a CLIA waiver for glucose meters using capillary specimens with critically ill patients, advocating for a moderate-complexity status. However, panelists noted that unnecessary burden should not be placed on healthcare providers and acknowledged that capillary testing is currently integrated into hospitals and other clinical settings.

Combined Data for Glucose Concentration ≥75 mg/dL

FDA

Study	N	Within ±5%	Within ±10%	Within ±12%	Within ±15%	Within ±20%	Exceeds ±20%
Study 1 – meter A	567	(48.9%)	(79.4%)	(85.4%)	(91.0%)	(96.8%)	(3.2%)
Study 2 – meter A	14884	(50.2%)	(74.5%)	(86.0%)	(92.1%)	(96.4%)	(3.6%)
Study 3 - meter B	333	(50.8%)	(81.7%)	(86.5%)	(92.5%)	(97.3%)	(2.7%)
Meter A: Venous / Arterial	200	(67.5%)	(93.0%)	(95.5%)	(98.0%)	(100%)	(0.0%)
Meter B: healthy population	145	(71.7 %)	(94.5 %)	(96.6%)	(100.0 %)	(100.0 %)	(0.0%)
Statstrip: venous	268	(63.8%)	(91.8%)	(97.0%)	(99.6%)	(100%)	(0.0%)
Statstrip: arterial	1267	(66.6%)	(92.7%)	(96.3%)	(98.2%)	(99.3%)	(0.7%)

One glucose meter has CLIA waived status, which it received in 2007 as a result of its accuracy validation having been performed on hospitalized patients by the manufacturer. Other glucose meter manufacturers performed accuracy validation for their meters through an over-the-counter (OTC) pathway on generally healthy, non-hospitalized patients. As it stands today, and reinforced by the panel discussion, the OTC pathway for waived clearance is no longer an option, and manufacturers must validate meter performance with waived users and each intended use patient population. Otherwise, hospitals must perform this extensive validation themselves, or create alternative testing processes with other devices that are CLIA waived for the intended use patient population.

Following a day of discussion and deliberation, the panel reached consensus that because of the strong need for rapid, POC capillary testing with glucose meters in critically ill patients, along with the meters' current widespread level of use, the benefits of capillary testing outweigh the potential risks. Even with the difference in performance of capillary versus arterial or venous whole blood specimens, the panel concluded that capillary testing can be safe and effective in this patient population if performed with a meter that has been cleared by the FDA to be safe and effective for use with critically ill patients.

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Keeping the laboratory environment clean and safe

By Dan Scungio, MT(ASCP), SLS, CQA (ASQ)

The clinical laboratory is an inherently dangerous place. Laboratorians face a variety of dangers working in an environment that contains biohazards. Utilizing standard precautions and correctly employing Personal Protective Equipment (PPE) are essential keys to ensure laboratorians' safety. Maintaining a clean and orderly environment and employing good disinfection practices are vital as well. A cluttered workspace and an area contaminated with biohazards threaten the safety of both employees and visitors.

General disinfection tips

Lab directors should conduct audits of their department's physical environment to identify safety hazards specific to their lab. Such audits typically do not need to interfere with the day-to-day lab processes, and they should be performed on a regular basis, at least monthly. Many changes can occur in a laboratory at any time, such as the movement of instruments, the placement of new equipment, or even the movement and stocking of lab supplies, and the implications of such changes for safety should be recognized.

When checking for physical environment safety, look to see that aisles are clear of boxes or other obstructions, especially if the pathway leads to a fire evacuation route. Loose wires from computers and keyboards need to be properly tied up. Make sure that lab floors are cleaned regularly; the U.S. Centers for Disease Control and Prevention (CDC) recommends that lab floors be wet-mopped at least daily in a biohazard area.[1] Also, make sure anti-fatigue mats on the floor are replaced on a regular basis so that wear does not create slip or trip hazards. In histology areas, be sure to keep paraffin wax build-up from occurring on walkways in order to prevent dangerous falls. Use scrapers or other implements to remove any wax build-up as it occurs.

Ensure that laboratory safety equipment such as emergency eyewashes, showers, and fire extinguishers are unobstructed at all times. It is important to make sure there is easy access to bloodborne pathogen and chemical spill response kits. Electrical panels in the department should have three feet of clearance in front of them. Check all lab electric cords as well for fraying or other damage. Simple movement of equipment can easily damage a cord, and exposed wiring can be a cause of laboratory fires. Ensure that compressed gas tanks are secured to prevent tipping.

Cluttered lab work benches can also include hazards for workers. A messy workspace can contain hidden dangers such as contaminated sharps, infectious materials, and even unknown chemical hazards if there are unlabeled materials. Lab areas should be dusted regularly as well. Dust may contain molds and other air contaminants that can potentially interfere with laboratory testing, particularly in a microbiology laboratory. That is one reason why electric fans should not be used in a lab setting. Fans in the lab can circulate those air contaminants. Fans also interfere with safety airflow devices such as chemical fume hoods or biological safety cabinets, and they can even interfere with the lab room air flow that is maintained for staff protection.

Disinfection protocols

Because of the nature of the biohazardous materials used in laboratories, lab benches should not only be orderly; they should be disinfected after every work shift and after any spill occurs.[2] This disinfection should take place with the use of an intermediate-level chemical germicide. While the CDC recommends the use of a 10 percent bleach solution as the disinfection standard,[3] there are other products that can be used in the lab setting.

Commercially available lab cleaning products can be purchased in the form of pre-filled spray bottles, large containers of fluids, or even canisters of single-use wipes. Be careful when selecting any commercial product to make certain it is effective enough to eliminate most bacteria (including *Mycobacterium tuberculosis*) and all fungi and that it inactivates viruses. Many products that are sold cannot perform all of those disinfection functions, and labs that use insufficient products may inadvertently place their staff at risk for infection. According to the CDC, some commercially available germicides can rapidly kill ordinary vegetative forms of bacteria such as staphylococci and streptococci, but only select brands are effective against more resistant organisms such as *Mycobacterium tuberculosis*, non-lipid viruses, and most forms of fungi. Check the information provided by the manufacturer to make sure that the disinfectant selected is potent enough for complete lab disinfection.

Some laboratory instrument manufacturers recommend the use of specific cleaners on their equipment because bleach products may harm instrument surfaces. These cleaners may not be effective for biohazard control in the lab setting, and while they may be used on the equipment, they should not be used for general counter or work bench disinfection as well. One good way to avoid harm to surfaces from repeated bleach use is to rinse the surface with water or even ethanol after the bleach has been used.

It is important to pay attention to the contact time needed for disinfectant chemical products to work effectively on laboratory surfaces. Whether using sprays or wipes, the disinfectant action does not occur immediately, and the wet product should be left on the counter or surface for a prescribed amount of time as designated by the manufacturer. Some products can take up to three to four minutes to kill the pathogens they are designed to eliminate. A common lab cleaning mistake is to wipe a disinfectant-treated area down with water or even paper towels to dry the area long before the contact time needed to complete disinfection has elapsed. This is a potentially dangerous practice that can lead to a laboratory-acquired infection. Staff education about the proper use of germicidal chemicals is critical for proper infection prevention in the work place.

Regular cleaning and disinfection of lab surfaces apart from those involved in testing *per se* are also necessary to maintain the safety of the physical environment. Routinely wipe down chairs, telephones, computers, and other small items such as timers and pens. These items can become contaminated when laboratorians handle them with gloves

that were worn during patient sample handling. While PPE is designed for staff protection, studies have shown that contaminated surfaces and items also can lead to lab-acquired infections. A *Salmonella typhimurium* outbreak occurred in clinical and academic laboratories across the United States in 2017 that caused illness for 24 people. When affected lab staff were interviewed by the CDC, some stated they had not worn gloves or lab coats, and some said they used pens and notebooks at home that were used in the lab setting.[4]

Being prepared for an accident

To complete an assessment of the physical laboratory work space, correct any issues discovered and educate staff to prevent reoccurrences. Provide a review of good disinfection practices and the proper use of products if necessary. Putting those pieces together is important in creating a strong lab safety culture. Then, once the physical lab environment is in safe order, it is time to ensure that features are in place that will help staff to maintain safety in the event of an accident.

Accidents and spills of chemicals or biohazardous materials do occur, and it is necessary to have adequate spill clean-up supplies ready. Every clinical laboratory should have materials ready in the event of a spill of blood or body fluids. The spill kit should include absorbents, implements for handling broken glass, PPE, and disposal containers or bags. Place signs indicating the location of spill kits and check kits periodically to make sure all needed supplies are present. Chemical spill kits should also be available. Make sure sufficient amounts of absorbents and neutralizers are kept, based on the amount of chemicals stored and used in the department. All staff should be adept at spill clean-up procedures. Regular training is necessary, and conducting spill drills will enable staff to respond quickly and appropriately when an accident occurs.

A career in a laboratory setting involves working with complex procedures and hazardous materials. Regulatory agencies—and common sense—demand that staff be able to work safely every day. That can be accomplished by maintaining a clean and safe physical environment and by providing work practice procedures and education. Watch for physical hazards and for practices that are unsafe, and make immediate corrections so that a safe environment can be maintained.

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New advancements in cassette and slide printing help prevent errors

By Mark D. Strobel

It is difficult to overstate the tremendous role that medical lab tests play in the U.S. healthcare system. Hundreds of millions of tests are ordered each year. Physicians and allied health professionals rely on laboratory test results to provide accurate data that is then used to make critical treatment decisions. Mistakes are a major concern because they waste money and, more important, because they can jeopardize patients' health and lives. Patient and/or specimen misidentifications are a major category of lab errors.

Risks for errors

Despite the best efforts of laboratory professionals, conditions exist in many labs that make it difficult for them to avoid errors. Slides are often exposed to harsh chemicals. Handwritten cassettes can become illegible, and chemicals can erode the labels that are applied to the slide prior to the staining process. Opportunities for errors are multiplied when laboratorians attempt to label cassettes and slides manually or match specimens with printed labels, especially when the labels are produced away from the work area. These types of procedures can result in the wrong label being applied, or in illegible handwritten cassettes or slide labels being matched with the wrong patient.

The consequences of mislabeled lab specimens can be disastrous for patients, for physicians, and for the reputation of the hospital or other testing facility. In an attempt to reduce the incidence of medical errors, to improve outcomes, and to control costs, many healthcare systems and regulating organizations have renewed their focus on process excellence at all points along the care continuum, including lab testing. One way to improve accuracy in labs is to find better ways to track and label specimens. The right printing technology can be an excellent solution.

Tracking and labeling specimens

Complete elimination of errors may be impossible, but error-free labeling should be the goal. Laboratories can take advantage of direct-to-slide and cassette printing technology to reduce the risk of specimen misidentification—and to improve internal efficiency. Cassette and slide printing technology, which enables high-resolution printing directly onto cassettes and slides, can provide the following benefits:

On-demand, color printing. On-demand printing can help labs reduce errors by enabling staff to print only the number of cassettes and slides they need, when and where they need them. Color printing capabilities can help labs operate more efficiently by eliminating the need to maintain inventory of cassettes and slides in multiple colors.

Chemical and heat-resistant inks. Harsh lab conditions may degrade the ink used in cassette and slide identification, which can increase the possibility of specimen identification errors. Advanced inks for cassette and slide printers can withstand xylene, alcohol, reagent, stain, heat, and chemicals, thereby reducing the incidence of errors.

Direct cassette and slide printing. Printers that let staff jprint directly onto cassettes and slides can reduce errors due to illegible handwriting directly on the cassette or slide face or slide labels. Direct-slide printing also eliminates the risk of labels falling off slides or becoming jammed in slide processing equipment.

Crisp printed text and barcodes. Using a direct-to-cassette or slide printer with a print resolution of 300 dpi ensures a high-quality, easy-to-read print. Error reduction is increased, as the print is legible and barcodes scan correctly.

Small printer footprint. When lab personnel send print jobs to a central printer, there's a risk of staff picking up the wrong cassettes or slides. Having the option of a small desktop-sized printer can enable labs to provide a printer for each workstation, eliminating that risk.

Customizable software options. Labs can operate more efficiently with customizable software that enables the use of templates to ensure the collection of all necessary data. Customizable software also allows labs to generate data for laboratory information systems.

Cassette and slide printers that are on the market today offer these advanced features. They can also print high-resolution images to enable labs to use graphics, logos, and two-dimensional barcodes on cassettes and slides to enable clear identification and tracking.

Choosing what's right for your needs

When choosing a cassette and slide printer, it's important for lab managers to ensure that they procure a model that will help the facility meet its error reduction and efficiency goals. Features to look for include efficient, hands-free operation and cartridges that enable lab personnel to store cassettes and slides with minimal exposure to dust and contaminants that might affect specimen quality.

Another important consideration is cartridge design: Decision makers should look for cartridges that enable quick changes so that laboratory staff can easily switch cartridge types to accommodate both tissue and biopsy cassettes and standard and charged slide needs. It's also a plus if staff can quickly ascertain the number of cassettes or slides remaining in the cartridge to expedite printing.

Durability is also an important feature. Cassette and slide printers should be rugged enough to withstand harsh lab conditions, be water-resistant, and be capable of withstanding disinfection from standard hospital cleaning products.

Finally, price is important. Cassette and slide printers come in many sizes and configurations and offer a wide range of features. Before making a purchase, lab managers should ensure that they are getting the most value for their investment.

Nothing is more important than patient safety, which is why it makes sense for lab facilities to frequently evaluate their processes and ensure that they are managing risks effectively. When it comes to specimen misidentification, there are clear steps lab personnel can take to minimize the possibility of errors. Using advanced cassette and slide printer technology can be an excellent option.

Mark D. Strobel serves as Vice President of Sales & Marketing for **Primera Technology**, manufacturer of specialty printers including the Signature Slide and Cassette Printers.

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Reinvented HLA testing algorithm could reduce cost, expedite living kidney donation

By Sujata Gaitonde, MD, FCAP, Laurie Gillard, MS, MLS(ASCP)^{cm} SBB, and Jesus A. Gonzalez, MS

The major histocompatibility complex is comprised of integral membrane glycoproteins that function in the recognition of self vs. nonself antigens and presentation of nonself antigens to the immune system. It was discovered on leukocytes and hence is referred to as the human leukocyte antigen (HLA) complex. HLA antigens are encoded on chromosome 6p, and this is the most polymorphic system in the human body.

Several scientists contributed to the discovery of the HLA complex. The discovery of the first HLA antigen is credited to French immunologist Jean Dausset (1916-2009). Dausset studied sera of patients who had received multiple blood transfusions and observed that these sera agglutinated leukocytes of other individuals but not self. He hypothesized that these leukocyte antigens would become important in tissue and bone marrow transplantation. The first alloantigen on leukocytes was called MAC (later known as HLA-A2).[1]

HLA antigens are classified into HLA Class I, Class II, and Class III. Class I antigens are present on the surface of all nucleated cells and platelets. They present intracellular derived foreign peptides to the cells of the immune system. MHC Class I antigens play a key role in alerting the immune system of viral infection by presenting internal viral peptides to cytotoxic T lymphocytes. Class II antigens are found primarily on antigen-presenting cells such as macrophages, dendritic cells, monocytes, and B-lymphocytes and present extracellular-derived foreign peptides. During an immune response against a bacterial infection, macrophages that have ingested the bacteria utilize HLA Class II antigens to present the extracellular bacterial peptides to T-helper lymphocytes to expedite the immune response. Class III antigens play a role in the inflammation and complement system.

HLA Class I and II antigens are important in solid organ and hematopoietic stem cell transplantation. Each class has three loci. For Class I, these are HLA-A, HLA-B and HLA-C. For Class II, the loci are HLA-DR, HLA-DQ and HLA-DP.

HLA typing

HLA typing consists of identifying the unique combination of antigens present in an individual's tissue. This is done by either low- or high-resolution molecular techniques. In low-resolution typing, the patient is typed to the point where the HLA antigen is identified by the allelic group. A patient can be typed and identified as HLA-A*02, which states that this individual presents HLA-A2 antigen but does not identify the specific allele of A2. High-resolution typing, on the other hand, does identify the specific allele the individual possesses. For example, typing by high resolution methods could be identified as HLA-A*02:01, which states that this person possesses HLA A2 antigen coded by gene A allele 01.[2]

Antibody testing

In the same manner that an individual develops antibodies against bacteria, viruses, and parasites via natural exposure or vaccinations, an individual can develop antibodies against non-self HLA antigens found in the population through blood transfusions, previous transplantations, and pregnancy. Patients who become sensitized to allogeneic HLA antigens run the risk of antibody-mediated rejection (AMR) of the transplanted organ. Anti-HLA antibodies are either IgG or IgM. The IgM antibodies are usually non-complement-binding and therefore usually non-consequential. IgG antibodies, on the other hand, depending on their sub-type (IgG1, IgG2, IgG3, and IgG4), can be complement-binding, and can contribute to AMR of the transplanted organ, thrombi formation, and possible loss of the graft if not treated successfully and promptly. IgG3 binds complement the most, followed by IgG1, IgG2, and IgG4.

In order to determine whether the recipient is sensitized to allogeneic HLA antigens, the clinical HLA laboratory performs a panel reactive antibody test (PRA), a screening assay to detect anti-HLA antibodies. If the PRA is positive, a single antigen bead assay (SAB) is performed to detect the specificity of the anti HLA Class I and/or Class II antibodies. The basic principle for both tests (PRA and SAB) is the same. Antibody screening is performed using a solid phase bead assay platform. HLA Class I and II antigens are extracted and purified from cell lines, usually an EBV cell line, and bound onto microparticles. Patient serum is incubated with these microparticles. If the patient possesses antibodies against allogeneic HLA antigens, they will bind to the complementary antigens on the microparticles. This binding is detected by adding an anti-human monoclonal IgG antibody that is tagged with a fluorescent tag. When the specimen is examined on the analyzer, the laser detects this fluorescent tag as a positive signal and the results from this procedure are reported out as the percent PRA. The PRA is essentially the transplantability index of the patient. If there are no anti-HLA antibodies, the PRA is 0 percent. This means the patient's transplantability index is 100 percent minus 0 percent—that is, 100 percent.

The optimal result of a PRA would be 0 percent, which means that the patient does not have detectable preformed HLA antibodies. A high PRA percentage does not necessarily indicate an abundance of antibodies. The patient could have low titers of an antibody that is cross-reactive with shared HLA epitopes. Likewise, a low PRA does not necessarily indicate that a recipient is suitable for transplantation. Even though the PRA is low, if the antibody that the recipient possesses is donor-specific, it could cause rejection. The PRA establishes the probability of a positive crossmatch with donor antigens, therefore indirectly giving an estimate of how appropriate a donor is for a recipient. The most important aspect of antibody testing is the specificity of the antibodies identified and, in the case of solid organ transplantation, whether the anti-HLA antibodies detected in the recipient's serum are against donor HLA antigens (also called donor-specific antibodies or DSA).

Crossmatch and virtual crossmatch

A crossmatch is a procedure in which serum of the recipient is incubated with lymphocytes of the donor, to determine

whether the recipient has DSA. Even if the PRA/antibody identification tests determine that the recipient has HLA antibodies, this does not mean that the immune system of the recipient will attack the donor kidney. If the donor does not possess the HLA antigens that the recipient has antibodies against, the recipient's immune system won't readily attack the transplanted organ. However, if the donor does possess this antigen, then the crossmatch would show that the recipient has a DSA.

HLA crossmatch is performed via flow cytometry, utilizing donor lymphocytes and recipient serum. T cells possess HLA Class I antigens, whereas B cells possess both Class I and II HLA antigens. Donor lymphocytes are allowed to react with recipient serum. Positive crossmatches indicate that the recipient possesses DSA that can potentially cause rejection, and therefore a more suitable donor must be found. If both the T and B cell crossmatch are positive, then the recipient has anti Class I DSA and/or both Class I and Class II DSA. If only the B cell crossmatch is positive, then the recipient probably has anti Class II DSA or weak anti Class I DSA.

A virtual crossmatch is an assessment of the immunological compatibility between recipient and donor based on the recipient's HLA antibody profile and the HLA antigens of the donor. In order for a virtual crossmatch to be acceptable, there needs to be sufficient, current data on the recipient's antibody profile.

Developing a new testing algorithm

Tambur et al studied the effects of altering the testing procedure on the transplantability of sensitized recipients and the success of transplantation. Two groups were established by the researchers. Group I consisted of individuals whose percent PRA was determined via solid phase-based testing with limited antibody identification. Group II consisted of individuals who had gone through more complete testing, including antibody identification, strength assessment, and use of pronase for crossmatch.[3] The researchers hypothesized that more in-depth and complete analysis of antibody makeup would increase the transplantability of sensitized patients. The more complete antibody testing allowed the center to "define the specificity of HLA directed antibodies for patients in Group II," which led to an increase in the number of sensitized patients who were transplanted, in comparison to patients in Group I. Although the percent PRA was determined in the same manner for both groups, they differed in the methods used to identify the specificity of the antibodies. The transplantability of sensitized patients in Group II was higher than that of Group I (49 percent vs. 40 percent), though the viability of the kidney at one year after transplantation was similar for both groups.

Bostock et al wanted to determine the probability of receiving a kidney transplant from a deceased donor waiting list based on the percent PRA. The group conducted a retrospective study, looking at the deceased donor waiting list of their institution, specifically looking at ABO type, lymphocyte crossmatch results, percent PRA, and time on the wait list. Potential recipients were classified into four groups based on the percentages of their PRA: 0 percent, one percent to 19 percent, 20 percent to 79 percent, and 80 percent to 100 percent.[4] The research group found that the probability of receiving a kidney decreases as the percent PRA of the potential recipient increases. The group also discovered that a higher risk of no transplantation is apparent once the PRA increases above 20 percent.

Mazuecos et al conducted a study in which kidney transplantations were constructed on a protocol based on virtual crossmatch analysis with a final crossmatch before transplantation for highly sensitized patients (PRA > 80 percent). Out of the 52 patients who were transplanted, five patients experienced AMR, and DSAs developed in ten patients. These results were compared to 35 patients who were not classified as highly sensitized. In terms of acute rejection, no significant difference was observed, but highly sensitized recipients were more prone to develop DSA. Virtual crossmatch assessment with a final crossmatch before procedure increased access to kidney grafts in highly sensitized patients with a low probability of AMR and high survival rate.[5]

A highly sensitive solid-phase bead assay for HLA antibody detection has recently become available and should be appropriately used to improve laboratory resource consumption by avoiding unnecessary testing and associated costs. Currently, all donors are HLA typed by low-resolution molecular methods and crossmatched with potential recipients. We propose a change in this testing algorithm for living kidney donation. For a recipient with 0 percent PRA detected by assay (and hence who is virtual cross match-negative), HLA typing and crossmatch should be performed on the donor only if he or she is approved for donation, since the process of finding a suitable donor includes several clinical and laboratory parameters other than PRA and crossmatch results. As laboratory professionals, we must emphasize appropriate test utilization. Testing should be performed only when necessary. High-complexity testing is expensive, and HLA laboratories should continuously look for ways to improve efficiency and reduce cost without compromising patient safety.

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The most kindest cut of all?

CRISPR gene therapy makes its mark

By John Brunstein, PhD

One of the Holy Grails of molecular processes—either for clinical utility, or to serve as the premise for science fiction plots—has been the quest for genetic engineering, that is, the specific alteration of selected genome nucleotide(s) in a target organism easily and with high efficiency. There is a high level of interest in this topic among the general public (and a lot of misunderstanding, too, but that's another article).

"But wait!" you say. "Haven't we been cloning genes and so on for years? Isn't this the sort of thing that undergraduates do in first-year labs now?" Well, yes it is, but that sort of genetic engineering is restricted to single-cell organisms (bacteria and yeast), and while there are many simple tools for cloning something into one of these systems at a unique location with high efficiency, the choices for where to make these modifications is limited. That is, even in those systems, until recently one couldn't just choose any specific genetic region at will to modify.

Efforts at gene therapy in humans have similarly been restricted, primarily to conditions where the underlying problem is something—usually a non-functional gene copy—whose function can be replaced *in trans* from some other location. Imagine, though, if there were a tool that allowed for correcting or modifying a non-functional gene *in situ*, meaning that all of its innate control regions remain active. That would also avoid any risk of possible insertional inactivation such as can occur with viral vectors (that is, where they insert at some unselected genetic location and by doing so disrupt a critical gene).

In this month's Primer, we're going to give an overview of a system, now a few years old and well developed, which allows for just this at-will selection of any unique genetic locus and its modification. This system is Clustered Regularly Interspaced Short Palindromic Repeats, or CRISPR.

The restriction enzyme

In the simple forms of genetic engineering referred to above, a major tool is the restriction enzyme. This is a bacterially derived enzyme which recognizes a fixed, usually palindromic, short (~4-8 bp) DNA sequence and provides a pair of cuts in the sugar-phosphate backbone. These cuts can then be used as sites to introduce ("splice in") exogenous DNA with compatible ends, with DNA ligase to reform the covalent backbone bonds. Of course, based on purely statistical considerations, such short recognition sequences must occur multiple times in a large genome, and we are stuck with the inherent sequence specificity of the enzyme. The sequences are believed to act as a form of bacterial immune system, paired with enzymes that protect or mask the bacteria's own DNA but leave invading viral (bacteriophage) DNA susceptible to multiple cleavage events rendering the virus inactive.

The CRISPR system is also believed to act as a bacterial immune system, but with a higher degree of complexity and, indeed, an ability to adapt to new viral challenges. In this regard it's conceptually analogous to the antibody system we have, but with the added benefit that a bacteria exposed to a new virus and developing a CRISPR-based defense passes this resistance on to its progeny.

In effect, CRISPR also acts like a restriction enzyme except that, rather than the DNA target sequence to be cleaved being determined by a fixed amino acid sequence of the enzyme, CRISPR binds to an interchangeable ~20 base RNA known as a crRNA (which is homologous to the DNA target sequence to be cut) and a second activating RNA known as a tracrRNA. It's this homology to target being coded for by the crRNA that allows the host bacteria to use this as an adaptive immune system; by capturing short segments of viral sequence in the genetic element which codes for crRNAs, new target specificities (crRNAs) are developed without having to change the amino acid sequence of the CRISPR protein itself. Equipped with the crRNA and tracrRNA, CRISPR is able to selectively bind to the sequences identified by the crRNA. While the CRISPR protein lacks any direct endonucleolytic activity of its own, it acts via the tracrRNA to recruit in a nuclease activity (most commonly, in the form of an enzyme called Cas-9) which binds to the complex and makes cuts in the DNA strands, much like a classical restriction enzyme.

If you're a bacterium defending yourself from an infecting virus, that's the end of the story; with the virus chopped up and inactivated, you can go back to your busy metabolic life. For our purposes in using this as a selective genetic engineering tool, we'll want to make a few changes, and add some subsequent steps.

Single guide RNA

The first change we'll make, for sake of ease, is combining the crRNA and tracrRNA into a functional single molecule (called an sgRNA, or "single guide RNA"). This has been worked out, and with the bulk of the sgRNA predefined, choosing a target sequence specificity is almost as simple as adding in the ~20 base region of homology to an sgRNA template. Cloned into an expression vector, the sgRNA is now expressed. As it happens, this molecule can now both bind to an intended target via homology, and directly recruit in Cas-9; no CRISPR protein is needed to hold the crRNA and tracrRNAs together. Thus, if we simultaneously co-express Cas-9 and sgRNA in a cell, it will generate site-specific DNA cuts at our targeted locus.

You probably noticed the phrase "almost as simple" in the preceding paragraph. Yes, it's true, not even the CRISPR system can actually just cut any sequence with no rules; it turns out that it must absolutely have a DNA motif called a PAM (protospacer adjacent motif) directly adjacent to the target region. Fortunately, a PAM is any element of the form "NGG," where N is any nucleotide, and on average we can therefore expect a PAM motif about every 16 base pairs in any random DNA sequence. So while CRISPR is not strictly allowing us to cut any DNA sequence, it's generally rare that we can't get within a few nucleotides of where we want to cut.

This, then, relates to the second part of using CRISPR in doing gene editing. We've made a couple of cuts; now how do we put this back together (and ideally, with our intended alterations)? To do this, we take advantage of the cell's natural

DNA double-strand breakage repair machinery, which will try to fix any double stranded cuts of this type. One such mechanism—non-homologous end joining, or NHEJ—is error-prone and useful if our intent is to shut off a gene; it will act to close up the Cas-9 cuts but usually will introduce a frameshift, rendering the rejoined gene non-functional. In the second repair method—homology-directed repair or HDR—the cell looks for homologous DNA elements to the break region, and in effect "copies" these into the cut. If we provide the cell with an excess of a short DNA element with both homology to the cut region and our intended changes at the cut site, the cell will have a high probability of using this as its repair template, and we'll have succeeded in making a site-specific modification of our target.

Note that with this HDR mechanism, the length of our "repair template" is variable, which in effect compensates for the need for a PAM sequence. While we may have had to move our cleavage site a short distance away from where we want the exact change, the HDR will allow us to engineer back down across the exact nucleotide(s) of interest.

In summary

To summarize, this system allows us to select (almost) any DNA target element of about 20bp; design an sgRNA and possibly an HDR element to direct the repair; and then use these to go into our cell of interest and make efficient, highly site-specific modifications. There are a number of variations and improvements on this (such as alternatives to Cas-9 with more desirable behavior), but the basic approach is the same.

This CRISPR-Cas9 system thus provides us with a very powerful tool with which to target our genetic editing—but how do we actually apply this to a large multicellular organism such as ourselves? We obviously can't edit all the cells in a multicellular organism, and editing one or two somewhere in isolation would just be a form of somatic mutation—of impact on the cell or few cells changed, but not of widespread impact on the whole organism. This hurdle in gene therapy is, of course, by no means unique to the CRISPR approach, and it's for this reason that diseases impacting, for example, bone marrow are most readily addressable. A sample of cells can be taken, modified *in-vitro* through an engineering method such as this, and then the selected reengineered cell(s) can be reintroduced into a suitably prepared host, where they can then clonally expand and provide the needed or repaired gene function. To date, CRISPR-based methods have been used successfully in some animal models, and the first human clinical trials (in contexts of sickle-cell anemia and thallasemia) are expected to begin in Europe some time this year.

While the CRISPR-Cas9 system is thus no magic wand for treating genetic diseases, it is a powerful tool in the genetic engineering kit and one which starts to bring ease of targeted manipulation of complex eukaryotic cells more in line with the tools we have for bacterial systems—those that we put in the hands of first-year undergrads. Combined with improved ways to selectively deliver these genetic tools to the critical cells of interest in a given disease, this method and its emerging derivatives will start to allow for direct treatment of an increasing spectrum of diseases. Laboratorians can expect to hear more of this system and its applications in the future.

John Brunstein, PhD, is an *MLO* Editorial Advisory Board member. He serves as President and Chief Science Officer for British Columbia-based **PathoID, Inc.**, which provides consulting for development and validation of molecular assays.

How healthcare CRM can drive profitability and efficiencies in toxicology labs

By Diane Janowiak, BS, MT(ASCP)

The head of business development at a leading toxicology lab had an "ah-ha" moment when he compared two of the lab's accounts. He was surprised to discover that the account with 50 percent fewer test orders was highly profitable, while the larger account actually lost money for the lab. This isn't an unusual scenario in toxicology labs, which often move so quickly to win business from competitors that they aren't taking the time to see if the accounts will generate profitable revenue.

According to IBIS*World*, the toxicology laboratories industry is poised to grow at a rate of 3.5 percent through 2022, propelled by rapidly changing technologies and the trend among employers and law enforcement to demand toxicology tests.[1] Even so, servicing unprofitable accounts isn't a sustainable business model in any industry; in healthcare it's particularly risky. Decreasing reimbursement rates, ongoing regulation shifts, and more discriminating healthcare consumers are putting downward pressure on profitability, making it essential to have access to the data needed to make informed decisions.

In order to gain this insight, some labs are installing healthcare-focused customer relationship management (CRM) systems to drive profitability and increase efficiencies, while delivering exceptional patient care. Often called HRM (healthcare relationship management) systems, these platforms consolidate the clinical and business data that reside in various lab systems into the platform, organize customer profiles, analyze data, and deliver business insights on dashboards for easy viewing.

With a HIPAA-compliant HRM system, all data from LIS (laboratory information systems), billing, supply, payer, and other sources is easily available to deliver a holistic view of all lab customers. HRMs then enable lab managers to measure and report on almost any metric, and set workflows based on insights that are uncovered.

Are all accounts the same?

	Account #1	Account #2
Accessions	231	477
Revenue (Cash)	$32,495	$8,701
PPA	$140.67	$18.24
Freight	$1,176	$1,680
Processor	$0	$0
Commission	$1,300	$261
Lab Labor	$2,384	$5,896
Lab Supplies	$2,729	$6,525
Billing Labor	$1,970	$1,547
Contribution Margin (Profit)	**$22,936**	**$(7,208)**
Contribution Margin %	**70.1%**	**-83.8%**

0% 10% 20% 30% 40% 50% 60%

Figure 1. If you are looking just at test volume, at first glance Account #2 seems to be the winning account. However, Account #1, with half the volume, is more profitable when you factor in revenue and expenses.

Facing strong headwinds

Lab managers today are looking to HRM systems to help them thrive in an increasingly difficult operating environment.

Downward pressure on reimbursements is chief among the challenges. The new year ushered in deep cuts to reimbursement rates for some lab tests. The cuts made by the Centers for Medicare & Medicaid Services (CMS) for clinical lab tests issued under the Protecting Access to Medicare Act (PAMA) aim to save the government around $670 million annually, a 10 percent reduction from the $7 billion that it pays annually for lab tests.[2]

At the same time, high-deductible healthcare plans are forcing consumers to foot a higher percentage of the bill for their testing. According to TransUnion Healthcare, patients paid 11 percent more in out-of-pocket costs in 2017 than the previous year.[3] This is creating more savvy and discriminating healthcare consumers who are more likely to question the necessity of healthcare tests and procedures. (That's a good thing for everyone in the long run, but a challenge for labs nonetheless.)

HRM systems can be instrumental in helping laboratory managers identify ways to counterbalance lost revenue by making labs more profitable and efficient, while better serving providers and patients.

Better insight and decision making

One way such systems do that is by enabling laboratory sales managers to quickly identify the profitability of both current and prospective customers.

The sales team at DRUGSCAN, a Pennsylvania-based toxicology lab, typically looks at three cost components to determine profitability: insurance (network vs. out of network, Medicare, HMO vs. PPO), test mix, and premium services, such as EMR interfaces and in-office phlebotomists. Then, the team calculates the profitability of a customer account by comparing these revenue drivers with the cost to service the account, including testing labor/supplies and overhead costs. **(Figure 1)**

The lab also sets up an internal review and approval process for new accounts to help determine profitability on the front end, before the customer account is even live. The new account approval request is documented in the HRM system, and it includes an estimate of the payment mix by percent, which is provided from the physician's billing manager or practice manager. This allows sales managers to forecast pipeline opportunity with estimated volume, revenue, and start date, so the lab is prepared to absorb the account.

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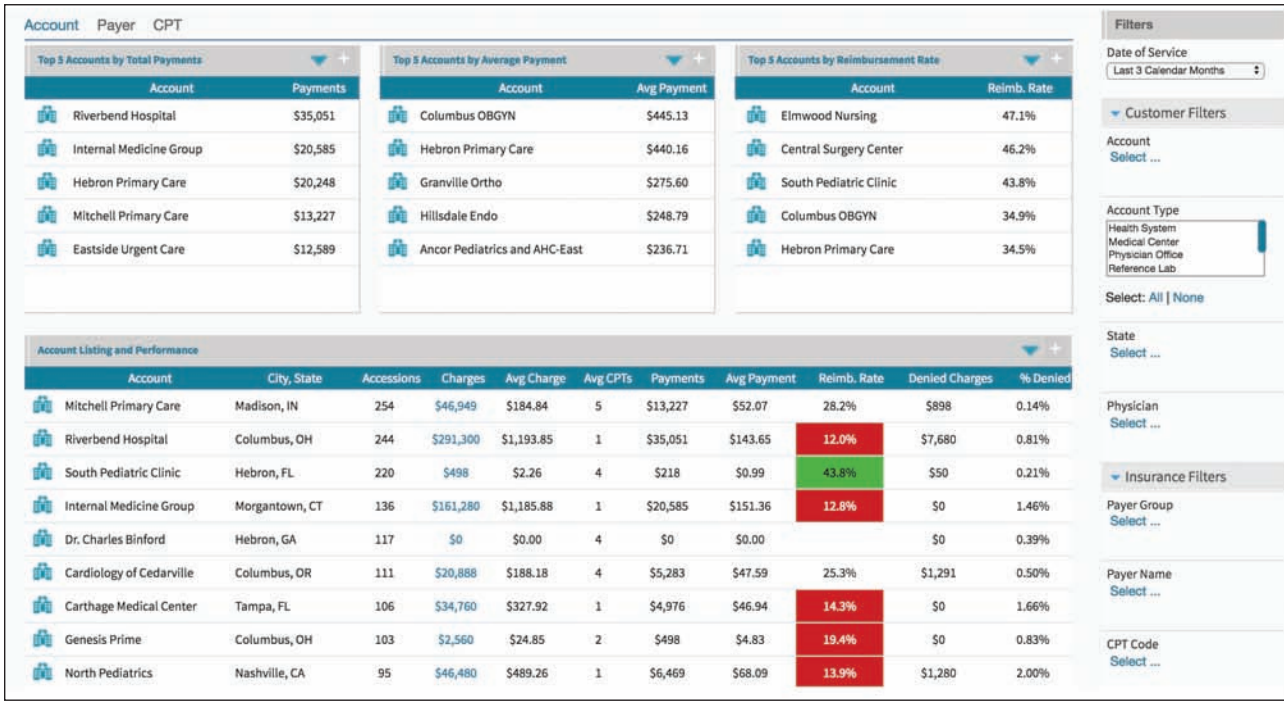

Figure 2. A revenue calculator can anticipate the profitability of clients by looking at the insurance payer mix, denial and reimbursable rates, and average payment. This enables sales managers to ensure that the lab can scale to the needs of the customer and that the customer will be profitable.

It also allows management to identify non-profitable or marginally profitable accounts before they are activated and have important conversations with providers. For example, if a new client plans to use the lab just for HMO patients, sales reps can initiate discussions with providers to even up the payment mix by including some higher reimbursable patients. **(Figure 2)**

The client activation process was previously driven with emails, which often took up to three days to approve. Using the workflows built into the HRM system, new account approvals are now completed within 24 hours of the request, enabling the lab to bring in new profitable business faster.

Finally, DRUGSCAN's lab management uses its HRM to align its sales compensation plans with company growth goals, rewarding reps for securing more profitable business.

Doing more with less

The insight generated by HRM also helps labs streamline documentation processes across departments and locations, proactively identify and address issues, and increase efficiency and productivity.

Cordant Health Solutions is a nationwide toxicology lab that includes a full-service pharmacy specializing in the complex management and dispensing of controlled substances. Lab managers adopted an HRM system to save costs by driving efficiencies. As a result, the company was able to proactively expand into several new industries while decreasing the number of sales reps by half and reducing customer support reps by 70 percent.

Before the HRM implementation, Cordant's six labs operated independently, and each had its own IT systems, including LIS, CRM, and billing systems, which didn't communicate between locations or departments. As a result, management had no visibility into real-time data or trends and lacked reliable information about client account health, revenue shifts, or sales activities. To get at this data, managers had to manually pull reports. Decisions were based more on anecdotes than data.

Executives knew that in order to expand their toxicology and pharmacy services, the lab needed all locations to be under the same IT umbrella to support lab services and other business segments, including medication monitoring, treatment, employee compensation, criminal justice, health plans, hospitals, and pharmacy.

Cordant's HRM platform integrates real-time data from each separate location and provides complete operational transparency into each area of the business. Now, sales managers have detailed insight to set realistic benchmarks for reps and track forecasting timelines for new accounts. The platform also enables reps to be more proactive by analyzing ordering patterns and reaching out to clients who have not ordered supplies in the last 30 days to reduce unnecessary overnight shipping costs.

Customer support staff can quickly identify accounts with spikes in billing questions, low specimen inputs, or volume variances, allowing the team to get in front of issues before they become larger problems. **(Figure 3)**

The HRM also enables teams to use email messages to instantly build cases, improving time management by not having to manually type information from emails into case files.

Activity with insight

It's been said that there is nothing so bad for business as activity without insight. In today's challenging healthcare, environment this is certainly true. To survive and benefit from the growing demand for drug testing, toxicology labs must be lean and make decisions based on relevant data, not assumptions or "this is how we've always done it." HRM systems can help pull data together from throughout the lab and deliver insights needed to make better decisions.

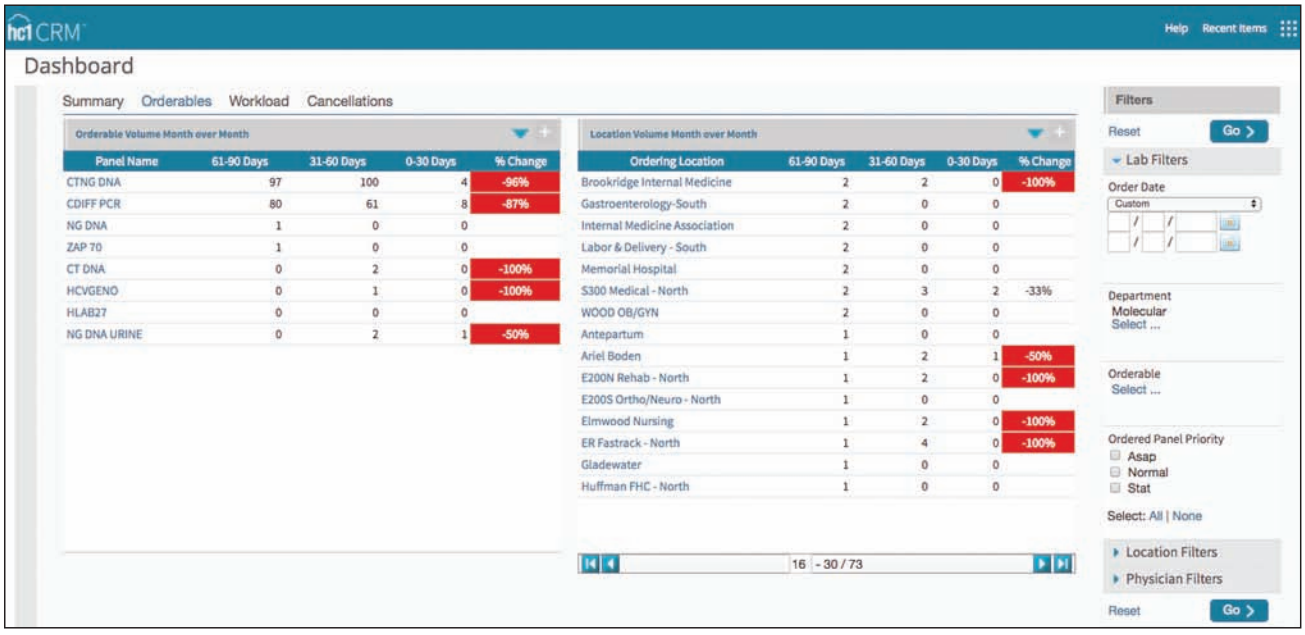

Figure 3. HRM dashboards allow lab managers to easily see when orders drop off, so sales or support reps can take proactive action.

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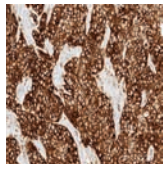

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Roche Diagnostics develops healthcare solutions with a focus on personalized healthcare

What are Roche Diagnostics' primary areas of expertise? At a high level, Roche's expertise is in developing healthcare solutions in pharmaceuticals and diagnostics, with a focus on personalized healthcare. On a practical level, the patient is at the center of everything we do, so in our diagnostics business our aim is to help laboratories empower healthcare professionals to make the right decisions for their patients at the right time. In parallel with that, we also enable patients to have better control over their own health and well-being.

What are the major categories of solutions that Roche provides for the clinical lab? We offer a variety of test platforms and assays for research and IVD use in the areas of chemistry and immunochemistry, tissue diagnostics, molecular diagnostics, hematology, and point-of-care testing. We've also been developing solutions in the area of digital diagnostics, most recently introducing a tumor board software solution that acts as a comprehensive dashboard for oncology care teams. We also have several solutions for target enrichment and library prep in next-generation sequencing (NGS) research and are developing an end-to-end sequencing workflow solution for clinical applications.

What are some of the major trends in clinical diagnostics that affect Roche's customers? How is the company responding to these? Some of the trends we see affecting our customers the most are the shift from fee- to value-based healthcare, declining reimbursement, the growth of service line structures in IHNs, the centralization of routine testing in parallel to the growth of point-of-care testing, and the increasing importance of data-driven healthcare. These are not new, of course, and we have been working with labs and healthcare institutions to develop solutions for several years. Some of them are technology-focused, such as automation solutions that allow the integration of routine chemistry and molecular testing in the core lab, and the development of lab-quality PCR testing for the point of care. Other solutions we have developed are at a higher level, such as lab workflow consulting, cross-portfolio solutions that address clinical focus areas like antibiotic stewardship and women's health, and the tumor board software solution that I referred to earlier.

How are changes in reimbursement for and regulation of U.S. labs affecting Roche's business and its plans for the future? Do you anticipate further changes? The conversation about lab regulation is still very fluid, but in general we fully support the efforts to modernize the regulatory framework for clinical diagnostic tests. We believe the reforms being discussed are important to ensure that patients and providers have access to innovative and clinically validated tests. We don't expect regulation of LDTs to have an impact on our diagnostics business.

On the other hand, the implementation of PAMA and declining reimbursement levels are having a significant impact on laboratories. But we are finding that partnering with labs and IHNs to look at broader cost management solutions, such as platform and menu consolidation, integrated core lab automation, workflow consulting, and health information management, is enabling them to address the increasing cost pressures effectively and even enhance their patient care in the process.

Companion diagnostics is a growing discipline. How does Roche use its expertise to lead in this area? Companion diagnostics and targeted therapies are a core component of personalized healthcare, and having both pharmaceutical and diagnostic expertise under one roof has certainly helped advance this discipline at Roche. In fact, two-thirds of our pharmaceutical research and development projects are being developed with companion diagnostics. But we also partner with many other pharma companies to develop companion and complementary diagnostic tests for their drugs, especially in the oncology space.

Beyond the actual science of the diagnostic test, we've found that one of the key ways we can lead in this area is to help pharmaceutical companies understand the difference between the development and implementation process for a diagnostic test as opposed to a drug and why partnering early in the development process is critical. For example, completing testing-access readiness and getting a test up and running can take a lab four to nine months. And there may be extensive pathologist training required as well. If you calculate in these and other factors, you should really be starting discussions with a companion diagnostic company 18 months to two years before the anticipated launch of a drug.

Can microbiology and molecular be friends? How can they be used together to enhance patient management? I think the tension is understandable, as molecular tests are increasingly replacing traditional microbiology tests in a growing number of applications because of their faster turnaround time and ease of use. We see that trend continuing in some areas, but we are also looking into new microbiology technologies that can complement PCR-based tests to help clinicians provide optimal patient care. We have one technology in development, for example, that has very exciting potential, not only in traditional infectious disease detection applications but also in assessing antibiotic susceptibility.

This interview continues online at www.mlo-online.com

Jack J. Phillips
President and CEO
Roche Diagnostics North America

Professional
As head of Roche Diagnostics in North America, my goal is to develop a culture that fosters excellence in science, innovation and wellness, along with a passion to improve the lives of patients. I serve as Board Chairman of the Central Indiana Corporate Partnership and am a member of the Executive Committee for AdvaMedDx.

Education
BS in Marketing from Northern Kentucky University

Personal
In addition to enjoying spending time with my wife and children, I am an avid cyclist and endurance athlete.

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