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MEDICAL LABORATORY OBSERVER

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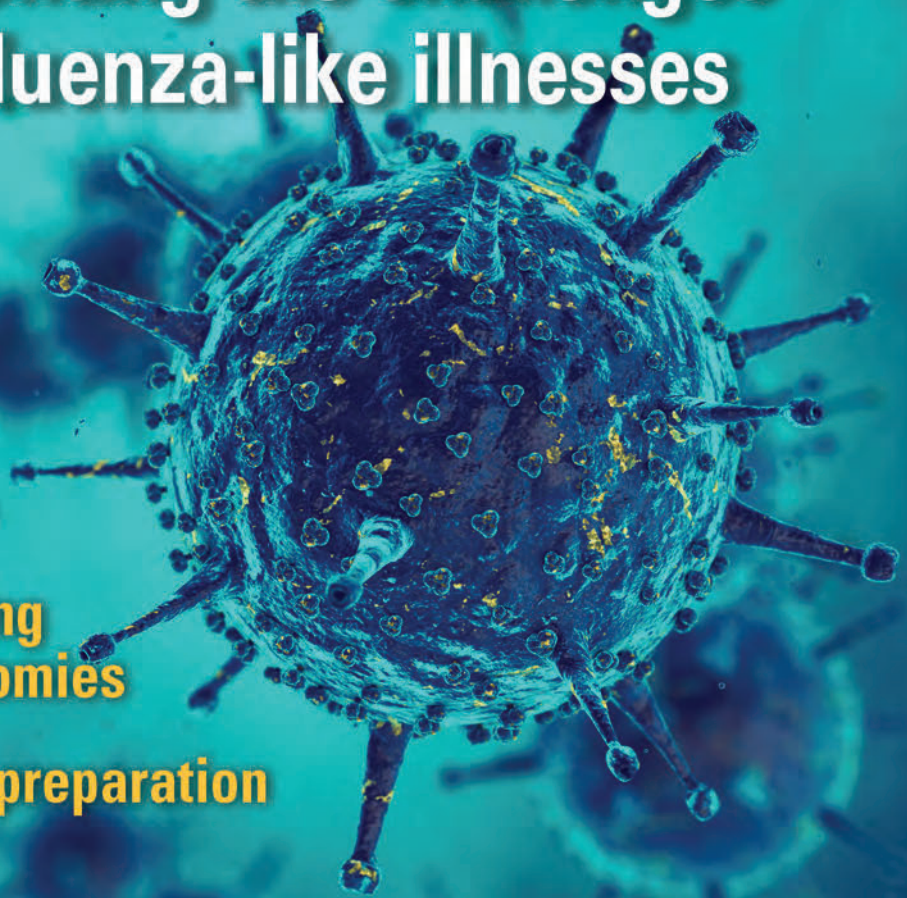
CE Confronting the challenges of influenza-like illnesses



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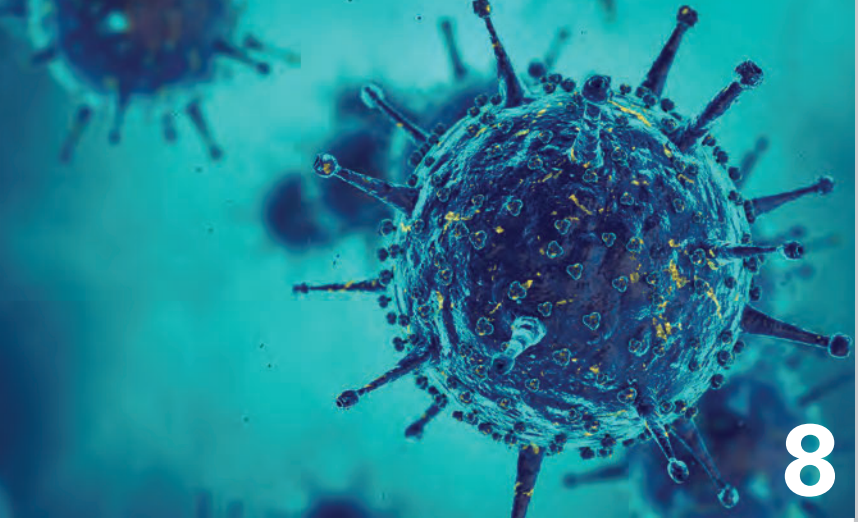

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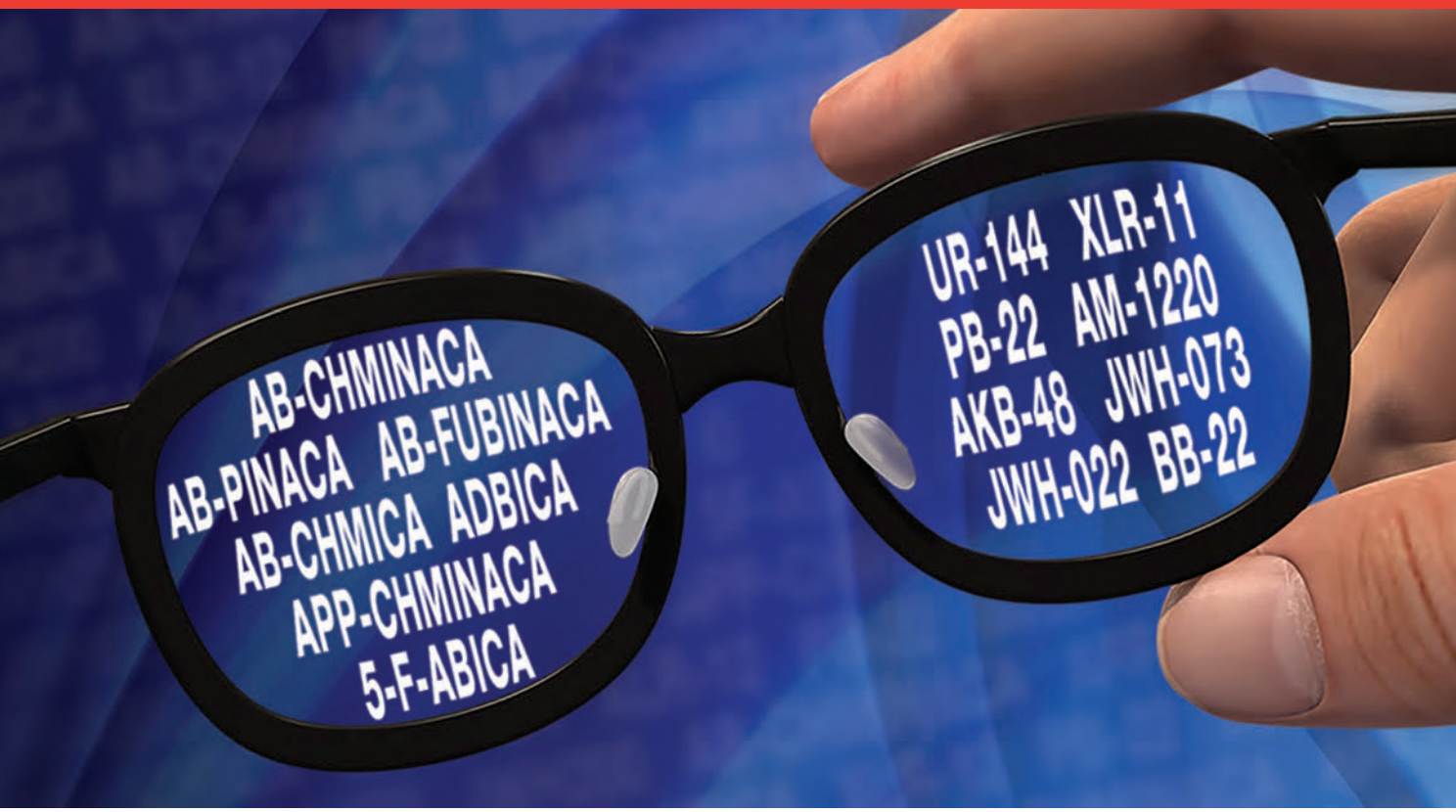

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CMS proposals on Medicare payment cuts worry industry leaders

As a lifelong Chicago Cubs fan, and as a journalist for, well, more than a few years, I used to joke with friends that I had an article "How the Cubs finally won the World Series" all ready to go, just hadn't had a chance to use it yet. Then, last year, the Cubs finally did win the World Series, and I had to put my money where my mouth was. In fact, I did write about that, albeit not for the pages of *MLO*.

During the past few months, a somewhat similar situation has begun to occur with regard to the long-awaited (with anticipation or dread, depending on your politics) repeal of the Affordable Care Act by the U.S. Congress. As you know if you've been reading the papers, the Republican-led Congress has promised to repeal Obamacare for years, as soon as there was a Republican president in the White House to sign a repeal-and-replace bill into law. But the Senate has failed several times since President Trump took office to cobble together a majority to pass a bill for him to sign.

And several times, I planned to write a "From the Editor" or "Washington Report" on the effects of the demise of Obamacare, and its replacement with another law, on the clinical lab—but I've had to scrap those plans each time. You can't write about the effects of something that hasn't happened.

But another important federal regulatory event *did* happen, on September 22, and industry experts are writing about the ramifications it may have for clinical labs. On that day, the Centers for Medicare and Medicaid Services, as required under the Protecting Access to Medicare Act (PAMA) of 2014, released the 2018 Clinical Laboratory Fee Schedule. PAMA directs the CMS to collect data from labs about private insurance reimbursement for lab tests. CMS is then to use data submitted by certain laboratories to set Medicare payments for specific tests.

It sounds fair, but many lab leaders—including the accrediting organization COLA—say that there are flaws in the methodology that cause CMS to set its Medicare payment amounts too low. The pricing schedule does not accurately reflect the market, and if it goes into effect, many labs will have to cut services or risk going under.

COLA has asked Congress "to consider how the anticipated steep cuts will harm access to critical, rapid, life-saving clinical laboratory testing for Medicare beneficiaries, especially in rural communities." COLA continues: "The proposed CMS reimbursement cuts for lab tests, which are commonly performed in patient care settings including physician offices, nursing homes, rehabilitation centers and urgent care centers, will impose burdens on the frailest Medicare beneficiaries and will harm patient care. Near patient testing offers many benefits, including rapid, accurate results in the treatment of diabetes; same day laboratory information for oncologists to treat their patients undergoing chemotherapy; and the quick detection of infectious diseases."

Among laboratory stakeholders, COLA is far from alone in decrying the CMS cuts. In fact, many individuals and organizations have spoken out against the new fee schedule. The formal comment period to CMS on the Clinical Laboratory Fee Schedule ended October 23.

You can read a more detailed analysis, with facts and figures, in this month's "Washington Report," written by three members of the CLMA Legislative Compliance and Regulatory Committee (LCRC).

MLO will continue to cover this ongoing story. And, if and when the Affordable Care Act is repealed and replaced, we will cover that too, and consider the implications for the clinical lab community.

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Influenza by the numbers

5 to 20
is the percentage of the U.S. population that gets the flu each year.

2 to 7
is the number of days influenza symptoms last.

$10.4 billion
is the annual cost of influenza in direct medical expenses.

$16.3 billion
is the annual cost of influenza in lost earnings.

31.4 million
is the annual number of outpatient visits due to flu in the U.S.

200,000
is the annual number of hospitalizations due to flu in the U.S.

50
is the annual percentage of children aged 6 months to 17 years who receive an influenza vaccination.

32
is the annual percentage of adults aged 18-49 who receive an influenza vaccination.

45
is the annual percentage of adults aged 50-64 who receive an influenza vaccination.

67
is the annual percentage of adults aged 65+ who receive an influenza vaccination.

• **Sources:** https://www.cdcfoundation.org/business-pulse/flu-prevention-infographic, https://www.cdc.gov/nchs/fastats/flu.htm, and http://www.who.int/mediacentre/factsheets/fs211/en/

Molecular Diagnostics

Doctors can now predict the severity of disease by measuring molecules. An international team of researchers has found a way to diagnose disease and predict patient outcomes simply by measuring extremely small changes in interactions among molecules inside the body. The new technique could offer vastly superior predictions of disease severity in a huge range of conditions with a genetic component, such as Alzheimer's, autism, cancer, cardiovascular disease, diabetes, obesity, schizophrenia, and depression.

Gene mutations that cause disease physically alter the interactions of molecules that cells use to communicate with one another. Until now, scientists have had no easy way to measure the subtle changes in these interaction forces. But researcher J. Julius Zhu, PhD, of the University of Virginia School of Medicine, and his collaborators have developed a method to accurately and efficiently calculate these tiny changes. It's a feat that requires incredible precision: Force is typically measured in newtons—the amount of force needed to accelerate one kilogram of mass one meter per second squared—but Zhu's technique measures on a scale of piconewtons—that is, one trillionth of a newton.

Zhu and colleagues have used the new technique to show that gene mutations responsible for mental-health diseases change molecular interactions by a few piconewtons. These small changes then have a tremendous ripple effect. The researchers found the molecular changes lead to harmful changes in how the cells communicate—and ultimately, in cognitive ability. By measuring the molecular changes, the scientists could predict the resulting cognitive impairment.

Zhu's approach represents a new use for a high-tech scientific instrument called "optical tweezers" that uses a highly focused laser to hold and move microscopic objects. Using the optical tweezers, scientists can measure the force required to break up intermolecular bonds among the signaling molecules inside the body, allowing them gauge the effects of gene mutations in patients.

Quality Control

CAP releases 2017 Laboratory Accreditation Program checklists to improve laboratory quality. The College of American Pathologists (CAP) has released the 2017 edition of its Laboratory Accreditation Program checklists. The checklists contain approximately 3,000 requirements that are used during laboratory accreditation inspections to help laboratories stay in compliance with the Centers for Medicare and Medicaid Services (CMS) regulations.

The CMS regulates all laboratory testing, except research, performed on humans in the United States through the Clinical Laboratory Improvement Amendments (CLIA). The CAP is a CMS-approved accreditation organization with deeming authority to inspect laboratories under CLIA.

The CAP's program is based on rigorous accreditation standards that are translated into detailed checklist requirements. CAP inspection teams use the checklists as a guide to assess the laboratory's overall management and operation. The program is internationally recognized and is the largest of its kind that utilizes teams of practicing laboratory professionals as inspectors.

As with each yearly checklist edition, the CAP reviews all 21 discipline-specific checklists to maintain program stringency and the highest standards of patient care while reflecting advancements in medicine, technology, and laboratory management. The CAP Checklists Committee, made up of practicing pathologist members, leads the annual review and updating of checklists, seeking input from experts in pathology and laboratory medicine.

In the 2017 accreditation checklist edition, the "Team Leader Checklist" has been renamed "Director Assessment Checklist," to better reflect the checklist's intent of assessing the laboratory director. The CAP made some of the most significant changes to checklists for the sections on personnel, specimen collection and handling, laboratory director responsibility and oversight, anatomic pathology, and molecular-based testing.

Diabetes

Variation in genetic risk explains which people develop type 1 diabetes in later life. Having certain genetic variants could explain why people can develop type 1 diabetes at markedly different ages, including later in life, says new research recently presented at this year's annual meeting of the European Association for the Study of Diabetes (EASD) in Lisbon, Portugal. The study is the first to suggest there is a specific genetic predisposition for late onset type 1 diabetes.

Type 1 diabetes (T1D) is caused by an autoimmune attack in the body killing off the insulin-producing beta cells in the pancreas, eventually leaving most people with a lifelong dependency on insulin. It typically affects children and young adults but can affect patients after the age of 30 years (referred to as late onset T1D).

Certain groups of genes associated with regulation of the immune system in humans are known to be linked to the risk of developing T1D. The major genetic determinants are the DR3 and DR4 alleles (or variants) of a group of genes called the HLA complex. The strongest risk occurs when these risk alleles occur in pairs which can either be homozygous (DR3/DR3 or DR4/DR4), or compound heterozygous (DR3/DR4) genotypes.

The research team, from the University of Exeter UK, aimed to investigate whether the increased risk of T1D that is observed in children and young adults with the DR3 and DR4 genotypes persists into adulthood. The scientists analyzed the development of T1D diabetes in a population of 120,000 individuals from the UK Biobank from birth to age 60 in subjects selected from the highest risk HLA groups. They found that although the highest risk genotypes made up just 6.4 percent of the population of the United Kingdom, they contributed 61 percent of all cases of T1D. Within these high-risk groups there were marked differences in both the likelihood of developing T1D and the average age of diagnosis.

In the high-risk HLA groups DR3/DR3, DR3/DR4, and DR4/DR4, there were marked differences in likelihood of developing T1D during a person's lifetime: 1.2 percent, 4.2 percent, and 3.5 percent respectively. For the DR3/DR3, DR3/DR4, and DR4/DR4 genotypes, the mean age of diagnosis was 17, 28, and 38 years old respectively, with 71 percent of T1D cases associated with the DR4/DR4 genotype being diagnosed in individuals over 30. For DR3/DR3/ just 26 percent were diagnosed over 30, while for DR3/DR4 the figure was 40 percent.

Drugs of Abuse

CDC awards $28.6 million to help states fight opioid overdose epidemic. The U.S. Centers for Disease Control and Prevention (CDC) is awarding more than $28.6 million in additional funding to 44 states and the District of Columbia to support their responses to the opioid overdose epidemic. The funds will be used to strengthen prevention efforts and better track opioid-related overdoses. This builds upon the July 2017 announcement that CDC was providing $12 million to states to support overdose prevention activities.

Increased funding for opioids in the fiscal year (FY) 2017 Omnibus Appropriations bill is allowing the CDC to support all states funded under its Overdose Prevention in States (OPIS) effort, which includes three programs that equip states with resources needed to address the epidemic. The programs are Prescription Drug Overdose: Prevention for States (PfS); Data-Driven Prevention Initiative (DDPI); and Enhanced State Opioid Overdose Surveillance (ESOOS).

Under the PfS program, $19.3 million in funding will go to 27 states in program expansion supplemental awards. Under the DDPI, $4.6 million in funding will go to 12 states and Washington, DC. Funds will be used by states to scale up prevention activities that include increasing the use of prescription drug monitoring programs and improving clinical feedback from these systems, expanding the reach of messages about the risks associated with opioids, and other practices such as conducting overdose fatality reviews to improve prevention efforts.

Under the ESOOS program, $4.7 million will go to 32 states and Washington, DC, to better track and prevent opioid-involved nonfatal and fatal overdoses. Funds will be used by states to directly support medical examiners and coroners, including funds for comprehensive toxicology testing and for enhancing their surveillance activities.

Genetics/Genomics

A new genetic marker for schizophrenia? Schizophrenia is a complicated disease that often appears in early adulthood. Although scientists have not traced the genetic causes, more than 80 percent of schizophrenia cases are considered to have a hereditary cause. In a new report published in *Translational Psychiatry*, Japanese researchers report that a rare genetic variant, RTN4R, may have a fundamental role in the disease.

"Schizophrenia is a disease caused by disturbances in neural circuits. Myelin-related genes are associated with the disease," explains Osaka University Professor Toshihide Yamashita, one of the study authors.

Myelin acts as a conductor of signals for the neural circuits. Yamashita hypothesized that myelin-related genes could contribute to the pathology of schizophrenia. RTN4R is a subunit of RTN4, which regulates crucial functions for neural circuits, namely, axon regeneration and structural plasticity. Moreover, "RTN4 is a promising candidate gene for schizophrenia because it is located at chromosome 22q11.2, a hotspot for schizophrenia," he says.

Rare variants describe mutations that have low frequency but a large effect. Yamashita and colleagues searched for rare variants of RTN4. Screening the DNA of 370 schizophrenia patients, they found a single missense mutation, R292H, that changed the amino acid of this protein from arginine to histidine in two patients.

R292H is located in the domain of RTN4R that binds to ligands, so a change in even a single amino acid could have profound effects on RTN4 function. To test this possibility, the scientists expressed the mutation in chick retinal cells, which only weakly express the gene, finding a significant change in myelin-dependent axonal behavior. Computer simulations showed that the mutation reduced the interaction between RTN4 and its partner protein, LINGO1, by increasing the distance between the two.

"There is growing evidence that rare variants contribute to neurodevelopment diseases," says Yamashita. The R292H mutation was not found in any existing databases. Our findings strengthen the evidence that rare variants could contribute to schizophrenia."

Personalized Medicine

Researchers identify potential biomarkers of age-related macular degeneration. Patients with any stage of age-related macular degeneration (AMD) carry signs of the disease in their blood that may be found through special laboratory tests, according to a new study led by AMD researchers based at Massachusetts Eye and Ear. The study uses metabolomics to identify blood profiles associated with AMD and its level of severity. These potential lipid biomarkers in human blood plasma may lead to earlier diagnosis, better prognostic information, and more precise treatment, as well as potential new targets for treatment.

"The study utilized metabolomics, or the study of the tiny particles called metabolites in our body that reflect our genes and environment," explains first author Ines Lains, MD. "The metabolome—the set of metabolites present in an individual—is thought to closely represent the true functional state of complex diseases. This is why we used it to test 90 blood samples obtained from participants with all stages of AMD (30 with early-stage disease, 30 with intermediate-stage and 30 with late-stage) and 30 from patients without AMD."

Their study revealed 87 metabolites that were significantly different between subjects with AMD and those without. The team also noted varying characteristics between the blood profiles of each stage of disease. Of the 87 molecules identified as associated with AMD, most belonged to the lipid pathway. In fact, six of the seven most significant metabolites identified in the study were lipids. Previous research has suggested that lipids may be involved in the development of AMD, although their exact role in the disease process is unclear.

Confronting the challenges of influenza-like illness

By Stefan Juretschko, PhD, D(ABMM)

Influenza-like illness (ILI) is a substantial clinical and economic burden on patients, healthcare providers, and the broader healthcare system. Depending on the pathogenicity of the viral strain and the effectiveness of the vaccine, there are typically between nine million and 36 million influenza cases annually in the United States, resulting in 140,000 to 710,000 hospitalizations.[1] However, influenza represents a small percentage of the hundreds of millions of upper respiratory infections (URIs) that occur annually in the U.S. alone.[2,3] This broader group of infections accounts for more healthcare provider visits than any other acute condition annually and results in almost 50 million lost days from work and school.[2,4,5] While the literature lacks reliable, contemporary data on the economic costs of URIs and more specifically ILI, it is estimated that direct and indirect costs combined likely exceed $100 billion each year.[3,6]

In addition to its high social and economic costs, ILI can lead to severe health consequences for individual patients, particularly among at-risk populations including the very young, the elderly, and the immunocompromised. Influenza alone is responsible for 12,000 to 56,000 deaths annually in the U.S.[1] And beyond the measurable mortality and morbidity of ILI, accurate, rapid diagnosis of the patient's condition also has substantial implications for key institutional quality metrics such as antimicrobial stewardship and infection control. This article will review the diagnostic challenges associated with ILI, the implications of missed or delayed diagnosis, and new diagnostic tools that may help address these challenges. Finally, areas for future research with respect to ILI diagnosis and patient management will be discussed.

Clinical presentation and differential diagnosis

ILI is a condition that presents with fever, cough, sore throat, shivering, chills, malaise, body aches, and/or nausea and is often associated with rapid onset. Frequent causes include the common cold and influenza, but ILI can be caused by more than 20 different viral and bacterial pathogens with overlapping and non-specific presentations. This complicates accurate, timely diagnosis.

More than 200 subtypes of viruses cause the common cold. While rhinoviruses represent a plurality of causative pathogens (30 percent to 50 percent of colds), other infectious agents are also implicated: coronaviruses (10 percent to 15 percent); influenza viruses (five percent to 15 percent); respiratory syncytial viruses (RSV, ~10 percent); parainfluenza viruses (PIV, ~ five percent); enteroviruses (< five percent); and human metapneumovirus (hMPV).[7] Additionally, the cause of 20 percent to 30 percent of common colds is unknown. Given the similar presentation associated with these viruses, it is not possible to establish the causative pathogen based on clinical diagnosis alone.

For instance, the differential for RSV in adults includes influenza and PIV. In infants it is even broader, including influenza, PIV, hMPV, rhinovirus, coronavirus, human bocavirus, and adenovirus. Studies have shown that RSV infection develops annually in three percent to seven percent of healthy older adults, may contribute to excess wintertime mortality previously attributed to influenza, and is a leading cause of hospitalization in young patients.[8-10]

Even the diagnosis of influenza can be confounded by the overlapping syndromes of ILI. A meta-analysis that reviewed the precision and accuracy of symptoms and signs of flu in adult patients over 60 years of age concluded that "clinical findings identify patients with influenza-like illness but are not particularly useful for confirming or excluding the diagnosis of influenza."[11]

Rapid and accurate diagnosis of the causative pathogen(s) for ILI is critical for informing patient management and selecting proper treatment, particularly in high-risk and hospitalized patients. Beyond direct patient impact, appropriate management of ILI can also help address key quality metrics such as infection control and antimicrobial stewardship.

High-risk patient populations

ILI poses a significant risk in immunosuppressed and immunocompromised patients, including hematopoietic stem cell transplant patients, solid organ transplant recipients, and patients receiving chemotherapy. Influenza, RSV, PIV, hMPV, adenovirus, and rhinovirus are associated with increased morbidity and mortality in these patient populations.[12-14] Rapid, accurate diagnosis is an important component of patient management in these populations as it helps direct appropriate antiviral and/or antibiotic therapy and can inform decisions about timing of transplant or additional therapy.[15] Current practice guidelines support testing for a wide range of suspected respiratory pathogens in these high-risk populations.[16-18]

Patients in the intensive care unit (ICU) setting are also particularly vulnerable to complications from ILI. Viral pathogens including influenza, RSV, PIV, hMPV, coronavirus, and rhinovirus and are all associated with severe pneumonia, requiring management in the ICU.[19] And while guidelines for respiratory virus testing in the ICU population

continued on page 10

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LEARNING OBJECTIVES

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

1. **Describe the healthcare cost burden of ILI in the United States and identify the symptoms and at-risk populations.**
2. **Identify the advances in multiplex molecular testing for ILI that have improved healthcare outcomes.**
3. **Describe past studies and their outcomes as related to improved rapid multiplex testing.**
4. **Discuss future opportunities for research in rapid multiplex testing.**

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continued from page 8

are undefined, a recent study showed that fewer than half of ICU patients with hospital- or community-acquired pneumonia were tested for viral pathogens. Among the patients that were tested, overall prevalence of viral infection was 28 percent, with 63 percent of the identified pathogens being other than influenza or RSV.[20]

The pediatric population is also at higher risk of adverse outcomes from ILI, as respiratory tract infections account for increased mortality and morbidity in patients who are less than five years of age.[21] RSV and PIV are the two leading causes of hospitalization for respiratory tract illness in young children, and RSV is estimated to cause more deaths in patients less than one year of age than any infectious agent other than malaria.[9,22]

Infection control

As healthcare payment models in the United States continue to shift away from fee-for-service and toward more capitated structures, managing overall cost-of-care is becoming increasingly important for providers who carry financial risk associated with avoidable readmissions and treatment of healthcare-acquired infections. These shifting financial incentives are leading to increased emphasis on and investment in infection control practices within the hospital. The U.S. Centers for Disease Control and Prevention (CDC) guidelines related to ILI recommend infection control practices that include patient isolation, targeted triaging, cohorting, and barrier protections.[23,24]

For infection control with suspected or confirmed influenza patients, the CDC recommends adherence to standard contact and droplet precautions as well as isolation and/or cohorting.[24] RSV is highly contagious and associated with serious healthcare-acquired infections. Infection control measures, including patient isolation or cohorting, limitations on patient transport, and contact and/or droplet precautions, are recommended to limit nosocomial spread, particularly in an outbreak scenario.[25,26] Similar precautions are recommended for hospitalized patients with PIV infection, particularly if exposure to immunocompromised patients is possible.[13,23] Accurate, rapid diagnosis of the causative agent of ILI is required to appropriately inform these various infection control practices and to manage limited isolation bed space, particularly during peak respiratory virus season.

Antimicrobial stewardship

The CDC reports that annually more than two million illnesses and 23,000 deaths are caused by antimicrobial-resistant (AMR) bacteria in the United States.[27] Pervasive inappropriate use of antibiotic therapy is a major contributor to the growing public health crisis of AMR. A recent large, population-based study assessed antibiotic prescribing patterns for more than 185,000 elderly patients who presented in the outpatient setting with a confirmed nonbacterial acute upper respiratory infection. The study showed that 46 percent of patients received an antibiotic prescription, with 70 percent of those receiving broad-spectrum therapy, despite a confirmed nonbacterial infection.[28] The literature demonstrates similar results related to misuse and overuse of antibiotics in varying care settings and across diverse patient populations, sometimes resulting in adverse patient outcomes and progressive antimicrobial resistance.[29-33]

The CDC's 2013 report on Antibiotic Resistance Threats in the United States led to the creation of a National Strategy for Combating Antibiotic Resistant Bacteria (National Strategy) which noted that one-third to one-half of all antibiotics used in inpatient and outpatient settings are either unnecessary or incorrectly prescribed.[34] Inappropriate use of antimicrobial therapy not only contributes to growing AMR, but also places an unnecessary economic burden on the healthcare system, with more than $1.1 billion in annual domestic spending on unnecessary antibiotic prescriptions for respiratory infections in adults.[35]

One objective of the CDC's National Strategy is to "develop new diagnostics, including tests that rapidly distinguish between viral and bacterial pathogens and…that can be implemented in a wide range of settings."[34] The CDC report notes: *Presently, most diagnostic tests take 24 to 72 hours from specimen collection to results….Thus, treatment decisions are typically required and made before laboratory results are available. As a consequence, patients may be initially treated with antibiotics when none are needed, prescribed an inappropriate antibiotic, or treated with multiple antibiotics when a single antibiotic would have been effective….However, the technological landscape is changing at a rapid pace. The current trend is moving towards clinical presentation or point-of-need diagnostic tests suitable for use during a healthcare visit because they require only minutes.*[34]

Prevalence of ILI episodes with Detected Respiratory Viruses

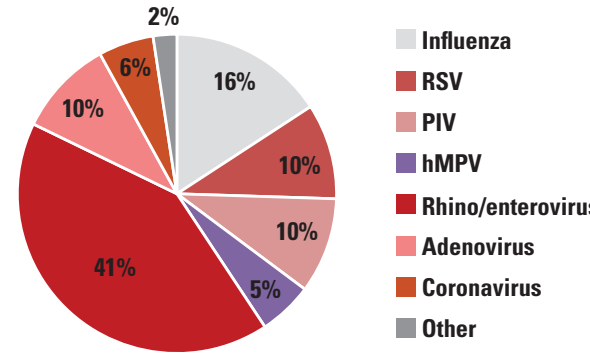

Figure 1. Respiratory viruses and influenza-like illness in a pediatric population

New diagnostic tools

Consistent with the CDC's National Strategy objective of developing new, flexible diagnostic capabilities, multiplex molecular testing is one tool that is now available to help resolve the overlapping clinical presentation of ILI and to address the need for rapid, accurate diagnosis of the causative pathogen. Previously, this type of multiplex molecular testing required advanced technical skills and equipment and was primarily restricted to the high-complexity laboratory setting. However, recent advances by multiple vendors have resulted in the commercialization of FDA-cleared, sample-to-answer platforms that significantly reduce the laboratory and staffing requirements needed to generate highly sensitive molecular results for the wide range of pathogens that are implicated in ILI. These diagnostic platforms have achieved both CLIA moderate complexity and waived status, making them accessible in a range of different care settings.

The past several years have shown rapid growth in the publication of studies reporting on the impact of sample-to-answer, multiplex molecular diagnostics for ILI. These studies have demonstrated the impact this technology can have across multiple care settings and on multiple clinical, quality, and economic outcome measures. Almost all of the studies have shown that multiplex molecular testing provides a more definitive diagnosis through a higher positivity rate while also delivering this result in a significantly shorter turnaround time, providing data in a clinically actionable timeframe.[36-39]

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Study	TAT Reduction	Relative Positivity Rate (Rapid Sample-to-Answer vs. Conventional)	LoS Reduction
Rogers, et al.[36]	12.3 hours	78% vs. 60%	0.3 days***
Rappo, et al. [37]	6.0 - 12.0 hours	N/A*	N/A****
Martinez, et al. [38]	30.4 hours	24% vs. 17%	2.1 days
Xu, et al. [39]	5.4 hours	+26%**	Not reported
Brendish, et al. [40]	34.7 hours	45% vs. 15%	1.1 days

TAT = Turnaround Time; LoS = Length of Stay
* - Study only reported on patients with positive results for both influenza-positive and non-influenza-positive results
** - Relative positivity rate not reported, but author noted that in an additional 660 (26%) of 2,537 specimens, the sample-to-answer platform detected viruses that would not have been detected with conventional methods
*** - Patients with positive test results only
**** - Sample-to-answer group had trend toward shorter LoS, but result was not statistically significant due to study size. Multivariate logistic regression found that a diagnosis of influenza was associated with significantly shorter length of stay (p=0.04).

Figure 2. Summary of clinical studies on sample-to-answer, multiplex molecular testing for respiratory virus diagnosis

Rogers et al reported that the implementation of a rapid, multiplex molecular assay in a major children's hospital led to a significantly higher positive test result rate (77.9 percent vs. 59.8 percent) while also providing a 65 percent reduction in time-to-result compared to a batch, PCR assay.[36]

Martinez et al reported on their experience with a rapid, multiplex molecular assay for ICU patients compared to conventional batch testing. They reported an average 30.4 hour reduction in mean time from sample collection to reported result. This shorter time-to-result contributed substantial clinical and economic outcome improvements with a reported 10 percent increase in the relative survival rate among the rapid, multiplex testing group. These patients also experienced a three-day reduction in ICU stay, contributing to a more than $8,000-per-patient reduction in the total cost-of-care.[38]

In perhaps the most rigorously designed study completed to date on rapid, multiplex molecular testing for respiratory pathogens, Brendish et al recently reported the results of a prospective, randomized controlled trial on the use of this technology at the point of care in the emergency department (ED). Consistent with prior reports, this study showed that rapid, accurate results impacted patient management, reduced cost-of-care, and contributed to appropriate infection control precautions. For patients with a positive test result, clinicians were able to stop antibiotics earlier, rather than completing a standard five-to-seven day course. With respect to antiviral therapy, 91 percent of influenza-positive patients in the rapid, multiplex testing group received appropriate, guideline driven antiviral therapy, compared to only 65 percent in the control group. For patients who were admitted to the hospital from the ED, the rapid, multiplex testing group experienced a 1.1-day shorter overall length-of-stay (LoS), contributing to an estimated $500 net cost savings per patient. And twice as many patients in the rapid, multiplex testing group with confirmed respiratory viral infections were isolated compared to the control group.[40]

These results in the ED have been confirmed in other studies that have shown higher rates of results reported to the patient while still in the ED (51.6 percent vs. 13.4 percent),[36] lower hospital admission rates,[37] reduced time in the ED by up to 23 percent,[38] reduced time to appropriate therapy[39] and reduced overall LoS for patients subsequently admitted to the hospital.[38]

In addition to these direct clinical and patient benefits, many of the studies also show improvements in key quality metrics. Multiple studies have shown reductions in the inappropriate use of antibiotics across a wide range of care settings and patient populations, consistent with CDC guidelines and the public health goal of reducing AMR.[36-38] These studies have shown that during peak respiratory virus season, when isolation facilities are at a premium, rapid, multiplex respiratory testing can be used successfully to inform cohorting strategies.[39,40] This use of multiplex testing in support of infection control measures is consistent with clinical guidelines and best practices that recommend the "application of rapid diagnostic tests to support clinical decisions involving patient treatment, room selection, and implementation of control measures."[23]

Opportunities for future study

The development of multiplex molecular diagnostic tools for ILI continues to accelerate at a rapid pace. And while the literature supporting the adoption of this technology also continues to grow, several gaps remain to be addressed. For example, there is strong evidence to support broad use of this technology in certain patient populations, such as pediatrics, the immunocompromised, and those in intensive care, but the clinical utility of rapid multiplex testing is other patient populations that are vulnerable to complications from ILI (e.g., the elderly), is not as well established. Studies focused on establishing the impact of multiplex testing in these patient populations should be areas of future investigation. Additionally, larger prospective studies appropriately powered to assess the clinical and health economic impact of these technologies would also be beneficial. The current literature suggests that providing rapid, accurate diagnostic results for ILI translates into improved outcomes, better quality metrics, and lower overall cost-of-care, but more robust studies to validate these results would benefit the laboratory community.

For now, what we know for sure is this: ILI is a high-prevalence condition that afflicts all patient populations and results in significant clinical and economic costs. The diagnosis of ILI is challenging, given the overlapping clinical presentation and the broad differential diagnosis that includes both viral and bacterial pathogens. Implementation of guidelines-driven infection control and antimicrobial stewardship interventions are predicated on rapid, accurate diagnosis of the causative agent. This definitive diagnosis is particularly important in high-risk populations such as patients with a suppressed immune system, patients in intensive care, and infants.

Sample-to-answer, multiplex molecular testing is a technology that can help address the challenges associated with the

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management of ILI. This testing has been shown to improve patient outcomes, reduce total cost-of-care, and support key quality measures such as appropriate antibiotic use and infection control. While there remain opportunities to further strengthen the evidence supporting adoption of this technology, sample-to-answer, multiplex molecular platforms are increasingly viewed as an essential tool in the diagnostic laboratory for the management of ILI. As the American Society for Microbiology (ASM) concluded in its recent white paper on the clinical utility of multiplex tests for respiratory pathogens: "There is no question that multiplex molecular panels provide superior diagnostic performance when compared to conventional methods, and there is a small, but growing, body of evidence that supports their positive impact on patient care and reduction in overall healthcare costs."[41]

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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. **How many cases of influenza are estimated to occur each year?**
   - a. two to fifty million
   - b. five to thirteen million
   - c. nine to thirty-six million
   - d. ten to forty-two million

2. **Which group of infections accounts for more healthcare provider visits than any other acute condition annually?**
   - a. influenza-like illness (ILI)
   - b. upper respiratory infections (URIs)
   - c. multiple drug-resistant infections (MDRIs)
   - d. none of the above

3. **What are the estimated direct and indirect costs of URIs each year?**
   - a. > $100 billion
   - b. > $100 thousand
   - c. > $100 million
   - d. > $100 trillion

4. **What population(s) is/are considered more at risk for developing an ILI?**
   - a. very young
   - b. elderly
   - c. immunocompromised
   - d. all of the above

5. **An accurate and rapid diagnosis of ILI has considerable implications for healthcare institutional quality assurance metrics such as antimicrobial stewardship and infection control.**
   - a. True
   - b. False

6. **ILI can be caused by more than _____ different viral and bacterial pathogens, which have similar clinical presentations.**
   - a. 5
   - b. 10
   - c. 20
   - d. 50

7. **Which virus contributes to the majority of cases that cause ILI?**
   - a. influenza
   - b. coronavirus
   - c. RSV
   - d. rhinovirus

8. **Accurate and rapid diagnosis of the causative agent of ILI isn't very important, because infection control practices and guidelines are not emphasized by the CDC.**
   - a. True
   - b. False

9. **CDC guidelines on infection control for ILI include:**
   - a. patient isolation, targeted triaging, cohorting, and barrier protections.
   - b. patient isolation, cohorting, and barrier protection only.
   - c. patient isolation, targeted triaging, and barrier protection only.
   - d. patient isolation, targeted triaging, and cohorting only.

10. **According to the article, a large number of patients still receive antibiotic therapy after receiving a confirmed diagnosis of a nonbacterial infection.**
    - a. True
    - b. False

11. **What task force was formed in response to the CDC's 2013 report on Antibiotic Resistant Threats in the United States?**
    - a. Agency for Combating the Misuse of Antibiotics
    - b. Agency for Delivering a Better Guide for the Use of Antibiotics
    - c. National Strategy for Combating the Misuse of Antibiotics
    - d. National Strategy for Combating Antibiotic Resistant Bacteria

12. **A main objective of this task force is to develop new rapid diagnostic tests for identification that include**
    - a. viral pathogens.
    - b. bacterial pathogens.
    - c. both a and b.
    - d. neither a nor b.

13. **What is the estimated annual domestic spending on unnecessary antibiotic prescriptions for respiratory infections in adults alone?**
    - a. $1.1+ thousand
    - b. $1.1+ million
    - c. $1.1+ billion
    - d. $1.1+ trillion

14. **The advancements in multiplex molecular testing have provided a tool to help resolve the overlapping clinical presentation of ILI and to provide a rapid diagnosis in a wide range of settings.**
    - a. True
    - b. False

15. **The benefits of the improved multiplex molecular testing include**
    - a. CLIA waived.
    - b. highly sensitive.
    - c. rapid result time.
    - d. all of the above

16. **What have studies shown with regard to the improved multiplex molecular diagnostic tests for ILI in the past several years?**
    - a. less sample volume needed and significantly shorter turnaround time
    - b. less sample volume needed and a more definitive diagnosis
    - c. significantly shorter turnaround time and more definitive diagnosis
    - d. less specificity and a more definitive diagnosis

17. **For which patient population is the clinical utility of rapid multiplex testing not yet established?**
    - a. infants
    - b. immunocompromised
    - c. elderly
    - d. patients in ICU

18. **What types of studies on rapid multiplex testing are lacking and would be beneficial?**
    - a. studies that assess the clinical economic impact
    - b. studies that assess the health economic impact
    - c. studies that assess turnaround times in different settings
    - d. a and b

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Urinalysis quality control at the point-of-care

By Brian Fernández

The goal of any clinical diagnostic test procedure is to provide critical information in a timely manner so that appropriate actions may be taken, ultimately improving patient outcomes. Point-of-care testing (POCT) is a term that has come to describe a multitude of rapid medical tests that can be performed at or near the site of patient care. The most compelling benefit of these tests is that, as opposed to having to wait hours or days for results to arrive from an outside laboratory, clinicians can obtain the results immediately, allowing for clinical management decisions to be made while the patient is still at the care facility. While the implementation of rapid diagnostic tests dates back to ancient history (sweet-tasting urine was once commonly used to diagnose diabetes mellitus), it was not until the 1950s that these rapid diagnostic methods gained any real predictive value. Today, the popularity and demand for POCT are increasing rapidly. TriMark Publications estimates that the global market for POCT was $14.5 billion in 2016, and is expected to grow by seven percent over the next five years.[1]

Urinalysis dipsticks at the point-of-care

Urinalysis using multi-analyte dipsticks is a point-of-care test performed at any hospital, clinical laboratory, doctor's office, health clinic, and nursing facility. Various iterations of these tests have existed for decades, and they continue to be among the most commonly performed tests of any kind. Urinalysis dipsticks contain discrete reagent pads to semi-quantitatively test for the presence of bilirubin, blood, creatinine, glucose, ketones, leukocytes, nitrite, pH, protein, specific gravity, and urobilinogen in a urine sample. Some urinalysis dipsticks contain reagent pads to test for the presence of creatinine and microalbumin. These tests may be read visually by comparing the colors that develop on each reagent pad to a chart provided by the strip manufacturer, or by an automated urine dipstick analyzer which helps to provide consistency in the timing and color interpretation regardless of lighting conditions or personnel.

Overview of QC for urinalysis dipsticks

Running daily Quality Control (QC) for POCT is critical. When measuring any kind of patient sample for indicators of disease, stable controls must be used to validate instrument performance and ensure accurate patient diagnosis. Using QC materials is not only good practice for labs that test human samples, but is also the law per the regulations outlined by the Clinical Laboratory Improvement Amendments (CLIA). Per CLIA 42 CFR section 493.1256 – Standard: Control Procedures: *a) For each test system, the laboratory is responsible for having control procedures that monitor the accuracy and precision of the complete analytical process.*[2]

Every facility in the United States that performs testing on human specimens for health assessment or the diagnosis, prevention, or treatment of disease is regulated under CLIA. Clinical tests are categorized as either waived, moderate, or high complexity. What category a test falls under depends on the amount of training required to perform the test, the degree of interpretation and judgment required, the difficulty of calculations, calibration and quality control requirements. Generally, CLIA-waived tests are considered the least likely to give an erroneous result. In the event of an erroneous result, they are the least likely to pose serious harm to the patient.

There is no guarantee that CLIA-waived tests will be completely error-free, however, and a bad result

QC Format	Pros	Cons
Dipper Style (Multi-use) 	• Simulates patient sample testing by utilizing a full immersion of the test strip into the control fluid • Reagent pads become fully saturated • QC method in full compliance with CLIA regulations	• Requires a large volume of control fluid to execute a test • Increased risk of contamination with repeated use • Increased risk of chemicals from reagent pads leading to erroneous QC results
Dropper Style 	• Requires very little volume to execute a test • Minimal risk of contamination from repeated use • No risk of chemical leaching from reagent pads leading to erroneous QC results	• Potential conflict with CLIA regulations as dropping does not represent the method by which patient samples are tested • Reagent pads are not as easily saturated and may lead to erroneous QC results
Dipper Style (Single-use) 	• Simulates patient sample testing by utilizing a full immersion of the test strip into the control fluid • Reagent pads become fully saturated • No risk of contamination from repeated use • No risk of chemical leaching from reagent pads leading to erroneous QC results • QC method in full compliance with CLIA regulations	• Unitized format may be less cost-effective on a per test basis than multi-use dipper and dropper style QC formats

Table 1. Dipper vs. dropper: pros and cons

continued on page 20

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can lead to a misdiagnosis and mistreatment. In fact, a study conducted across three hospitals in the United Kingdom in 2009 and 2010 determined that POCT represented error rates that were considerably higher than central laboratory testing and that most of the errors occurred in the analytical phase.[3] The College of America Pathologists Laboratory Accreditation Program (CAP-LAP) states that all clinical laboratory tests, including CLIA-waived tests, should follow a routine QC program as per other moderate and high complexity tests.[4]

Urinalysis dipsticks fall into the CLIA-waived category and are generally very reliable, simple to use, and easy to interpret. There are, however, numerous potential scenarios where a competent user may obtain an erroneous result. For example, most manufacturers package urinalysis dipsticks in canisters with a desiccant to keep the reagent pads dry. Failure to close the canister correctly can result in ambient moisture affecting the performance of the test. The leukocyte reagent pad, for instance, is particularly sensitive to humidity, and a poorly stored dipstick can lead to a false-negative leukocyte result, thereby missing a diagnosis for a potential urinary tract infection. Prolonged exposure to high temperatures and light can also negatively affect the performance of the tests.

Dipper or dropper?

When it comes to selecting QC for urinalysis dipstick testing, the two main formats to consider are dipper- and dropper-style controls. As the names imply, a dipper style control is used by fully immersing the urinalysis dipstick into the control fluid to fully saturate the reagent pads, whereas a dropper-style control is used by dispensing the control fluid dropwise onto the reagent pads. Several manufacturers produce urinalysis dipstick controls in these two basic formats, each with a unique set of stability claims, features, and advantages. Dipper-style controls are typically delivered in tubes with 10-15 mL of fluid. The minimum amount of fluid required to execute a test in a standard 13 x 100 mm borosilicate test tube is about 8.5 mL. This is quite a large volume of control per test, but it is necessary in this format to ensure that reagent pads are immersed.

Single-use dipper-style controls in this format would therefore be rather cost-prohibitive, which is why many control manufacturers allow for multiple dips into the same control tube. There is, however, a limit to the number of tests that can be performed in the same tube, because a variety of chemicals leach out of the reagent pads, potentially leading to erroneous QC results. The blood analyte reaction is particularly sensitive to shifts in pH and exposure to oxidative compounds that become released from the reagent pads. This effect is exacerbated by repeated dips over extended periods of time. Repeated use of this style of control also increases the risk of microbial contamination from frequent handling and multiple testing events.

Dropper-style controls are the most cost-effective because very little volume is required to execute a test. As many of the new generation of urinalysis dipsticks are formulated with specialized reagent pads that help prevent carryover contamination to neighboring reagent pads, they may sometimes be more difficult to fully saturate using a dropper-style control. The drops of control fluid tend to sit on top of the reagent pad until enough material has been delivered to fully penetrate. Failure to thoroughly wet the reagent pad with the control fluid may lead to an erroneous QC result. The glucose reagent pad on some brands of urinalysis dipsticks is particularly difficult to saturate using a dropper-style control because manufacturers have taken steps to prevent the reagents from carrying over to other pads. More specifically, the peroxidase enzyme from the glucose reagant pad can trigger a false-positive result on the blood reagent pad.

This issue can be mitigated by implementing proper training when utilizing dropper-style QC for urinalysis. There may be some confusion and/or lack of consensus as to how to interpret CLIA regulations when using dropper-style QC. CLIA 42 CFR section 493.1256 states, "*(8) Test control materials in the same manner as patient specimens.*"[2] Urinalysis dipsticks are intended to be dipped into the patient's urine sample, fully immersing the reagent pads. Nonetheless, there are some legitimate scenarios, such as in cases of low sample volume or with neonatal urine samples, that a dropping method may be utilized with patient samples.

Single-use dipper QC

Given the pros and cons of the dipper- and dropper-style controls (**Table 1**), the ideal control would be comprised of the best aspects of each: full immersion for pad saturation and CLIA compliance, and reduced risk for reagent pad leaching and contamination from repeated use. Since refrigeration is not always available near the site of patient care, many POCT devices are designed to be stored and operated at room temperature (RT). Consequently, QC materials that are used to verify the performance of the POCT devices would ideally also have extended RT stability. It follows that a single-use dipper style control, with extended RT stability, would be the ideal solution for urinalysis dipsticks QC performed at the point-of-care, particularly if the large volume requirement can be significantly reduced. A U.S. patent[5] has recently been granted for such a device whereby the control fluid is contained within a thermoplastic pouch that allows for the full immersion of a urinalysis dipstick in only 1.5 mL of control fluid, a mere fraction of the volume required for the traditional dipper style. Moreover, the single-use nature of the new format mitigates the risks associated with repeated use. Most important, this format directly simulates the analytical process used to test patient samples, thus providing the most robust and appropriate form of QC for urinalysis dipsticks for any clinical setting.

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Brian Fernández serves as Director of Research and Development for Redondo Beach, California-based **Quantimetrix**.

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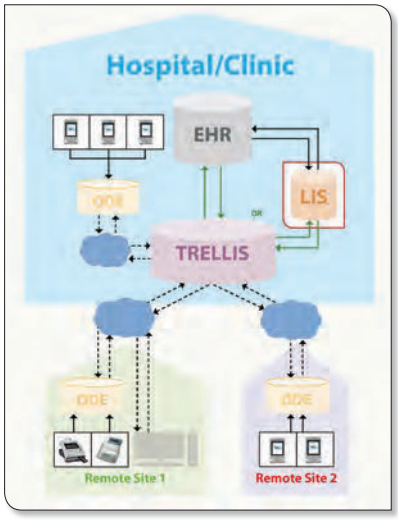

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Automated urinalysis in the clinical lab

Here are things to consider, and a look at some of the talent in the room…

By Stacy M. Kenyon, PhD, and Kendall W. Cradic, PhD

Because it is useful in the diagnosis and monitoring of renal and urinary tract diseases, urinalysis is one of the most commonly ordered laboratory tests. Basic urinalysis includes macroscopic examination, chemical analysis, and microscopic sediment examination. Although associated with significant labor and training requirements, manual microscopy remains the gold-standard methodology for sediment analysis; however, automated instruments are a valuable tool in the clinical lab.

Numerous studies have been performed comparing the performance of automated instruments to manual microscopy. Herein we provide a brief overview of the available technologies and conclusions from some of the recently published studies.

Overview of technologies

There are two types of technologies available for urine sediment analysis: flow cytometry and digital imaging techniques. These methods are summarized below, with additional technical specifications included in **Table 1**. A brief mention of the optional chemistry analyzers is also included for each sediment analyzer.

Flow cytometry can be used to identify and quantify cells, casts, bacteria, and other particles in urine sediment. Addition of fluorescent stain that binds to microbes in the specimen adds sensitivity and specificity for detection of smaller pathological elements. As particles pass through a flow cell, they are illuminated by a laser. The elements in the flow cell are classified according to impedance, light scatter, and fluorescence. Results are viewed as scattergrams with the numeric counts of each sedimentary element appearing as a distinct cluster. This method has been used for detection and quantitation of erythrocytes, leukocytes, hyaline casts, bacteria, and epithelial cells. Other elements such as crystals, yeast, oval fat bodies, sperm, mucus, and pathological casts are also detected but are not readily quantified. Specimens containing these elements are flagged for review and quantitation by manual microscopy.

Currently, Sysmex is the only company offering a flow cytometry-based instrument for clinical urinalysis. This family of analyzers consists of several instruments, the newest stand-alone sediment analyzer being the UF-1000i. A combined platform performing automated chemical and sediment analysis was recently released as the UX-2000.[1-3]

As for digital imagining techniques, identification of pathologic elements in urine sediment can also be accomplished using automated imaging. In this approach, a collection of high-resolution digital images are captured and then analyzed by image and pattern recognition software. There are two basic designs for collection of digital images in automated instruments; cuvette-based and flow cell systems.

In cuvette-based platforms, a urine sample is briefly and gently centrifuged in a specially-designed cuvette, resulting in a monolayer of particles. An automated, bright field microscope (typically with 400x magnification) then captures 10 to 20 images of the deposited particles on the surface of the cuvette. Images of the particles are displayed as whole-field views, similar to manual microscopy, allowing reviewers to visualize and manually verify the presence of pathological elements. Image processing software automates the process of identifying and categorizing particles with the help of a comparative reference image library.

The Hungarian company 77 Elektronika introduced UriSed (SediMAX in some market regions) in 2009. Since then, it has released UriSed 2, and more recently, UriSed 3. This latest iteration incorporates phase contrast microscopy in addition to bright field, to improve differentiation of elements such as hyaline casts, red cell membranes, crystals, and yeast. Images are evaluated in real-time using the company's Auto Image Evaluation Module (AIEM). UriSed 2 or UriSed 3 can be linked with the chemistry analyzer LabUMat 2 (or AutioMAX to SediMax) for full automation of urine chemistry and sediment analysis.[1,3,4]

Roche Diagnostics has entered the market for automated urine sediment analysis with the cuvette-based Cobas u701. However, the instrument is not yet available in the U.S. It provides quantitation of erythrocytes and leukocytes, semi-quantitative assessment of bacteria, epithelial cells, and hyaline casts, and qualitative evaluation of pathologic casts, crystals, yeast, sperm, and mucus. For complete automated urinalysis, the Cobas 6500 couples the u701 module with a u601 urine chemical analyzer.[2,5]

Flow cell digital imaging

Flow cell digital imaging techniques are also in clinical use. Flow cell analysis of urine sediment captures images in dynamic fluid rather than of a static surface as in the cuvette-based method. After images are collected, particles are identified and quantitated using image recognition software and comparison libraries. Urine is aspirated into the instrument and laminar flow is used to hydrodynamically orient particles as they pass through a flow cell. Digital images are captured and particles are classified based upon morphological features.

Beckman Coulter offers the Iris iQ200 family of instruments based on its proprietary Digital Flow Morphology for controlling flow characteristics and Auto-Particle Recognition (APR) software for identification and characterization of elements in urine. The camera captures ~500 frames per sample, and the instrument provides an interface for on-screen verification and review of results. The instrument is capable of differentiating erythrocytes, leukocytes, hyaline casts, unclassified casts, epithelial cells, bacteria, yeast, crystals, mucus, sperm, and amorphous substances. There are several iQ200 platforms available, iQ200SELECT, iQ200ELITE, and iQ200SPRINT, that can be linked with iChemVELOCITY to create the iRECELL platforms for total urine analysis.[5,6]

The Chinese laboratory diagnostics company DIRUI has been rapidly expanding its line of urinalysis instruments. The FUS-100 and FUS-200 urine sediment analyzers are also based on flow cell imaging technologies. The instruments use Flat Flow Digital Imaging technology, a trained neural network, and Artificial Imaging Identi-

System Product Name	Chemistry Analyzer	Sediment Analyzer - Technique	Sample Volume Requirements (Total urinalysis)	Maximum # Tests/ Hour
Sysmex UX-2000	Dry chemistry test strip	Flow cytometry	5 mL	150*
Elektronika LabUMat 2 & UriSed 2/3	Dry chemistry test strip	Cuvette-based imaging	2 mL	120
Roche Cobas 6500	Dry chemistry test strip	Cuvette-based imaging	2.8 mL	116
Dirui FUS-100/200 & H-800	Dry chemistry test strip	Flow cell-based imaging	3 mL	60 (FUS-100) 120 (FUS-200)
Beckman Coulter iRECELL	Dry chemistry test strip	Flow cell-based imaging	3 mL	101 (depending on configuration)

*50% particulate analysis

Table 1. Overview of technical specifications for total urinalysis systems

Instrument	Study	Number of specimens	Erythrocytes				Leukocytes				Bacteria			
			Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV
UriSed/SediMax	[3]	1454	80.3	87.4	59.5	95.1	76.7	88.2	47.7	96.4				
UriSed 3	[1]	277	Concordancea = 95.3				Concordancea = 95.0				59.2	95.4	88.4	79.8
UriSed	[4]	332	50.0	94.0	60.0	92.0	82.0	84.0	56.0	95.0				
Sysmex UX-2000	[3]	1454	92.7	77.1	42.6	98.3	94.3	94.7	71.7	99.2				
Sysmex UX-2000	[1]	277	Concordancea = 96.0				Concordancea = 96.8				74.8	90.2	81.9	85.8
Sysmex UF-1000i	[2]	300	Concordancea = 83.7				Concordancea = 93.8				62.1	90.4	90.5	61.8
Roche 6500	[5]	540	82.0	81.0	80.0	74.0	93.0	87.0	93.0	85.0				
Roche 6500	[2]	300	Concordancea = 86.0				Concordancea = 88.7				77.8	84.6	88.1	72.1
iQ200	[5]	540	90.0	63.0	65.0	76.0	92.0	71.0	83.0	75.0				
iQ200	[6]	209	75.8	97.7	86.2	95.6	77.7	93.9	91.2	83.7				
FUS200	[6]	209	72.7	94.9	72.7	94.9	68.1	95.7	92.8	78.6				
FUS100	[4]	332	73.0	86.0	47.0	95.0	68.0	89.0	60.0	92.0				

* Concordance was calculated as the number of cases matching within one grade.

Table 2. Analytical performance characteristics recently reported for various urine sediment analyzers.

fication (AII). A digital camera captures up to 820 images per sample (depending on the model) and AII identifies and classifies particles based on shape, contrast, texture, and frequency domain features. The FUS instruments can be combined with the H-800 chemistry analyzer for total urinalysis.[4,6]

Comparative instrument performance

Selecting the "best" design for urine sediment analysis is a complicated endeavor. Due to inherent differences in detection methodology, direct comparison between automated platforms is not straightforward. To overcome this challenge, element counts from an automated platform are generally compared to matched results from manual microscopy. Analytical performance characteristics for each class of particulate can then be reported. Sensitivity and specificity have been commonly used, as well as negative and positive predictive value. The agreement criteria often vary by element, and can be based upon presence vs. absence (e.g., patholgic casts) or agreement within a semiquantitative grade (e.g., RBCs of greater than 3, 4-10, etc.) Some studies instead report a more qualitatively derived concordance between methods, with an accompanying statistic such as Cohen's kappa or the intraclass correlation coefficient (ICC). When comparing results from different studies, it is also important to consider that individual labs establish cutoffs based on their patient population as suggested by CLSI guidelines.

Table 2 summarizes select studies between 2013-2017 reporting analytical characteristics for clinical automated urine sediment analyzers. The majority of reports focus on the accuracy of automated detection and quantitation of erythrocytes and/or leukocytes relative to manual microscopy. Performance characteristics from these reports show the analytical capabilities of these instruments.[1-6] Analytical sensitivity and specificity for erythrocyte and leukocyte detection was reported in four of six studies. The remaining two studies use concordance statistics in their analyses. Only two studies report statistics for detection of bacteria in urine sediment.

In Table 2, the analytical sensitivity and specificity for any given instrument can be widely variable when compared to manual methods. However, some generalizations can be made. In practical terms, identification rates of pathological features are clinically similar between flow cytometry and image-based methods. While images have the advantage that they allow operator review verification, this step does not appear to provide a substantial gain with regard to identifying erythrocites and leukocytes. Flow cytometry may have a slight advantage in recognition of bacteria due to the inclusion of bacteria-specific dye in reagents. In general, all automated instruments struggle with discrimination of crystals, yeast, pathological casts, and other pathological elements. Thus, algorithms are required to identify samples that need manual microscopy to detect them.

In summary, automated urine sediment analysis technologies continue to improve. As indicated by the relatively high specificity in most studies, automated analyzers are very useful for ruling out the presence of pathologic particles in urine. However, there remains a need for manual microscopy performed by experienced laboratorians to confirm abnormal findings. This is the practice that is followed at our institution, and in our patient population manual microscopy confirmation is required in 25 percent to 30 percent of samples, whereas the remainder can be reported based upon automated analyses.

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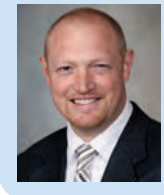

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Keeping up with POCT regulatory compliance

By Connie Mardis, MEd, and Daniel C. Gundler

Today, hundreds of tests once considered too complex for point-of-care testing (POCT) are routinely performed outside the laboratory.[1] Due to hospitals' decentralized structure, laboratory testing is performed on a multitude of POCT devices from various manufacturers in many hospital wards, critical care departments, clinics, and physician offices. Typically, POC devices in a hospital can include dozens of blood gas analyzers, urine chemistry and cardiac marker systems, and handheld coagulation instruments, as well as hundreds of glucose devices.

Perceived barriers to implementing POCT have been attributed to accountability factors such as quality control, adequate staff training, and oversight for accreditation purposes.[2] This article will review accreditation requirements and advances in open, vendor-neutral POCT data management to facilitate billing capture, regulatory compliance, and inspection preparedness.

Why POCT?

Because of its convenience, timeliness, and potential to improve patient outcomes, POCT's popularity continues to rise.[1] Near-patient testing increases the likelihood that healthcare professionals and the patient will receive test results faster, which may facilitate faster diagnoses, more timely treatment interventions, and improved patient compliance.

For example, a large, retrospective cross-sectional study of diabetic patients found that the availability of POCT not only lowers HbA1c in the short term (<1.5 years) but also in the longer term.[3] Reduced HbA1c indicates improved glycemic control and lowers the patient's risk of diabetic complications.[3]

Since diagnostic testing makes up two to three percent of healthcare costs and drives nearly 70 percent of clinical decision making, it is essential that laboratorians and POCT operators deliver quality results.[4]

The POCT regulatory environment

The clinical applications for POCT continue to expand, as does the identity of the staff who may conduct the testing and the regulatory requirements that apply.[5] For POCT devices operating under the central laboratory license, the single biggest challenge to the adoption of POCT is maintaining control, regulatory compliance, and training records for thousands of operators performing testing on hundreds of devices in anywhere from 30 to 50 locations within the hospital system (**Figure 1**).[6] As analysts and hospital associations predict no slowing of hospital and health system consolidation, POCT challenges are anticipated to continue.[7]

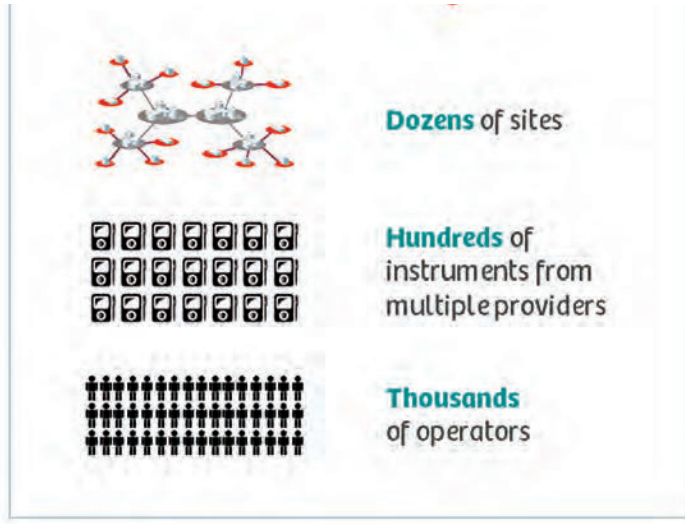

Figure 1. The challenges of POCT

In the United States, all clinical testing, no matter where it is performed, is regulated by the Clinical Laboratory Improvement Amendments of 1988 (CLIA).[8] POCT typically refers to CLIA waived or nonwaived laboratory tests performed at remote locations by non-laboratory personnel.[5]

Testing sites may choose to have CLIA inspections, or to be accredited and inspected by organizations including The Joint Commission (TJC, formerly JCAHO), College of American Pathology (CAP), or the Commission on Office Laboratory Accreditation (COLA). The professional organizations inspect laboratory members using their own standards, which the U.S. Centers for Medicare and Medicaid Services (CMS) have reviewed and found to be at least equal to CLIA standards.[8] The International Standards Organization (ISO) was introduced to healthcare organizations when the CMS approved Det Norske Veritas (DNV) as a deeming authority for Medicare certification and payments. DNV was the first new deeming authority named by CMS in more than 40 years.[9]

ISO 15189 specifies requirements for quality and competence in medical laboratories. It can be used by medical laboratories in developing their quality management systems and assessing their own competence in the laboratory and POCT. It can also be used for confirming or recognizing the competence of medical laboratories by laboratory customers, regulating authorities, and accreditation bodies.[10]

While Federal regulation of POCT is minimal, states and accrediting agencies often impose additional requirements on POCT.[5] Regulatory requirements for POCT generally focus on two areas: (1) training and competency of the personnel doing the testing and, (2) verification of strict adherence to the manufacturer-specified procedure for each test. The latter focus is particularly important because waived or moderately complex laboratory methods, both of which can be performed by non-laboratory personnel under certain circumstances, become highly complex if used in a manner that deviates from the FDA-approved manufacturer's protocol. Since high complexity essentially eliminates a laboratory test from consideration for POCT, it is essential that supervision of POCT includes verification that testing procedures do not deviate from the manufacturer's instructions.[5]

Managing POCT compliance

POCT supports the laboratory by delivering timely information—with the confidence of effective quality controls—to physicians at the most valuable touch points with patients. To achieve these controls, POC device manufacturers introduce connectivity systems to maximize efficiency and improve clinical outcomes through remote instrument and operator oversight.

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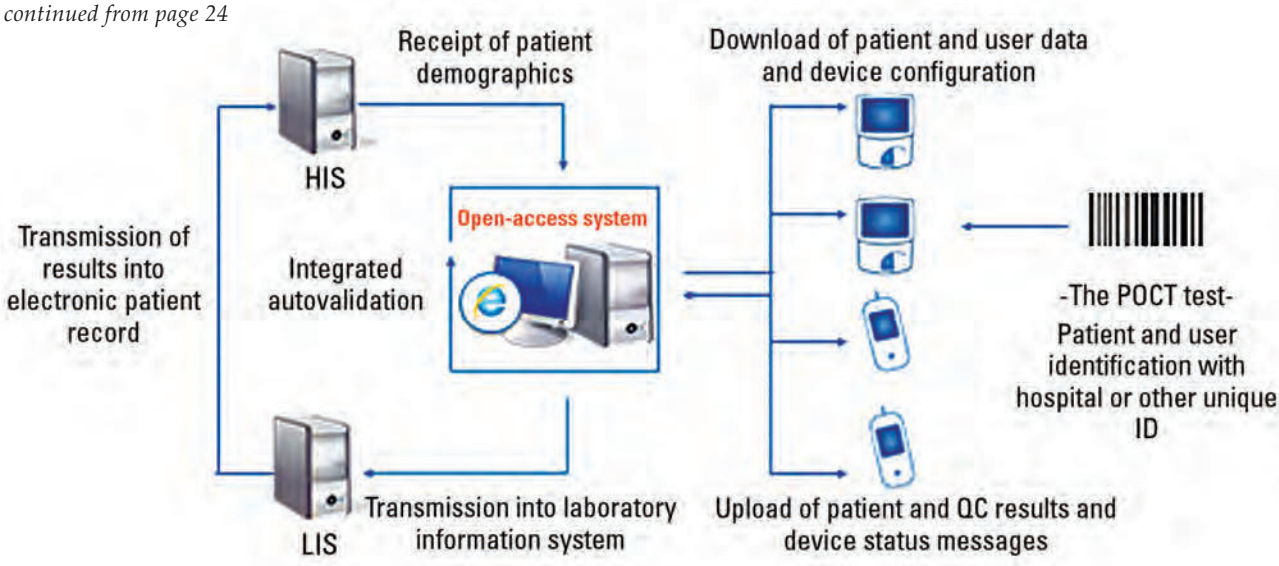

Figure 2. An open data-management solution connects POCT devices from all manufacturers to the hospital's IT system

In the past, the challenge of maintaining separate POCT data management systems for each manufacturer's products to interface with the hospital and laboratory information systems (HIS and LIS) has added complexity and increased software licensing costs. Today, hospitals can utilize an open-access data management system to connect more than 160 POC devices from all manufacturers to the hospital's IT system (**Figure 2**). A manufacturer-independent solution helps ensure IT investment protection in the event a hospital changes POC equipment vendors.

While the majority of POCT done today is performed using instruments, or is migrating to instrument reading to reduce subjectivity in result interpretation and transcription errors,[11] open-access data management systems are available that can support reporting of visual-read tests and facilitate billing capture.

An open-access data management system can automatically validate and transfer patient results obtained from POCT devices to the electronic medical record and monitor and manage data, POCT devices, and operators. POC coordinators can now proactively manage organization-wide EQA results according to accreditation requirements. Distributions and statistics are easily viewed and filtered with the familiar proactive traffic-light display that flags noncompliances in any connected POCT devices at any site.

The content of e-learning courses and tests, supplied by each POCT device manufacturer to the open-access data management system, guarantees that only approved content is used for training. When an operator passes the test, indicating successful completion of a course, the results are automatically documented in eLearning, and a message is sent to the data management system, which automatically extends the operator's certification for another year.

Advances in POCT connectivity offer capabilities to address accountability factors currently perceived to be barriers to adoption. The use of connectivity can greatly improve efficiency when managing different aspects of regulatory compliance. An open-access data management system is a key enabler for POCT coordinators, by connecting devices from any manufacturer and providing operator oversight so testing efficiency is maximized, clinical workflow is improved, compliance is adhered to, and costs are efficiently managed.

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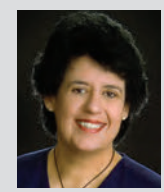

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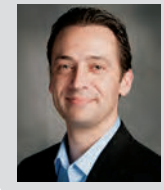

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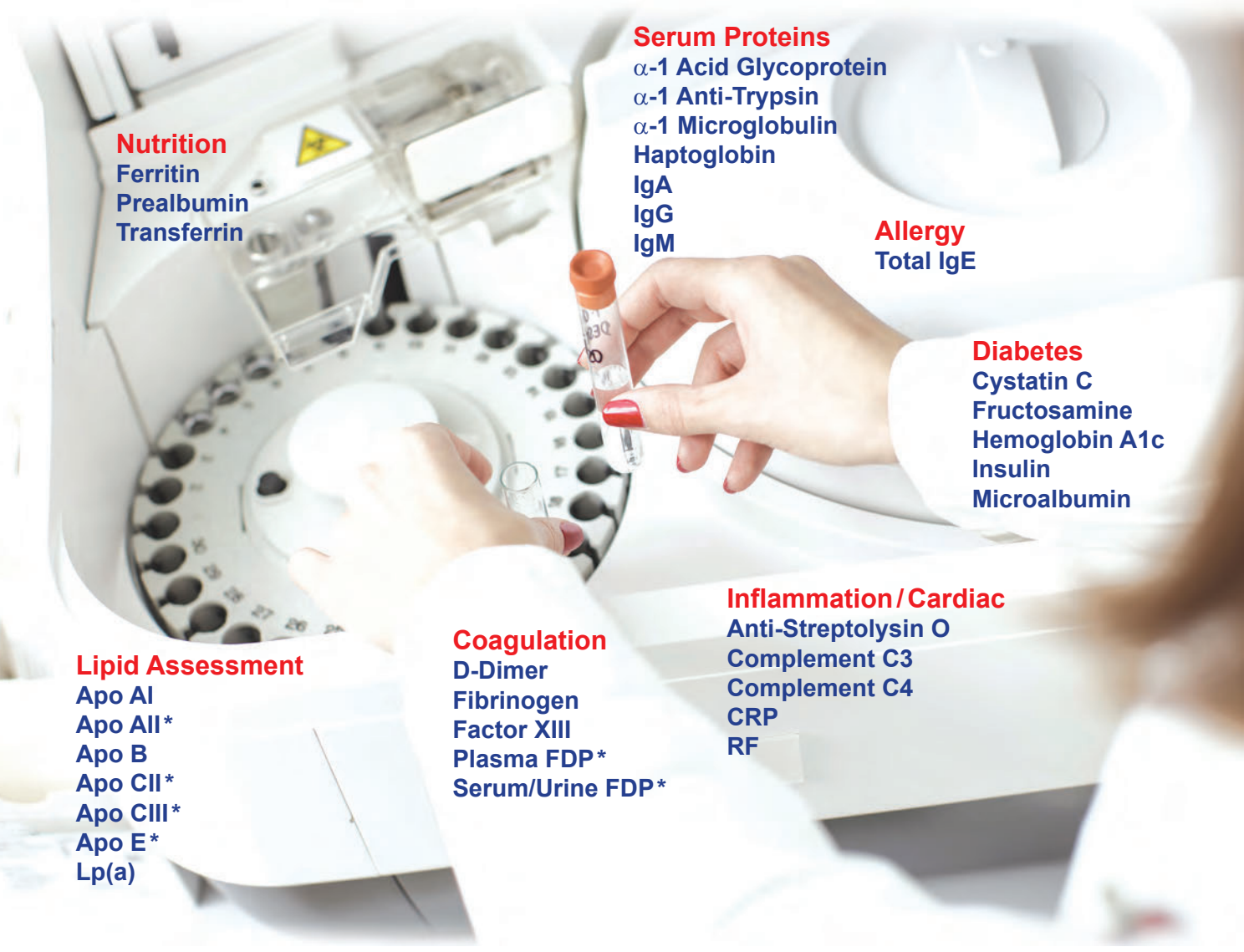

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The inspection-ready lab includes IT

By Jennifer Lyle

The majority of clinical laboratories undergo regular inspections by government and/or accrediting agencies. These inspections cover all aspects of the laboratory operations, including information technology (IT)—the laboratory information systems (LIS) and middleware that modern laboratories rely on for those operations. What does it take to make sure your laboratory IT is as ready as the rest of the lab when inspectors come to call?

Inspections are opportunities

Preparing for, anticipating, and undergoing an inspection can be stressful. The potential fallout of not doing well during an inspection—risk to patient safety, cost to respond to inspector findings, and, in extreme cases, even closure of the business—is too great to chance failure.

On the other hand, doing well on the inspections and being awarded continuing accreditation provide an opportunity to publicize the laboratory's contributions to the organization and community. Such communications may take the form of internal memos, news releases published on the organization's website, or coverage in local media. Such publicity will help to instill confidence in the laboratory's stakeholders—staff, administration, care providers and patients.

> ***"The facility eventually came to the realization that the inspectors were not the enemy; they were people who wanted to help make the company better, ultimately assuring the safest possible product was available for patient care."***

Clinical laboratories may be inspected by one or more agencies including CAP, CLIA, AABB, The Joint Commission, and even, depending on the types of services offered by the lab, the U.S. Food and Drug Administration (FDA). According to CAP's website, "the Centers for Medicare and Medicaid Services (CMS) has granted the CAP Laboratory Accreditation Program deeming authority, which allows CAP inspection in lieu of a CMS inspection. It is also recognized by The Joint Commission, and can be used to meet many state certification requirements."

The CAP inspection is "the gold standard" for many labs, so this article will refer to the checklists that CAP provides.

Tracking compliance internally

Laboratories have different ways of tracking the CAP requirements and their own compliance. Methods range from building a spreadsheet to using sophisticated computer databases or programs. One lab that this author is familiar with uses both methods: staff extract all the CAP requirements into a spreadsheet that includes the actual requirement information and add their own notes about the related records and their location(s). Armed with this tool, the lab or IT analyst can quickly locate the documentation for the inspector if asked to produce it. The lab's compliance tracking software program keeps detailed training and staff proficiency records, and the spreadsheet notes where to access the records related to the particular requirement.

CAP checklists for IT

Modern clinical labs are large, complex organizations with hundreds of supporting procedures covering all disciplines of the lab and many related aspects—for example, sample collection, quality management, workplace safety, and training. Even though many labs have multiple information systems in the form of an LIS and middleware, there is not an IT-specific checklist. Instead, the IT-specific requirements are a subset of the *Laboratory General* checklist.

The reason IT solutions have a place in the lab is that they integrate with and support the lab workflow. Thus, many non-IT specific requirements are impacted by IT. For example, CAP requirement GEN.40530 says the lab needs a way to track samples sent to it from a remote site. If the LIS has the capability to do this tracking, then the functionality provided will need to meet the stated requiremets; for example, recording the time of dispatch and receipt.

Meeting the requirements

Some requirements are out of the lab's direct control, such as those dealing with the facility maintenance, fire equipment, network security, and power sources. For these items, the laboratory can conduct internal inspections in conjunction with its IT peers to ensure compliance prior to outside inspections.

There are some CAP requirements which could be met by the lab's IT systems, but due to poor or lacking software design, they are not. If the LIS does not provide the compliance needed in an automated fashion, the lab will need to develop a manual process. Looking again at CAP checklist item GEN.40530 for Specimen Tracking, if the LIS does not have an adequate tracking system, the lab can design forms that are completed by hand and kept on file. In cases like this, the lab benefits from having a strong IT representative who can communicate the particulars of the requirement to the vendor and advocate for its inclusion in the vendor's software delivery plan.

There are several requirements related to system validation. All of these requirements state the need for validation upon initial software installation and whenever a modification is made. In addition, some require periodic validation even if no system changes have been made. They are:

- GEN.43022: LIS testing, no periodic revalidation required, records must be kept two years beyond the life of the system
- GEN.43450: calculated patient result values; every two years
- GEN.43875: auto verification, at least annually
- GEN.48500: interface result integrity, at least every two years.

Validating a new or a major LIS upgrade involves hundreds of hours of testing, with potentially thousands of test steps. While the listed requirements are only a small part of the overall checklist, the amount of work and record-keeping to demonstrate compliance is disproportionately large.

Capturing "test evidence"

After developing the test plans for the validation process, the laboratory or IT analyst captures "test evidence" for the record. The test evidence may be copies of patient reports, or screen shots that show the software has performed as expected. For smaller projects, such as the two-year interface validation, it may not be an issue to print the screen shots and store them in a three-ring binder whose location is referenced in the spreadsheet mentioned above.

For a new laboratory or blood bank information system, the test evidence can easily amount to hundreds of pages of data. Labs with space constraints may choose to capture the information electronically using screen print tools or scan the printed pages so they can be stored electronically. The spreadsheet would be completed with the electronic file information necessary to find those records easily in cases where the inspector wants to review them.

More and more, labs are looking for automated tools to alleviate understaffed departments and provide efficiencies that free up time for their existing staff to focus on more complex issues. One such tool is the compliance tracking software solution mentioned above, a system that replaces manual tracking of personnel training records and employee proficiencies.

Another solution that's being used in more laboratories is automated testing software. This software performs the actual testing, and it also captures the records necessary to demonstrate that the system performs as expected. Robust testing software can also summarize the records in a way that shows the conditions tested and includes a cross-reference to the test case or cases in which the condition was demonstrated. The reports are available electronically.

A state of readiness

A colleague who at one time was associated with a blood bank software vendor recently told this author that when the company had its first few FDA inspections, there was an aura of fear and resentment over being judged by an outsider. However, the company eventually came to the realization that the inspectors were not the enemy; they were people who wanted to help make the company better, ultimately assuring the safest possible product was available for patient care.

Similarly, laboratory inspections can be used to educate the lab on process improvements that assure their practices are consistently reliable and safe. The inspection-ready lab and its IT staff should not "get ready" for an inspection so much as maintain a constant state of readiness through consistent, organized, and disciplined processes. That is the best way to enhance quality, increase stakeholder confidence, and assure patient safety.

Jennifer Lyle is CEO and Founder of Nevada-based **Software Testing Solutions, LLC.** She founded the company in 1999 to create innovative solutions which automate and accelerate the in-depth testing of healthcare applications.

Prenatal genetic screening goes beyond trisomies

By Kimberly Martin, FRCSC, FCCMG, FACOG, FACMG, and Trudy McKanna, MS

In the May 2015 issue of *MLO* [2015;47(5):14] we reviewed the evolution of prenatal genetic screening. The closing line of that article stated that "…rapid technological progress, particularly using SNPs, holds the promise of even greater improvements in test performance and safety."[1] Now, we revisit the status of prenatal screening for chromosome abnormalities and the continued advancements in this area.

Non-invasive prenatal testing or screening (NIPT/NIPS) using cell-free DNA (cfDNA) has been commercially available for over five years. A number of peer-reviewed journal articles have consistently demonstrated its superior positive predictive value for common trisomies (21, 13, and 18) compared to traditional maternal serum screening. Norton et al demonstrated a 79 percent sensitivity and 3.4 percent positive predictive value (PPV) for trisomy 21 using standard first-trimester screening in all patients. In comparison, cfDNA screening showed a 100 percent sensitivity and 81 percent PPV for trisomy 21 for all patients.[2] Most important, a total of 11,994 women in that study were < 35 years of age, without additional risk factors for trisomy 21, and cfDNA showed 100 percent sensitivity and 76 percent PPV in that "low risk" group.

Both the American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics (ACMG) have updated their screening statements to recommend that NIPT be made available to all pregnant woman, regardless of their prior risk for aneuploidy.[3,4] However, approximately half of women with private insurance and essentially no women with Medicaid are financially covered to choose NIPT as a first-line screen.

The fact that NIPT maintains its high performance standards in average-risk women in turn prompts continued discussion to "rethink screening." There are many conditions that are unrelated to maternal age, and usually without a family history. For what additional conditions *beyond trisomies* is it reasonable/responsible to offer screening to *all* pregnant women, if non-invasive screening is possible with acceptable sensitivity, specificity, and positive predictive values? How is SNP technology for NIPT uniquely positioned to provide sensitive and specific expansion of prenatal genetic screening?

A logical next step in prenatal genetic screening is to consider smaller genetic changes in a chromosome, called copy number variants (CNVs). CNVs are typically microdeletions or microduplications less than 10 Mb that are associated with clinically significant outcomes and are unrelated to maternal age. In 2012, Wapner et al reported that clinically relevant deletions and duplications were found in six percent of pregnancies with ultrasound anomalies and 1.7 percent of pregnancies without risk factors.[5] However, unlike the common trisomies, deletions and duplications are not part of routine serum screening or NIPT. There are different approaches to CNV screening: targeted and whole genome.

Targeted screening

Targeted CNV screening entails choosing specific deletion or duplication sites with known clinical outcome, and providing risk assessment particular to that associated condition. For example, the most common microdeletion condition, 22q11.2 deletion syndrome, has a published incidence at birth of 1/2000, though this may be an underestimate. It is a well-described clinical entity with known chromosomal breakpoints and clear diagnostic testing parameters. Therefore, screening for 22q11.2 deletion syndrome is a natural expansion of NIPT.

ACOG and ACMG guidelines are clear with respect to the necessary laboratory conditions that need to be met in order to offer CNV screening. ACMG states that "Laboratory requisitions and pretest counseling information should specify the DR, SPEC, PPV, and NPV of each CNV screened."[4] This requires analytical and clinical validation for each deletion or duplication, as well as accurate estimates of population incidence of each condition. Currently, different NIPT laboratories offer a range of microdeletion syndromes, but their reports often do not follow these guidelines.

A recent publication on the clinical experience of SNP-based microdeletion testing addresses the issues of expanded screening and the need for transparent follow-up. This publication extends the initial reporting of SNP-based NIPT screening for 22q, and highlights outcome data for the remainder of the microdeletion panel currently offered (1p36 deletion, Angelman, Prader-Willi, and cri-du-chat). Performance improvements to this SNP-based testing resulted in a decrease in false positive test rate (0.07 percent for 22q) and an increase in PPV (44.2 percent for 22q; 31.7 percent combined for others). While some publications have questioned the expansion of NIPT into microdeletions due to concerns about positive screen rate and low detection rate, the targeted nature of SNP-based NIPT screening is shown to have a higher sensitivity than other NIPT methodologies.[6]

Whole genome

The concept of targeted CNV screening can be further expanded to other less common and less well-described microdeletions and microduplications, as well as rare autosomal trisomies (RATs), by looking at genomic information across all chromosomes. However, ACMG specifically recommends not screening for genome-wide CNVs, stating (among other reasons) that "If this level of information is desired, then

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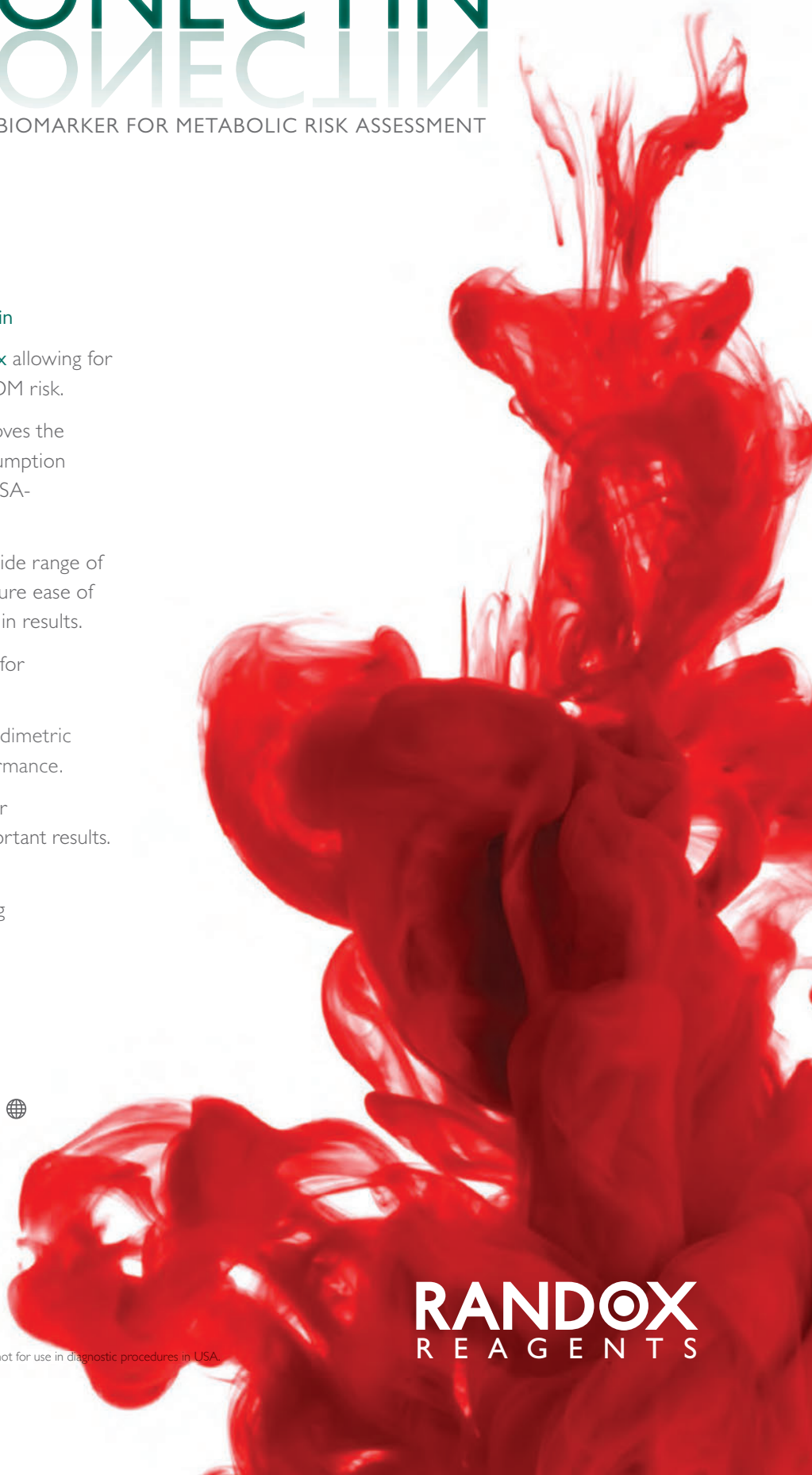

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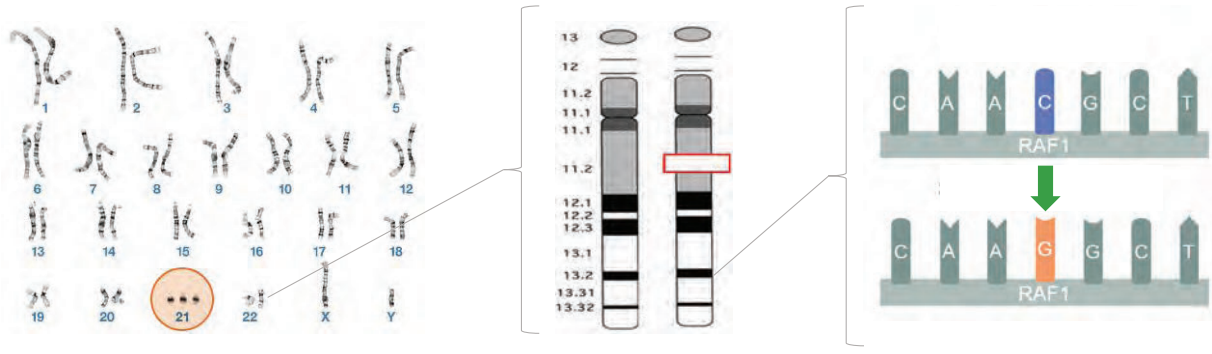
The evolution of NIPT

Whole chromosome aneuploidies → Copy Number Variants (CNVs) → Single-gene disorders (SNVs)



The evolution of NIPT

diagnostic testing followed by CMA is recommended."[3] The clinical performance of such test options is not clinically well validated, and reporting does not follow test metric guidelines, as described earlier.

Single gene disorders

As the precision of non-invasive prenatal screening using SNP technology has narrowed in on CNVs, this screening can then focus even further to single gene disorders.

The first commercially available screening test for single gene disorders was launched in early 2017. This panel screens for single nucleotide variants (SNVs) in 30 genes responsible for a variety of genetic conditions. These genes can be generally categorized by clinical phenotype: Noonan spectrum, Craniosynostosis, Skeletal, and Syndromic disorders. Many of the conditions have no ultrasound findings early in pregnancy, are typically *de novo*, and may be associated with advanced paternal age. The combined incidence of these conditions in the general population is approximately one in 600.

Each of these genes are screened in the cfDNA of a pregnant woman using next-generation sequencing technology. Pathogenic or likely pathogenic variants are reported, with the recommendation for confirmation by diagnostic testing.

The view from here

Currently, prenatal screening options are typically limited to trisomies 13, 18, and 21, even though the general population incidence of other genetic conditions may be higher. Unfortunately, despite significant published data regarding the superior performance of NIPT over conventional screening, many women are denied access due to lack of insurance coverage.

Advances in SNP-based NIPT technology have allowed for the expansion of these prenatal genetic screening options for conditions unrelated to maternal age such as targeted microdeletions and single gene disorders. Indeed, the promise of greater improvements in non-invasive prenatal test performance has held true. It is exciting to consider the next advancements on the horizon. However, one critical fact remains unchanged: regardless of the kind of prenatal screening performed during pregnancy, the results are not diagnostic, and no irreversible decisions should be made on the basis of screening results. Confirmatory testing, either prenatally by chorionic villus sampling or amniocentesis or postnatally by peripheral blood draw, is required.

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HPV in the news

By MLO Staff

Human papillomavirus (HPV) continues to be a hot topic to laboratory scientists, to clinical practitioners, and to the general public. The relationship between HPV and cervical and other cancers, advancements in diagnostics and screening, and issues related to vaccination are frequent areas of study by researchers. Here are summaries of three recent HPV studies that could have far-reaching significance.

HPV vaccine and improved fertility

More than 40 percent of American teens are now getting vaccinated against human papillomavirus. But, despite HPV infection being associated with reduced semen quality and lower pregnancy rates, there is still public concern about whether the HPV vaccine itself could affect future fertility.

Now, the first prospective cohort examining the relationship between HPV vaccination and fertility, led by a Boston University School of Public Health (BUSPH) researcher, has found that the vaccine can actually improve chances of conception in some women.

The study, published in the journal *Paediatric and Perinatal Epidemiology*, shows little overall association between HPV vaccination and the chances of conceiving for men and women—except among women with a history of sexually transmitted infections (STIs). STIs are associated with lower fertility, but vaccinated women with an STI history had about the same chance of becoming pregnant as unvaccinated women who had never had an STI.

"Our study found no adverse effects of HPV vaccination on fertility and indicated that it may, in fact, protect fertility among individuals who have had other STIs," says BUSPH doctoral student Kathryn McInerney, the study's lead author. "Our study should reassure those who are hesitant to vaccinate due to fertility concerns."

The study used data derived from the Pregnancy Study Online (PRESTO), a preconception cohort of North American pregnancy planners. The ongoing study enrolled 3,483 women and 1,022 men aged 21 to 45 years who were actively trying to conceive. Couples were followed for 12 months or until pregnancy, whichever came first. At enrollment, 33.9 percent of women had been vaccinated against HPV, compared to 5.2 percent of men.

"Internationally, parents have chosen not to vaccinate their children due to concerns about the vaccine's effect on future fertility," McInerney says. "We hope this study will be useful for health providers who counsel individuals and families about HPV vaccination."

Screening for cervical abnormalities

HPV testing detects a higher number of precancerous cervical lesions than cytology-based Pap smears in a female population including a proportion offered HPV vaccination, according to a new study conducted by Australian researchers and published in *PLOS Medicine*.

Many countries are currently considering switching from classic Pap tests to primary HPV tests for cervical cancer screening, based on strong evidence linking cervical abnormalities and infection with certain HPV types and data suggesting that HPV tests detect more high-grade precancerous lesions. However, no study has yet compared the different methods in a population in which younger women had been offered prior HPV vaccination.

In the new study, researchers randomized cervical samples from 4,995 women aged 25 to 64 in Australia, in a 1:2:2 ratio, to be analyzed by either cytology (with HPV testing of low-grade abnormalities); HPV testing with partial genotyping of the virus for the highest-risk types HPV16 and 18, and cytology (for participants with other high-risk HPV genotypes); or HPV testing with partial genotyping and dual-stained cytology. In the first screening round of the trial, the authors assessed the rates of women being referred for further testing and of detection of CIN2+ (high-grade cervical intraepithelial neoplasia) precancerous lesions.

For the cytology group, the overall referral and detected CIN2+ rates were 27/995 (2.7 percent and 1/995 (0.1 percent); for the HPV testing and cytology group, they were 75/1992 (3.8 percent) and 20/1992 (1.0 percent); and for the HPV and dual-stained cytology group, they were 79/2008 (3.9 percent) and 24/2008 (1.2 percent). The researchers found that, in the first round of screening, detection of CIN2+ was significantly increased with HPV testing as compared with cytology, while referral was non-significantly increased. Adverse events were rare, and the one case of early-stage cervical cancer (in the HPV testing plus cytology group) was detected as appropriate by screening.

"These findings provide initial confirmation of an improved performance of primary HPV screening compared to cytology screening in settings with HPV-vaccinated populations," says lead author Karen Canfell, PhD, of Cancer Council New South Wales, Australia. These findings support the planned introduction of cervical screening by HPV testing in Australia, which will occur at the end of 2017.

HPV testing and cervical pre-cancer

Women who receive human papillomavirus (HPV) testing in addition to a pap smear receive a faster, more complete diagnosis of possible cervical precancer, according to a study of more than 450,000 women by Queen Mary University of London (QMUL) and the University of New Mexico Comprehensive Cancer Center.

The study, published in *JAMA Oncology*, used data from the New Mexico HPV Pap Registry in the United States. It is the first comprehensive evaluation of HPV testing on the long-term outcomes of women who had received a borderline abnormal Pap test result.

A total of 457,317 women were included in the study. Of these, 20,677 women (4.5 percent) received a borderline abnormal result through a Pap smear and were followed in the study for five years. Some of the women with borderline abnormal Pap smear results had an HPV test.

HPV testing led to a 15.8 percent overall increase in the detection of cervical precancers, and time to detection was much shorter (a median of 103 days versus 393 days).

Virtually all cervical pre-cancers were detected in women who tested positive for HPV, suggesting HPV testing is a good additional screening method after the Pap smear. Colposcopy (a medical examination of the cervix) could then be focused on women who would need it most: those with a positive HPV test.

At the same time, however, HPV testing of women resulted in 56 percent more biopsies and a 20 percent increase in surgical treatment procedures performed. Most of the additional biopsies were for low-grade lesions which could have regressed, indicating some overtreatment due to HPV testing.

Professor Jack Cuzick from QMUL says: "This study shows that knowing a woman's HPV status can help determine her likelihood of needing additional procedures, and prioritize immediate treatment and medical resources to the women who need them most."

The authors warn that, as this was an observational study, the use of HPV testing was not randomized. Thus there could have been socioeconomic or other relevant differences among healthcare facilities that have not been measured.

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Back to Basics: Array diagnostics

By John Brunstein, PhD

In this month's column, we are going to continue with our "Back to Basics" theme by reviewing what underlies a common molecular diagnostics (MDx) laboratory method, microarray-based diagnostics. We will also take the opportunity to see how its use has changed in the few years since it was last covered in this space.

What's an array, anyway?

First, let's remind ourselves what an "array" is in this context. It's most accurately described as a spatially distinguishable set of interrogatable probes for specific short nucleic acid targets. If that seems like a rather meaningless juxtaposition of words, you've come to the right place: read on. The most traditional format for a microarray is a small silica (glass) piece or "chip," perhaps about the size of a small postage stamp, held in a defined orientation in some sort of carrier. This chip provides a piece of spatially referenced real estate, divided into a grid of rows and columns; within each referenced location, many identical copies of a user-defined nucleic acid oligonucleotide are tethered down at one end via a linker molecule so that they project up, rather like tiny hairs.

Each of these oligonucleotides is thus free to hybridize to its complementary target sequence, assuming something along the lines of a Southern blot is performed. That is, the chip surface is immersed in a suitable buffer at an appropriate annealing temperature for the hybridization reactions in question, and thermodynamics is allowed to assert its authority. This drives hybridization between any in-solution nucleic acid strands which are the complement (or at least close match) to tethered probes. It is then followed by a few rounds of washing to remove any extraneous weak binding nonspecific interactions, and the result is an array chip where any grid spots which had a matching nucleic acid molecule in solution have captured and localized this to a unique, known grid address.

Probes can have variety, too

Let's pause for a moment there to consider some of the potentially useful variations we might do on the chip-bound oligo side. Above, we only referred to the spatially fixed items as oligonucleotides. The exact chemical nature of these oligonucleotides is up to us at time of chip manufacture, and while they are commonly made of "garden variety" DNA, we can employ tools such as degeneracy (that is, a mix of more than one nucleotide at a position in a probe sequence, allowing for perfect match to more than one sequence variant at that nucleotide position) or non-canonical bases such as inosine (again, allowing for controlled degeneracy in hybridization matching). Other useful tools might be the kuse of peptide nucleic acid (PNA) or locked nucleic acids (LNA) as probe components, as these provide for stronger (more specific) target binding than purely natural bases.

What can be spotted down as the captive probe at each grid point is open to a great deal of imagination. One factor that tends to limit wild flights of fancy is the fact that really short probes don't work very well; mathematically, they just don't have much sequence specificity, and they require awkwardly low temperatures for hybridization and washing. Really long probes also don't work well; they have increased likelihood of binding to partial matches, and they can start to have physical steric hindrance or homodimer interactions, such as hairpin formation, that make them poorly available to interact with sample in the liquid phase. In addition, if we expect to use the array at a single hybridization and wash temperature for all targets, then within a certain small window of variation (probably less than 1°C) all probes should have matching annealing temperatures.

Spot detection

The next thing to contemplate is how to detect which array grid spots have bound to targets from the liquid sample they were immersed in. The most common methods here are photonic- (optical-) based, and are most easily achieved if we pretreat the liquid test sample so as to add some form of fluorescent label to all of the nucleic acids it contains. Using this method, our array readout methods are straightforward digital image capture of the array area, and spatial detection and differentiation of the glowing spots which indicate captured, labeled target material. An inherently helpful aspect of this approach is that optical readout resolving power permits very close spacing of individual array grid spots, or, put another way, very high spot density.

Fluorescence detection is also amenable to limited multiplexing, meaning that we can differentially label multiple (usually, two) samples and detect them independently on a single array. For these most traditional silica microarrays with fluorescent readout, the number of distinct grid spots (probes) per chip area is limited by mechanical aspects in the chip production process, not readout resolution. If we want to ask how many indexed spots or grid reference points can we fit on a microarray of this type, it gets a bit into how the array is made. The simplest method mechanically spots tiny droplets of the desired pre-made full length probes at their intended grid points, and these chemically adhere; in this method, the density is limited by the mechanical step size of the spotting or "printing" instrument (and in placing the tiny spots far enough apart that they don't bleed to each other and intermix during printing). A second approach uses photolithography to define and chemically activate array grid spots for in-situ synthesis of desired oligonucleotide probes right on the silica surface; as this is optically driven rather than a purely mechanical approach, it's at least theoretically capable of higher grid densities than direct spotting. In reality, the end user probably has little concern about which method was

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used; suffice it to say methods exist to reliably create two-dimensional silica chips with well over a million discrete spots or "features" present.

(As an aside, now that we have a feel for the number of features we could have on a microarray, it starts to become apparent that while we could introduce things like degeneracy within a single spot, it probably makes more sense to just have two or more spots as needed to represent each sequence variation uniquely; then we can actually identify which of the possible sequence forms is present, rather than lumping them together. It's up to the array designer to decide, though, demonstrating the sort of flexibility one can have with microarray methods.)

If traditional microarrays are fixed oligonucleotide spots on silica wafers with spatial indexing and fluorescent detection of target capture, what are some of the variations on this? While space limitations restrict us from going into all of the other microarray formats and approaches possible, it's worth mentioning at least one other common format. This is the fluid-phase bead array approach, where rather than attaching oligonucleotide probes to a flat silica surface, we attach them to differentiable microscopic beads. Different bead types can be told apart either by color code, or actual tiny monochrome barcode-like markings; each bead type is then coupled to a single probe.

These types of arrays are also generally read out by optical methods based on fluorescence, but tend to be limited to a few hundred features at most (it becomes hard to differentiate many more bead types than that). While that's a disadvantage compared to 2D silica arrays for feature density, liquid phase hybridization kinetics can make bead type arrays faster than their competitors. It's also possible to rapidly customize a bead-based array by adding or removing one bead type with its probe, while 2D silica arrays, once printed, are fixed. On the detection side, one variation is in use of electrochemical methods for spot readout rather than fluorescence. This approach is used in some clinical service array-based devices, but a caveat here is that limitations to detection spatial resolution by this method mean these forms of 2D arrays have very low feature densities.

Common types of array assays

So we've reminded ourselves of what the common forms of a microarray are, and how they're read out; what is it that we can do with them, and has that changed (or its practical utility changed) in the past few years? First, let's summarize the list of some of the most common microarray applications:

Expression arrays. These work by collecting and labelling expressed mRNAs in a sample, and then hybridizing to an array with probes for various genes of interest. Probes can be specific for individual isoforms or splice variants; data obtained is not just presence or absence of particular mRNAs, but also relative abundance.

Array CGH. As covered in detail in the June 2014 installment of this column ("Array CGH: mechanisms and applications," *https://www.mlo-online.com/array-cgh-mechanism-and-applications.php*) this technique in a nutshell differentially labels whole genome DNA from a "control" source and a "sample" source, then attempts to hybridize for markers evenly distributed across the genome. Competition for hybridization between sample and control means that duplications and deletions in the sample are readily detected by this method.

Resequencing arrays. These arrays represent selected, limited regions of the genome in a series of oligonucleotides which both "tile" (overlap in sequence coverage) and collectively represent possible sequence variations. By measuring which of these possible sequence versions hybridize to the sample, the sample sequence from the region of interest, such as the whole ~16 kb mitochondrial genome, is read out.

SNP arrays. These interrogate large numbers of (ideally) uniformly, randomly distributed single nucleotide polymorphisms (SNPs) across the genome. These are helpful in detecting issues such as loss of heterozygosity (LOH; for example uniparental disomy of a chromosome).

Use as a detection method for highly multiplexed PCR assays. Conventional real-time PCR systems can multiplex a handful of targets—possibly up to as many as six, although three or four are more frequently feasible—but imagine being able to set up a PCR reaction for the detection of possibly hundreds of targets at once. Microarrays and, in particular, smaller ones such as the liquid phase types described above, provide an excellent approach for detecting which of the possible reaction products are formed in such a test. Note that since this is an endpoint PCR detection, it provided qualitative data only, but such may be of use, for example, in infectious disease settings where any detection is diagnostic.

In general, this summary list of what we can do with microarrays hasn't really changed in the past five years or so. Their practical utility in some contexts, however, has changed, primarily in those applications where arrays were (are) used to screen large amounts of genetic information such as whole genome expression studies or array CGH. When microarrays first started becoming popular in clinical applications, they represented the most cost-effective approach to genome-wide measurements of a range of selected targets. The biggest change in that over the past few years has been the steady declines in cost and technical difficulty for next generation sequencing (NGS), and the increasing accuracy and throughput of those methods.

For labs currently equipped with microarray instrumentation and with established operational workflows for sample processing and data interpretation, microarray methods will likely remain competitive for some years to come. For a lab just looking now to establish tools for genome- wide/high throughput analyses, consideration of NGS as an alternate platform is warranted, however, as it may be more flexible or cost-effective, depending on intended application. As NGS systems continue to become cheaper and easier, they are likely to further become the method of choice for these sorts of studies. Until then, however, the molecular laboratorian is likely to see both methods in use and of practical utility.

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

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Automated slide preparation and interpretation can enhance lab efficiency

By Ann Ludwig

With technological advancements in automated hematology analyzers, why do we continue to look at blood smears through a microscope? A thorough review of the blood smear in conjunction with the patient's clinical picture and automated hematology analyzer results becomes invaluable in the diagnosis and clinical care pathway determination of many disease states, including leukemias and anemias. In order to perform a thorough blood smear analysis, we must start with an impeccably made blood smear.

Reviewing a consistently uniform blood smear throughout a patient's course of treatment is essential to clinicians as they assess treatment efficacy. Improving consistency in review of manually made blood smears may lead to improved messaging to the clinician, potentially impacting patient care.

There are many challenges to mastering the art of the manual smear and that sought-after feathered edge. One must consider many factors in the process. A quicker push at a higher angle results in a thicker smear. A larger drop of blood results in a longer smear, jeopardizing the quality and location of the feathered edge. The hematocrit of the sample (viscosity) can impact the thickness of the smear, resulting in variation of cell distribution whether a smear is too thick or thin. While the goal is to have the smear cover approximately two-thirds of the slide with a feathered edge at the end, the slightest adjustment of the hands vs. the size of the drop of blood and viscosity of the sample may lead to inconsistencies on the part of even the most practiced laboratorians.

Teaching the manual method requires starting with the basics. This practice can be very laborious and often requires multiple attempts to adjust the blood drop size, angle, and speed of the push. Inconsistencies from length to width and thickness still remain (**Table 1**).

The benefits of automation

With today's ever-changing healthcare environment, laboratory managers and directors are challenged to find ways to optimize the utilization of laboratorians and support staff while maintaining and improving turnaround times, and continuing to provide the highest quality patient care. Continuing to perform manual tasks such as preparing manual blood smears takes laboratory professionals away from tasks that require critical thinking that they were trained to do and are relied upon to perform.

Today, there are automated and semi-automated slide makers and slide maker/stainers on the market that can ease the burden on the laboratorian while providing consistency in the smear preparation process.

Semi-automated smear preparation units are designed to provide an improved method of preparing peripheral blood films using the push or wedge technique. They can relieve laboratorians of some of the labor burden, but not all. These units tend to be user-friendly and require very little maintenance. What they are unable to do is self-adjust based on the sample viscosity, creating the possibility that the smear length and thickness may still be inconsistent.

Automated slide makers/stainers with closed tube sample processing provide hands-free, walk-away smear preparation and staining. Automated units drive the consistency needed to ensure uniformly made smears meeting quality, safety and turnaround time requirements. In addition, with direct-to-the-slide printing and barcode reading capabilities, automated smear preparation units are able to imprint the patient's sample ID and other interfaced demographics directly on the frosted end of the glass slide. This ensures positive patient identification and reduces the chance of transcription and tube mismatch errors that may occur with the manual methods.

Once the slide is identified, the sample is mixed and aspirated. The mixing is consistent from sample to sample, resulting in a uniform cell suspension each time. A drop of blood is then added to the glass slide. Wedge prep/push smear technology is incorporated into the automated smear preparation units so that the smear covers approximately two-thirds of the glass slide, ending in that desired feathered edge.

A step further

Some automated slide makers/stainers take the wedge prep smearing process a step further. With patented technology,

Parameters	Smear Conditions Summary			
	Increase Parameter		Decrease Parameter	
	Smear Length	Smear Thickness	Smear Length	Smear Thickness
[SAMPLE VOLUME]	LONGER	THICKER	SHORTER	THINNER
[ANGLE]	SHORTER	THICKER	LONGER	THINNER
[SPEED]	SHORTER	THICKER	LONGER	THINNER
[SMEAR START POSITION]	LONGER	THICKER	SHORTER	THINNER

Table 1.

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Hybrid power in laboratory instrumentation

By Jennifer L. Schwedler, PhD, and David A. Basiji, PhD

Ongoing pressure to reduce healthcare costs, combined with a shortage of qualified histotechnicians, is driving testing labs to increase slide staining throughput and efficiency. Enhancing throughput is a multifactorial problem that weighs the tradeoffs among process speed, process reliability, and available equipment and labor, all with an overarching constraint that staining quality must be upheld. In the current challenging reimbursement climate it is essential that equipment manufacturers continually develop higher-throughput automated staining systems to improve diagnostics and, ultimately, patient care.

The first wave of fully automated slide stainers revolutionized the way in which clinical and research pathology labs organized their workflow, made staffing decisions, and reduced their sample turnaround time to meet increasing workloads. Automated staining instruments showcased the value of online deparaffinization and heat-induced epitope retrieval (HIER), leading to enhanced staining consistency in less time and with less labor. However, the first-wave instruments imposed a ceiling on the number of slides, typically 30, that could be processed in parallel even if more slides could be loaded into the machine.[1,2]

Once initial workflow improvements were realized with full automation, the parallel processing ceiling forced labs to deploy more instruments as a means of further increasing laboratory throughput. Labs without the space or budget for additional instruments could still increase overall throughput by performing deparaffinization and HIER of large numbers of slides offline. However, this strategy increased the labor burden and could fragment workflow due to the mismatch between high-capacity offline steps and lower-capacity stainers. Increasing numbers of labs, particularly those specializing in gastrointestinal, dermatological, and breast samples, require large-scale batching capacity to keep up with their constantly growing workload. As a result, full automation is no longer sufficient in and of itself. It is also imperative that manufacturers increase the number of slides that can be automatically processed in parallel.

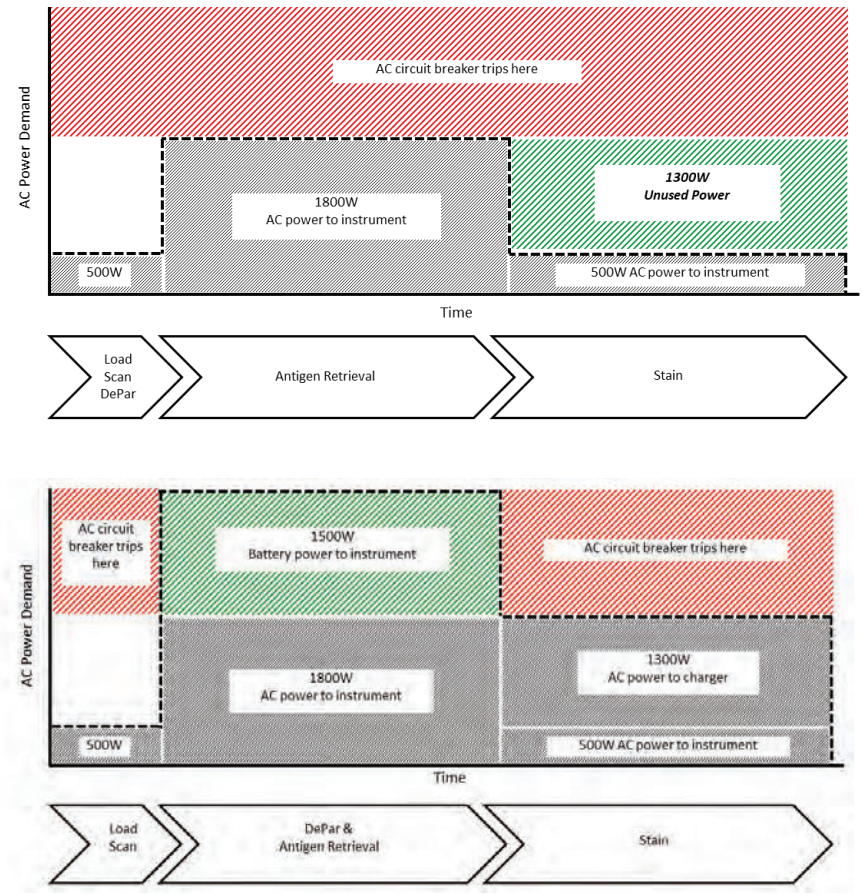

Figure 1. Breaking the 30 slide HIER barrier with battery hybrid power. Following the power-intensive HIER phase of slide processing, excess power is available but unused in most instrument designs (top). In a stainer incorporating a battery and inverter/charger, the battery's stored power is combined with AC wall power during HIER. During low power operation following antigen retrieval, the excess power available from the AC circuit is used to recharge the battery completely before the end of the run (bottom). Using this strategy, 48 slides can be processed in parallel, a 60 percent increase over the typical 30 slide parallel processing limitation.

The origin of the 30-slide parallel processing ceiling can ultimately be traced to the use of under-slide heaters for HIER. The poor thermal conductivity of the microscope slide itself, combined with the need to rapidly heat and hold temperature during HIER, necessitates the use of relatively powerful heaters that can exceed the power available from standard electrical circuits when more than 30 slides are in HIER at the same time. Under-slide heating also makes it difficult to determine the antigen retrieval (AR) solution temperature at the tissue level, limits the volume of AR solution (which leads to evaporation issues), and makes the heaters vulnerable to corrosion and failure due to the hostile under-slide environment.

Over time, manufacturers have minimized some of the issues associated with under-slide heating. For instance, evaporation can be controlled via the use of individual plastic cover tiles or liquid cover slips. Heater reliability has also been improved over time with better heater sealing techniques and improved fluid management. However, the fundamental issues of high power consumption and poor thermal control due to indirect tissue heating persist.

In order to achieve the goal of staining more than 30 slides in parallel and more slides per day, it is necessary to increase the amount of power available for the heating of AR solution from a standard

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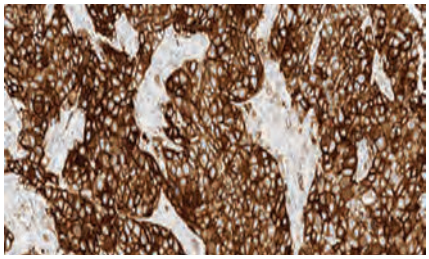

VENTANA PD-L1 (SP263) Assay staining in urothelial carcinoma with membranous and cytoplasmic staining of the tumor cells, and with immune cell staining within the stroma.

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some units can incorporate the hematocrit results from the integrated hematology analyzer(s) and apply fine-tune adjustments to each smear. With the hematocrit results driving the behavior of the slide maker, the unit adjusts the speed and the angle at which to push. Each smear produced is of uniform length, width and thickness.

Customizable stain times, another feature of automated slide maker/stainers, allow for the laboratory to achieve the desired coloration not only for whole blood smears but also for body fluid or bone marrow smears. The samples may be loaded while the laboratorian performs other assays. Upon return, completed labeled stained smears are ready for review, thus increasing laboratory efficiencies.

Digital imaging and more

Incorporating digital imaging is the last step to fully automating the slide making and staining process and features cell location and pre-classification. Cell image analyzers provide automation of manual white blood cell differential counts through automatic cell location. Merging results from multiple slides allows differential reporting on the lowest of white blood cell counts, virtually eliminating the need to perform buffy coat analysis. Red blood cell pre-characterization based on the laboratory's established review criteria and platelet estimate capabilities all drive tech-to-tech consistency while aiding in consistent reporting among staff on even challenging morphologic cases.

Body fluid software found on cell image analyzers is the last piece of the puzzle in automating the smear, stain, and review process in your laboratory. Analyzing cyto-spin smears, the cell image analyzer can automatically perform pre-classification of nucleated cells and captures a digital image of the entire sample area. The laboratorian may also tag areas of interest for follow-up by the pathologist or for collaboration, education, and training.

Remote review stations provide not only a more ergonomic workspace but flexibility for lab staff and for pathologists. Abnormal cells can be reviewed from any networked computer licensed with remote review software, allowing more frequent interaction between the laboratorian and pathologist. This increased collaboration opportunity may lead to faster interpretation and quicker result reporting, enabling the clinician to move forward with diagnosis and treatment.

The industry has come a long way in automating one of the most time-consuming tasks in the laboratory. It is no longer necessary for highly skilled and trained laboratory professionals to stand over the slide prep bench. Staff can now spend more time on the difficult cases that require careful analysis and assessment. Together, automated slide making and staining integrated with automated cell image analysis can enhance the level of service a laboratory provides its clinicians and patients.

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wall outlet. Without costly and sometimes unavailable high-power dedicated circuits, the maximum power available to an instrument is typically ~1800 watts, comparable to that of a hair dryer.

One strategy for increasing available HIER power relies on a common characteristic of most staining protocols for formalin fixed paraffin embedded (FFPE) tissues. The power-intensive HIER phase of FFPE tissue staining constitutes less than one-half the total slide processing time. Later protocol phases tend to require much less power since the slides are stained at room temperature. A high-capacity energy storage device (e.g., a lithium ion battery) can be used to boost the power available during the HIER phase, after which the storage device is recharged during the remainder of the staining protocol when excess power is available from the wall circuit. Such a strategy relies on a specialized device called an inverter/charger that converts the battery's direct current (DC) power to AC power and combines it with AC power from the wall circuit, thereby increasing the power available from ~1800W to over 3000W. Though inverter/chargers are not generally employed in biomedical or other instrumentation, they are well-proven and widely used in high reliability grid-tied and battery-backed power installations that rely on transient charging via solar panels and/or generators. Using such a strategy with a modestly-sized lithium battery, parallel slide processing capacity can be increased from 30 slides to 48 slides, a 60 percent increase (**Figure 1**).

The advent of fully automated stainers transformed the workflow of large and small labs alike and made automated immunohistochemistry, immunofluorescence, and *in situ* hybridization routine. Such developments have allowed labs to adopt more staining techniques and produce more consistent results with limited staff, thereby saving time and cost while improving patient care. As medicine and molecular diagnostics become increasingly more sophisticated, the throughput and performance of slide staining instruments will become critical. Hybrid power systems incorporated into the next generation of instruments will therefore become an important enabler for laboratories going forward.

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U.S. regulatory clearance for clinical flow cytometry is a breakthrough for leukemia and lymphoma patients

By Jeannine Holden, MD, MBA

By the time the FDA convened a Public Workshop on Clinical Flow Cytometry in Hematologic Malignancies[1] in February 2013, flow cytometry was already a well-established means of evaluating patients with known or suspected leukemia or lymphoma. Despite the lack of any FDA-cleared assays and the consequent requirement for laboratory-developed tests (LDTs), the rapid turnaround times and highly detailed and reproducible results produced by flow cytometry had driven widespread adoption by both clinicians and laboratorians,[2] beginning in the late 1980s. So why was the FDA holding a public workshop? What issues did the agency and other stakeholders find to be so urgent as to merit this effort?

Powerful but problematic

Flow cytometric immunophenotyping was first developed by immunologists in the late 1960s and early 1970s as a research tool that enabled their study of the immune system. Had the AIDS epidemic not required the urgent adoption of this technology by clinical laboratories in order to monitor patients' CD4-positive T helper cell counts in the mid-1980s, flow cytometry might never have been widely adopted for the assessment of leukemias and lymphomas. Once the technique was available to laboratorians, however, its potential utility was apparent. Initially working with reagents labeled for research use only (RUO), immunologists and hematopathologists in laboratories all over the world independently developed in-house assays and expertise that they shared via professional organizations. Vendors that had previously supplied the research market rapidly moved to provide easier-to-use reagents, cytometers capable of analyzing more parameters simultaneously, and more intuitive analysis software suitable for the clinical market.

In 1997, the FDA created a new regulatory category: Analyte Specific Reagents (ASRs).[3] Creation of the category was driven by the marked increase in clinical demand for high- complexity LDTs, particularly flow cytometric immunophenotyping and molecular diagnostics: RUO-labeled reagents were of uncertain quality and vendors could not bring IVDs to market quickly enough to meet the demand.

ASR labeling assured laboratories of consistent reagent quality and addressed concerns of patients and insurance companies over the use of RUO-labeled reagents, but also resulted in some unintended consequences as the result of two specific requirements of the labeling: vendors could not market ASR combinations, nor could they provide information that might assist laboratories in combining ASRs. Given that flow cytometric immunophenotyping's power relies specifically on the simultaneous multiparameter assessment of thousands of individual cells, these requirements presented a challenge to both laboratories struggling to design their own individual LDTs and to vendors possessed of abundant expertise but prevented from sharing it.

The FDA's position was understandable: high-complexity LDTs could only be performed by laboratories with sufficient expertise to develop and validate their own assays. If vendors assumed any of this burden, then the laboratory did not necessarily have the required expertise.

Guidelines and consensus

Recognizing the need for guidance in the absence of any FDA-cleared *in vitro* diagnostic (IVD) assays, expert panels published various guidelines and expert recommendations. The 2006 Bethesda International Consensus Recommendations on the Flow Cytometric Immunophenotypic Analysis of Hematolymphoid Neoplasia established a core set of antibody specificities but failed to reach consensus as to panel design—that is, which antibodies to combine together in which tubes; the many independently-developed LDTs could not be easily reconciled into a single assay.[4] Bethesda also established the clinical indications that warranted flow cytometric immunophenotyping for suspected hematologic malignancies.[5]

The 2013 ICSH/ICCS Practice Guidelines for Cell-based Fluorescence Assays reinforced Bethesda recommendations and described the fundamental differences in assay performance criteria between quasi-quantitative assays such as lymphocyte subset and stem cell enumeration assays (for which FDA-cleared IVDs existed) and qualitative assays such as leukemia/lymphoma.[6] These differences meant that the former could not serve as predicates for the latter.

2013 FDA Public Workshop

The stakeholders who participated in the Public Workshop on Clinical Flow Cytometry in Hematologic Malignancies included the FDA itself, laboratorians, and vendors. The issues highlighted included the increasing complexity of the LDTs in use by that time, many of which used eight- and 10-color flow cytometry (as opposed to the three- and four-color platforms in use when the FDA first established ASR labeling), as well as the inherent drawbacks of LDTs: labor-intense and error-prone manual workflows, lack of standardization, and wasteful duplication of effort among laboratories. The FDA subsequently summarized many of these concerns in its October 2014 Draft Guidance for Industry, Food and Drug Administration Staff, and Clinical Laboratories: Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs).[7]

An FDA-cleared IVD for leukemia/lymphoma

As a result of the 2013 workshop, the FDA engaged directly with flow cytometry vendors to discuss the possibility of developing an IVD test. On June 29, 2017, the FDA announced[8] approval via the de novo premarket review pathway of the first agency-authorized test for use with flow cytometry to aid in the detection of several leukemias and lymphomas. Alberto Gutierrez, PhD, Director of the Office of In Vitro Diagnostics and Radiological Health in the FDA's Center for Devices and Radiological Health, described this test as "…a major step forward for the hematology-oncology community. Laboratories and healthcare professionals now have access to an FDA-validated test that provides consistent results to aid in the diagnoses of these serious cancers."

Education within product labeling

Flow cytometric immunophenotyping for leukemia and lymphoma relies on pattern recognition by trained professionals. The FDA asked the vendor to include in its product labeling

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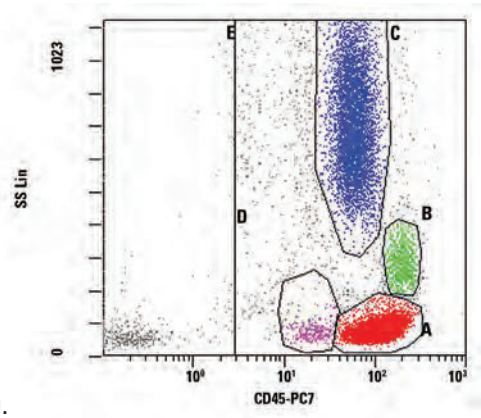

Figure 1.

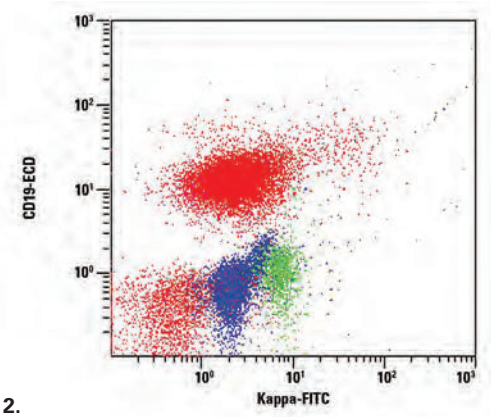

Figure 2.

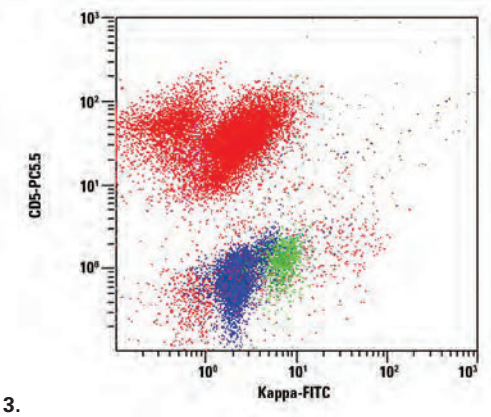

Figure 3.

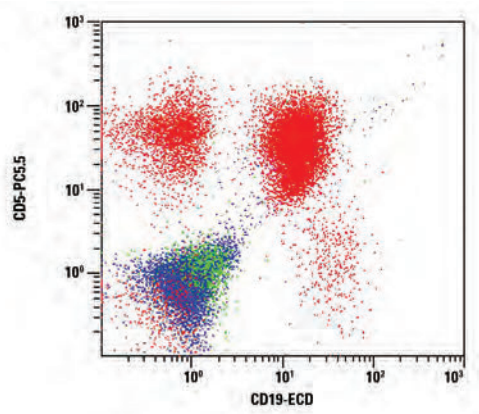

Figure 4.

Clinical vignette: chronic lymphocytic leukemia/small lymphocytic lymphoma

A 64-year-old male presents with lymphocytosis. A peripheral blood sample is submitted for flow cytometric immunophenotyping using 5 reagent tubes: CD2-FITC/CD56-PE/CD7 ECD/CD5-PC5.5/CD45-PC7.

The results were as follows: Flow cytometric immunophenotyping identifies a phenotypically distinct population of cells with low light scatter properties that express CD19, low density CD20, CD5, and CD45 and display Kappa immunoglobulin light chain restriction. CD38 expression is absent.

Taken together, the findings in this case are most consistent with chronic lymphocytic leukemia/small lymphocytic lymphoma. Note that correlation with clinical and laboratory data is recommended, and that additional immunophenotyping may be warranted.

The figures show examples of annotated dot plots

In **Figure 1**, the dot plot shows the characteristic distribution of the lymphocytes in red, the monocytes in green and the granulocytes in blue. This CD45 vs. Side Scatter dot plot is ungated and shows all events collected. Gate E includes all CD45 positive events and may be used to set a stop count gate during acquisition in order to ensure that sufficient non-debris events are collected. While Gate E may also be used to exclude CD45 negative debris from the analysis, these events should not be ignored when analyzing a case, as some aberrant populations are CD45 negative.

This dot plot permits distinction of the usual populations found in peripheral blood, bone marrow, and lymph node samples, including lymphocytes (Gate A, red), monocytes (Gate B, green), and granulocytes (Gate C, blue). Gate D (pink) is shown here in the area typically occupied by myeloblasts, but may be used to highlight other populations. By applying different colors to the events comprised by each gate, the various populations may be followed throughout the analysis. Gates should be adjusted by the analyst to conform to the naturally occurring separations among the populations, but where no separation is observed an estimate based on experience should be used.

In **Figure 2**, a Kappa vs. CD19 dot plot is gated on E and shows all CD45 positive events. The prominent CD19 positive population expresses low density Kappa immunoglobulin light chains. A small polyclonal population displaying slightly higher density CD19 is also present. Both populations are red.

In **Figure 3**, a Kappa vs. CD5 dot plot is gated on E and shows all CD45 positive events. A distinct Kappa/CD5 dual positive population is noted. The CD5 positive/Kappa negative population represents T lymphocytes.

In **Figure 4**, a CD19 vs. CD5 dot plot is gated on E and shows all CD45 positive events. The CD5 positive/CD19 negative population represents T lymphocytes. The CD19 positive/CD5 negative population represents normal B lymphocytes. The aberrant CD19/ CD5 dual positive population is consistent with the remainder of the analysis.

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a tool that would familiarize users with the expected staining patterns generated by these particular combinations of reagents. The resulting casebook includes 16 illustrative case studies with characteristic findings typical of various lymphoid and myeloid neoplasms as well as cases from patients with clinical and/or laboratory findings that suggest an underlying neoplastic process, but where no immunophenotypic abnormality is identified. Specimen types include peripheral blood, bone marrow, and lymph nodes. Casebook representative cases were selected from clinical trial data and were reviewed, annotated, and interpreted by the author.

Each case includes a clinical vignette that describes the patient demographics and clinical history, case-specific listmode data files for reanalysis by the user of the casebook, specific analysis protocols to be used with the listmode data, and a report showing the analysis with provided protocols. See **Figures 1** through **4** for examples of annotated dot plots (of which there are 60 for each case). Each case concludes with a summary that highlights the immunophenotypic findings as well as potential pitfalls. By independently analyzing the downloadable listmode files, users can further reinforce their pattern recognition skills.

Enhancing patient care

The development of the first preformulated IVD antibody cocktails for use in the clinical lab is a direct result of concerns highlighted by the FDA's 2013 Public Workshop; and their official statements have confirmed its significance for both the hematology-oncology community and patients.

Estimates from the U.S. Leukemia and Lymphoma Society show that approximately every three minutes one person in the U.S. is diagnosed with a blood cancer, and almost 143,000 people are expected to be diagnosed with leukemia and lymphoma alone in 2017. The availability of this new *in vitro* leukemia and lymphoma (non-Hodgkin's only) test is a major step forward for those suffering from these serious cancers.

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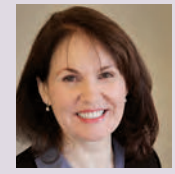

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Benefits of an instrument-compatible capillary blood collection microtube

By Dima Fouad Yassin, MT (Bsc), CPHQ, and Mousa A. Al-Abbadi, MD, FCAP, CPE, CPHQ, FIAC

Sheikh Khalifa Medical City (SKMC) provides a network of comprehensive healthcare services for the Island of Abu Dhabi, United Arab Emirates, which include acute care facilities as well as tertiary surgical and medical services. The laboratory processes 600 hematology specimens per day with 50 pediatric specimens.

At SKMC, pediatric specimens are currently collected into a capillary blood collection microtube, obtained mainly from infants and newborns. In adults, specimens are collected in capillary tubes when venous collection is difficult. The accessioning, labeling, and testing of specimens collected in capillary tubes pose many challenges due to the small size of containers. The inability to properly affix a full-sized barcode label at the bedside can cause specimen labeling errors. The specimens might arrive in the hematology laboratory without a label or even mislabeled. Often, the barcode label is wrapped around the tube, which makes it difficult to read the patient's information. Specimens with missing or illegible patient information are rejected and have to be re-collected, which contributes to additional work for healthcare personnel and subsequent delays in testing. Typically, there are restrictions on the amount of blood collected from these patients, and thus collection of a second specimen is problematic. In the context of these issues, hospitals and laboratory leadership try to find ways to improve service and workflow efficiency.

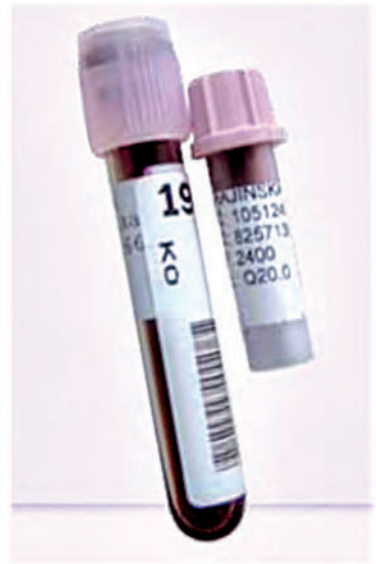

Figure 1. Present equipment

The capillary blood collection microtube with K2EDTA is used for the collection, transport, storage, and automated processing of capillary blood specimens for hematology testing. The instrument-compatible microtube has the same outer dimensions as a venous collection tube (13 x 75 mm) with a false bottom, which facilitates the collection of small volumes (maximum of 500 µL) of blood and the application of a full barcode specimen label. The tube has a pierceable cap, which allows it to be processed in the automated mode on hematology analyzers, thereby, minimizing laboratorians' hands-on time in processing capillary specimens.

Sheikh Khalifa Medical City Hematology Laboratory Profile
Test Volume:
• 600 hematology specimens per day
• 50 hematology microcollection specimens per day
• Hours of operation: 24 hours a day, 7 days a week

Hematology Staff
20 staff members, two shifts
Often, off-shifts may only have two laboratorians in the hematology department

Table 1

Conversion from the current equipment to the new microtube may enable the hematology lab to further improve efficiency and potentially reduce specimen labeling errors. Depending on lab specifics, conversion may result in significant time savings.

Facility analysis

Table 1 shows the SKMC hematology laboratory profile. This analysis was performed to evaluate the current microcollection process performed with the present equipment (**Figure 1**) and to determine if conversion to the new microtube (**Figure 2**) may aid in improving workflow. Methodically mapping the current procedure enabled identification of areas of waste that negatively impacted the process. Then, the processing was streamlined without compromising the essential collection steps or specimen quality.

Current workflow processes

Key findings: routing of specimens. The majority of specimens are received from the Pediatric Intensive Care Unit (PICU) between 3 am and 4 am, with additional specimens arriving throughout the day. During this period, these specimens are received in a batch of 10 to 15 tubes.

Current labeling issues. When specimens are received in reception, they are checked for labeling. If the tube is not labeled or is mislabeled, the specimen is rejected immediately and the nurse is then contacted to re-collect the specimen.

Presence of microclots. Tubes are inverted and checked for microclots manually, one by one. If a clot is present, a marker note is made on the label and the nurse is contacted to re-collect the specimen. The clot checking process includes the following steps:

- The cap is removed.
- Wooden sticks are used to check for clots.
- If clotted, the tube is recapped and is rejected, and the nurse is contacted to re-collect.
- If not clotted, the tube is recapped and is placed in the instrument running rack.

Specimen storage. After processing, the capillary tubes are stored in a box; the venous tubes are stored in tube racks. Retrieval of specimens from a box is more time-consuming than when the tubes are placed on a rack.

Specimen collection process flow. Hands-on (manual processing) capillary tubes (**Table 2**):

- Specimens are received.
- Tubes are uncapped.
- Tubes are checked for clots.
- Tubes are recapped.
- The instrument is set on manual mode.
- The specimen ID number on the barcode label on each tube is manually scanned using the instrument barcode wand or is manually entered into the instrument.
- Blood specimens in each capillary tube are manually aspirated.
- The tube is then placed on a rack for result verification.

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Day	# of capillary tubes in a batch	Hands-on time for processing capillary tubes in the batch (secs)	# of instrument-compatible microtubes in a batch	Hands-on time for processing instrument-compatible microtube in the batch	% time savings
1	9	594	0	N/A	
2	15	746	15	190	
3	20	1386	20	263	
	Total tubes: 44	Average time: 61.95 secs/tube	Total tubes: 35	Average time: 12.94 secs/tube	80%

Table 2. Hands-on time required for processing each batch of tubes

Hands-on (automated processing) instrument-compatible microtube:
- Specimens are received.
- Tubes are uncapped.
- Tubes are checked for clots.
- Tubes are recapped.
- Tubes are placed on rack.
- Labels are aligned/specimens are processed in the automated mode on the analyzer.

The hands-on time required for laboratorians to process the two tubes was evaluated on three separate days (**Table 2**).

On Day 1, a batch of nine capillary tubes was processed (two specimens were rejected due to clotting, seven specimens were analyzed); hands-on time required was recorded. The hands-on time included the time required to check all nine tubes for clots and for the manual processing of seven tubes on the hematology analyzer.

On Day 2, a batch of 15 capillary tubes and 15 instrument-compatible microtubes was processed, and on Day 3, a batch of 20 capillary tubes and 20 instrument-compatible tubes was processed. Hands-on time for processing each batch was recorded for each day.

Results

The hands-on time required to process each batch of tubes is presented in **Table 2**. When processing a batch of capillary tubes, the laboratorian first checked each tube in the batch for microclots. If clots were observed, the tube was discarded and not processed further. If no clots were observed, each tube was sequentially manually processed. The hands-on time was calculated for each batch of tubes using a stopwatch. The stopwatch was started from the time the staff member checked the first tube for clots until all tubes were manually processed on the hematology analyzer.

Figure 2. New microtube

When processing a batch of the instrument-compatible microtubes, the laboratorian first checked each tube for microclots and placed the tubes in an instrument rack. The rack was then placed on the instrument for automated processing. The hands-on time was calculated for each batch of tubes using a stopwatch. The stopwatch was started from the time the laboratorian checked the first tube for clots until all tubes were checked for clots, placed the tubes on the instrument tube rack, and then placed the rack on the analyzer for automated processing.

This analysis showed that a significant amount of laboratorians' hands-on time is saved when a batch of the instrument-compatible microtube is processed compared with a same sized batch of capillary tubes—an average savings of 49 seconds per tube or an average savings of eight minutes per batch of 10 tubes.

When processing current microtube specimens in a batch, a considerable amount of laboratorians' time is spent waiting for the instrument to complete specimen analysis on a tube and to reset, before the second tube can be manually aspirated. This time savings is especially impactful during peak hours when large numbers of specimens are received in the laboratory and there is only one laboratorian in the laboratory. Less hands-on time spent by the laboratorian processing a microtube specimen in the instrument-compatible microtube allows him or her to focus on other technical aspects, such as performing manual differential counts or other value-added work.

Conclusion

Converting to the instrument-compatible microtube can potentially offer the following benefits for the laboratory:
- An 80 percent reduction in the laboratorian's hands-on time, enabling the technical staff to focus on other value-added tasks.
- Reduced specimen identification errors, since a full-sized label can be placed on the tube.
- Establishment of a simplified and uniform process for all received specimens without workflow interruptions for manual processing.
- Improved laboratory efficiency and turnaround time.
- Better storage (tube rack versus box) and retrieval of processed specimens, facilitated by the 13 x 75 mm tube configuration.
- Smoother transition between shifts, since specimen processing can be completed more rapidly and less work may remain for the next shift personnel.

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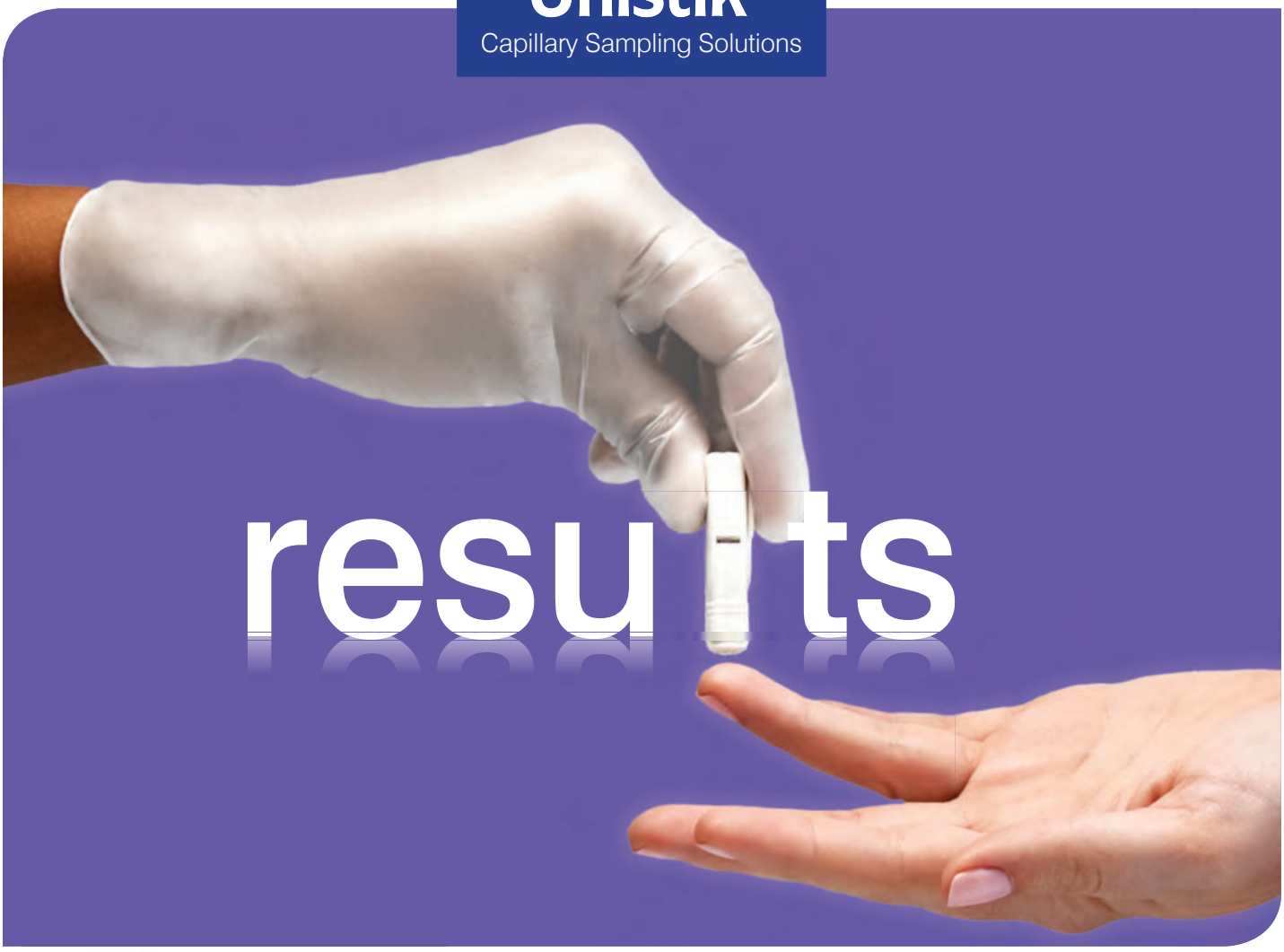

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Culture collections serve invaluable functions

By Robin E. Stombler

The names of infectious diseases that threaten the health of people worldwide have become part of the common vocabulary: Zika, MRSA (Methicillin-resistant *Staphylococcus aureus*), *Salmonella*, *N. gonorrhoeae*, *E. coli* O157:H7. New threats emerge regularly, and priority rankings shift. Behind these names are precious materials that commonly support the authentication and verification of these pathogens. These commodities are known as culture collections.

Culture collections, on the surface, do what their name implies: they collect microorganism cultures. These collections are living repositories that, when properly maintained, can provide an invaluable service to education, research, clinical, food, environmental, and industrial applications. Yet ensuring the availability, accessibility, and affordability of the biological reference materials held in these collections can be challenging, which can potentially impede global health solutions.

Culture collection demographics

The World Federation of Culture Collections (WFCC) developed an international database on biological reference materials globally. This World Data Center for Microorganisms, maintained at the National Institute of Genetics in Japan, states that it holds data on the organization, management, and services of 476 culture collections from 62 countries. The WFCC produces guidelines on the optimal operation of a culture collection.

In the United States, culture collections may be held by federal and state government agencies, such as the Centers for Disease Control and Prevention (CDC) and state health departments. Universities may also maintain libraries of biological materials for their research. Independent organizations, such as the American Type Culture Collection, serve as well-known repositories of tens of thousands of cultures.

Collections vary in size and content. Some may contain varieties of bacteria, fungi, yeast, viruses, and protozoa, while others focus on a specific area of scientific interest (e.g., Department of Insect Pathology in the Czech Republic). Still others may collect pathogens of immediate regional interest, as when a new pathogen emerges in one corner of the world and threatens the health of those inhabitants.

Culture collections may be certified to international standards for quality management systems (for example, ISO 9001). They may also be accredited for laboratory testing (as with ISO 17025) or for competence as a reference material provider (e.g., ISO Guide 34). Certification and accreditation are not mandatory.

The need for reference material availability

Biological materials from culture collections are used to ensure the authenticity, validity, and relevance of laboratory testing. Whether a laboratory needs to confirm the presence of *Vibrio parahaemolyticus* in seafood or test drug resistance to *Mycobacterium tuberculosis* in a compromised patient, it is imperative for the testing material to be reliable and accurate. Public Health England, which is the custodian of four culture collections, posts: "Authenticated reference strains are of paramount importance for clinical diagnostic testing, food, water, and environmental microbiology testing, and validation studies."

Yet, despite the need, these reference materials are not always available. Take, for example, a federal policy directive issued in September 2011. The USDA Food Safety and Inspection Service announced it would implement routine verification testing for six Shiga toxin-producing *E. coli* (STEC), in addition to the more prevalent *E. coli* O157:H7, in raw beef manufacturing trimmings. The directive stated that on June 4, 2012, raw, non-intact beef products or their components containing the STEC strains would be considered adulterated.

To perform the testing, test methods needed validation. To conduct method validation, food laboratories required qualitative and quantitative quality controls. Samples to assess the proficiency—or accuracy—of the test providers were also necessary. Yet, culture collections either did not have all the various strains or they did not have the rights to distribute them in time to meet the directive. In fact, it took more than a year from the announcement before the six STEC strains were available in convenient formats, enumerated derivatives, or certified reference materials.

The same concern surfaces in the clinical setting. Emerging microorganisms that create threats to public health require clinical laboratory testing. As demand for diagnoses increases around new strains, laboratories need to have proper test methods, quality controls, and proficiency testing. Culture collections must be able to respond.

More than a year ago, the U.S. Department of Defense, working in concert with the CDC, the Pennsylvania Department of Health, and local health departments, announced the discovery of the first *mcr-1* gene found in bacteria in a human in the United States. The *mcr-1* gene, which first emerged in China in 2015, makes bacteria resistant to colistin, which is a last-resort antibiotic used to treat patients with certain multi-drug-resistant infections. It has been discovered primarily in *E. coli*.[1] According to the CDC tracking, since the time of its discovery in the U.S., the *mcr-1* gene has been found in 16 additional states. Culture collections must be ready to share the isolate for testing, research, and development purposes.

Culture collections and health policy solutions

More than two years ago, the CDC, in collaboration with the Food and Drug Administration, launched an antimicrobial resistance isolate bank (AR Bank). This collection of microbial pathogens has set lofty goals: support development of diagnostic devices and antimicrobial drug products; advance diagnostic tests for the identification and characterization of resistant bacteria; and accelerate research and development of new antibiotics. The value is clear. With this precious resource, the agencies "will support earlier diagnoses and more effective treatment options that can slow antibiotic resistance."[2] Innovative approaches like this one deserve resource support and encouragement.

As federal directives and guidance are developed in response to a public health concern, more communication and advance collaboration with culture collections and the laboratory quality control and proficiency testing communities may assist in preparations. In support of the public health, culture collections should be accessible and responsive to appropriate recipients.

While there are hundreds of culture collections, it is possible that only one will hold a specific, rare strain. It is also possible that more than one will collect the same microorganisms. At times, a collection may refuse to distribute a strain for legal or financial reasons. From a policy perspective, it is important to recognize these constraints when addressing specific pathogens. Federal agencies should refrain from recommending only one source of distribution.

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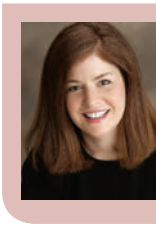

Robin E. Stombler is President of **Auburn Health Strategies, LLC**, a strategic and business development firm representing health and science organizations.

Molecular assay predicts *Neisseria gonorrhoeae* susceptibility

By Lao-Tzu Allan-Blitz and Jeffrey D. Klausner, MD, MPH

The emergence of untreatable *Neisseria gonorrhoeae* infections has caused great concern.[1-4] Untreated or inadequately treated *Neisseria gonorrhoeae* infection is associated with many health consequences including pelvic inflammatory disease and infertility, neonatal blindness,[5] and an increased risk of HIV transmission and acquisition.[6-8] And treatment with ceftriaxone may be a major driver of ceftriaxone resistance.[9]

Building on our previous discussion of multi-drug resistant gonorrhea and the potential utility of a laboratory-developed molecular assay to determine ciprofloxacin susceptibility [*MLO*. 2016;48(12):30], we here report on the implementation and outcomes, including costs and the frequency of clinical cure.

gyrA genotyping and targeted therapy

The use of antibiotics previously thought to be ineffective may slow the emergence of ceftriaxone resistant infections by alleviating the selective pressure.[10] The use of ciprofloxacin 500 mg orally as an alternative to a 250 mg ceftriaxone injection for the treatment of *Neisseria gonorrhoeae* infections has been made possible by the development of a rapid genotypic assay for the determination of mutation at codon 91 of the *gyrase* A (*gyr*A) gene of *Neisseria gonorrhoeae*; a non-mutated (wild-type) *gyr*A genotype reliably predicts full susceptibility to ciprofloxacin.[11]

In 2007, we developed a real-time polymerase chain reaction assay for *gyr*A genotyping remnant nucleic acid amplification DNA specimens,[12] which was verified in accordance with Clinical Laboratory Improvement Amendments.[13] In November 2015, that assay was implemented at the University of California Los Angeles for genotyping all remnant *Neisseria gonorrhoeae* positive nucleic acid specimens.[14] The results of the *gyr*A genotyping are available to clinicians within twenty-four hours and are reported in the patient's medical record.

At the University of California Los Angeles, the use of *gyr*A genotyping has decreased the use of ceftriaxone for the treatment of *Neisseria gonorrhoeae* infections from 94 percent prior to assay implementation to 78 percent after; there was also a concomitant increase in the use of targeted ciprofloxacin therapy.[14] Notably, the use of electronic reminder notification sent to providers with genotype results and treatment recommendations further augmented the use of ciprofloxacin targeted therapy, from three percent prior to reminder notifications to 18 percent after.[15]

Cost issues and other concerns

Given those results as well as the numerous potential benefits of *gyr*A genotyping and targeted therapy, further implementation of the assay in other health systems is warranted. An important consideration for future implementation, however, is the financial costs of *gyr*A genotype testing. A recent analysis of the direct costs of the *gyr*A genotyping program at the University of California Los Angeles noted that the costs vary by the prevalence of resistant infections, frequency of testing, and assay performance.[16] In settings where there is a high frequency of testing (an average of 17 tests per day), with a presumed ciprofloxacin resistance rate approaching 25 percent (the national estimate[17]), as well as a 30 percent rate of infections with indeterminate genotype results, the cost of *gyr*A genotyping with genotype-based targeted therapy was only $12.41 more expensive per case than recommended two-drug ceftriaxone and azithromycin therapy.[16] That cost difference may not be prohibitive given that there are other factors that must be considered.

For example, that analysis did not take into consideration the theoretical decrease in ceftriaxone-resistant infections expected with use of ciprofloxacin as an alternative regimen, nor did it take into account other potential benefits of oral therapy over injection therapy. Those benefits may include a reduction in the number of accidental needle stick injuries, an increase in the proportion of patients treated, and improved partner treatment.

One valid concern about the *gyr*A testing program is the lack of studies demonstrating effective treatment with ciprofloxacin among wild-type *Neisseria gonorrhoeae* infections. A prior study demonstrated ciprofloxacin to be 99 percent effective in treating phenotypically susceptible *Neisseria gonorrhoeae* infections,[18] but that was not done in conjunction with genotype analysis. A clinical trial is currently underway at the University of California Los Angeles, which is evaluating patient outcomes among those with wild-type *Neisseria gonorrhoeae* infections treated with ciprofloxacin 500 mg orally.[19] The preliminary test-of-cure data are unpublished, but are promising; between 7 and 21 days post treatment with ciprofloxacin 500 mg orally for wild-type *Neisseria gonorrhoeae* infections, 11 of 11 patients with repeat testing had a negative *Neisseria gonorrhoeae* nucleic acid amplification test result.

The view from here

In all, the use of *gyr*A genotyping to promote targeted oral ciprofloxacin therapy appears to be a promising strategy. The use of *gyr*A genotyping in conjunction with electronic reminder notifications successfully increased the proportion of patients treated with targeted oral ciprofloxacin therapy at the University of California Los Angeles. The costs of implementing the assay are considerable; however, in high frequency testing centers such as commercial laboratories, the costs of the assay may not be prohibitive given the other potential benefits. Finally, preliminary data on patient outcomes among those with wild-type *Neisseria gonorrhoeae* infection treated with ciprofloxacin are encouraging. Thus, *gyr*A genotyping for the promotion of targeted ciprofloxacin therapy is a step towards expanding our antimicrobial toolbox for treating an infection that is rapidly becoming untreatable.

Note: The print version excludes source references. Please visit *www.mlo-online.com*.

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Healthcare industry steps up security as cyber attacks increase

By Anil V. Parwani, MD, PhD, MBA, FASCP

Data breaches in the United States healthcare industry cost $6.2 billion each year. Over the past two years, roughly 90 percent of hospitals have reported a security breach.[1] Cyberattacks are on the rise. Between 2009 and 2013, the percentage of healthcare organizations that reported attacks rose from 20 percent to 40 percent.[2]

When a hospital experiences a cyberattack, the implications can be far-reaching, from impacting the hospital's finances and reputation to patient safety, availability of IT programs, and possible compromise of patient and employee information.[3] Accessing registration and demographic data can be used to steal patients' identity, financial data, credit cards, and social security numbers.

The worldwide WannaCry ransomware attack in May 2017, which targeted computers running the Microsoft Windows operating system, added a whole new perspective on the implications of a large-scale cyberattack. Although governments in the United Kingdom and the U.S. downplayed the effect that the ransomware attack had on patient care, the attack had a reverberating effect. Many doctors in the UK resorted to pen and paper for record-keeping, and some patients refrained from elective surgeries.

Expanding connectivity heightens risk

With expanding connectivity of information systems, laboratory work stations, and instruments to the Internet, the need to secure laboratory information is critical.

"A ransomware incident is a possibility in every hospital, clinic and outpatient facility," Paul H. Keckley, PhD, healthcare analyst, wrote.[4] "Preventing it is a high priority, and, if attacked, managing it quickly and efficiently is an absolute necessity to sustain patient care and protect the reputation of the organization."

Dr. Keckley suggests that hospitals encourage staff to follow measures to protect against ransomware and other cyber threats, such as:

- Regularly updating internet browsers, computer operating systems, and applications
- Using strong passwords
- Declining to open suspicious links or attachments
- Routinely backing up important files.

Protecting laboratory data is critical

The workflow in the pathology laboratory depends on the use of LIS, which acquires, generates, analyzes, stores, and manages electronic protected health information (ePHI). "Laboratories likely also store ePHI in software that run laboratory instruments and automation lines as well as in middleware such as auto-verification software," Ioan Cucoranu, et al, wrote. "Therefore, making sure that the data contained in laboratory software remain protected and secure at all times is critical to daily pathology practice. The same is true for interfaced devices such as chemistry analyzers that also store ePHI. Accordingly, security policies and procedures have to be in place and enforced in the laboratory."[5]

The U.S. Office of the National Coordinator (ONC) for Health Information Technology (HIT) suggested several steps are needed to perform a security risk analysis. They include reviewing current health information security, identifying vulnerabilities, minimizing security risks, and monitoring results.[5]

In the United States, the privacy and protection of medical information and health records is governed by the Health Insurance Portability and Accountability Act (HIPAA). The HIPAA Security Rule establishes national standards to protect individuals' electronic personal health information that is created, received, used, or maintained by a covered entity. The Security Rule requires appropriate administrative, physical, and technical safeguards to ensure the confidentiality, integrity, and security of electronic protected health information.

Symantec, an enterprise security vendor, believes the healthcare industry is prone to cyberattacks because it underfunds its cybersecurity investment. In comparison, the federal government spends 16 percent of its IT budget on security, and industries such as banking and finance spend 12 to 15 percent of their IT budget on security programs.[6]

More training for end users

Adding to the risk is the fact that healthcare companies encourage medical staff to use their own tablets, smartphones, and laptops at work. In one survey, 81 percent of healthcare providers indicated they allow their medical staff to use their own iPads and other mobile devices. Yet 46 percent of those companies said they had done nothing to secure the mobile devices.[2]

One of the reasons that cyberattacks are on the rise is the strong demand for patients' medical records in the black market. Electronic health records (EHR) have greater value than financial data, and can bring in $50 in the black market. In comparison, a stolen Social Security number or credit card number can bring in $1.[2] The wealth of data on EHRs—names of patients, birth dates, policy numbers, diagnosis codes, and billing information—can be used in myriad ways, such as buying medical equipment or medications to resell. Another scheme is to file false claims with medical insurers, using a patient number with a false provider. And, in an alarming trend, cyber criminals have discovered it is more profitable to ransom a hospital's data than to steal it.

Many security issues can be minimized by educating hospital personnel. A 2015 study by Wombat Security Technologies and the Aberdeen Group determined that employee training on cyber security can reduce the risk of a cyberattack from 70 to 45 percent.[6]

That study emphasizes that not enough companies pay attention to the greatest security threat—the end users. Although investing in IT security technologies can help minimize the threat of data theft and ransomware, healthcare systems should train their staff to be more cognizant of cyberattacks.

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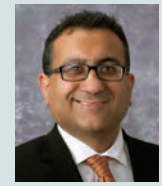

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The new PAMA CLFS

By Rodney W. Forsman, BS, Tim Murray, MS, MT(ASCP), and Paul Keoppel, MBA, MT(ASCP)

Medicare payments for clinical laboratory services have long been a target for cost savings. The Medicare Clinical Laboratory Fee Schedule (CLFS) was introduced in 1984 and was based on a percentage of the median of test prices surveyed in 1982. The Medicare SGR (Sustainable Growth Rate) legislation was enacted in 1997 with the intent to reduce Medicare reimbursements annually. Successful lobbying by physician groups halted reductions in physician fee levels and replaced them with increases. The SGR law mandated overall reduction and, in order to pay for the "doc fix," savings needed to be found elsewhere. The clinical laboratory represents only 1.6 percent of Medicare spending but has routinely been levied spending cuts to fund the SGR disparity. The Accountable Care Act (ACA), sequestration and the Middle Class Tax Relief & Job Creation Act of 2012 combined to produce a vastly disproportionate reduction in laboratory reimbursement compared to other providers. For the first time in 30 years, CMS invoked its presumed authority to call for the repricing of all tests based on new technology. Certain laboratory associations negotiated for repeal of this approach and claimed success when it was replaced by section 216 of the Patient Access and Medicare Protection Act (PAMA) in 2014. This statute called for a market based CLFS, and the rules that followed established a weighted median of individual private payor test reimbursements reported by "Applicable Laboratories."

The narrow definition of an Applicable Laboratory excludes hospital laboratories but includes 45 percent of all commercial and five percent of physician office laboratories. This results in data heavily weighted by discounted pricing by large commercial laboratories to major payors. The presumption is that the product of these calculations would yield market-based prices significantly lower than the current CLFS. Beginning in January 2018, existing prices would be lowered 10 percent each year for the first three years and 15 percent for the next three years or until the established weighted median price is reached. This could result in a 55 percent drop in payment in six years. A $3.91 billion savings to the Medicare program is projected in the first five years. These fees will be applied to all who are paid on the CLFS and will likely extend to private payors who pay using a function of the CLFS.

The Office of the Inspector General (OIG) has released the September 2017 analysis of Medicare payments for the clinical laboratory. It shows that reimbursements dropped $200 million in 2016 compared to both of 2014 and 2015. This, combined with the projected decrease of $670 million in 2018, will produce a reduction of $870 million. These changes far exceed the goals anticipated when PAMA was enacted.

The new fee schedule

The proposed 2018 CLFS was published on September 22, 2017, with a comment period ending October 23, 2017. The following analysis reveals major concerns and inconsistencies:

- CMS calculations would have resulted in an actual decrease in payment in 2018 of 21.9 percent if not for the 10 percent limit of decrease the first year. The decreases can be a bit deceiving. Almost all of the top 25 volume tests by revenue are decreasing by 10 percent. This will have a larger impact on laboratories than will lower-volume, high-priced tests going down by 10 percent.
- 1,942 labs reported with over 4.9 million lines of records. The OIG 2015 payment review states that there are 61,040 laboratories paid on the CMS CLFS, which means that only 3.2 percent reported data.
  - Only 21 hospital labs reported, resulting in only 1.0 percent of data submitted.
  - 1,106 physician office laboratories (POLs) were 7.5 percent of the data.
  - Only 36 labs were situated in rural areas.
- There are 1,360 codes listed.
  - 75 percent of the codes will have a fee decrease. 58 percent of those have more than a 10 percent decrease and will be phased in over the next six years.
  - 53 are going down by 50 percent or more, 826 are going down between 10 percent and 49 percent, 115 are going down between 0 and 10 percent, and 134 codes have an increased fee.
  - 232 codes have no National Limitation Amount (NLA). Fourty-four of those have an NLA of $0.00. **Any code that does not have an NLA is going directly to the full decrease the first year.** Two examples: 1) Lipid profile 80061 is a top 25 test and the average 2017 fee is $17.86. The annual phased-in fees would have been $16.07, $14.46, $13.02, and, in 2021, $11.23. Because it has no NLA ,the price is dropping to $11.23 in 2018 for a decrease of 37 percent; and 2) The acute hepatitis panel 80074 had an average CLFS fee in 2017 of $64.04 but will drop to $38.74 in 2018, for a 40 percent decrease.
- The drug screen codes are missing market prices because the codes have changed since the data reporting period and several are on the top 25 test list.
- The G0480 definitive drug code goes from $117.65 to $47.96.
- Some of the data is obviously wrong. Code 81341 has a submitted minimum price of $0.01.
- The biggest price drop is for 81435, which goes from $801.33 to $37.99 over the phase-in period.
- There are 93,728 lines submitted for CBC, and 1,536 labs reported a price of less than $0.25! On the other hand, there are 813 laboratories that reported a reimbursement over $100.

Current activity

Concerned organizations and individuals have contacted the CMS Administrator, Seema Verma, and legislators. Those requests take several forms to include an additional postponement and the expansion of the Applicable Laboratory definition in an attempt to include hospital or hospital laboratory outreach data. Curiously, since the passage of PAMA, most hospital laboratory payments made using the CLFS have shifted to the bundled Hospital Outpatient Prospective Payment System (OPPS) and are no longer being paid from the CLFS. Additionally, the OPPS payment offset adjustment by CMS for those bundled laboratory tests has been estimated by the American Hospital Association as being two-thirds too low to account for the increased testing costs. Inclusion of hospital laboratory data would likely not be significant, but generating and reporting the data would cause a major burden on hospitals.

On August 2, 2017, representatives from the Clinical Laboratory Management Association (CLMA), American Medical Association (AMA), American Osteopathic Association (AOA), American Academy of Family Physicians (AAFP), Commission on Laboratory Accreditation (COLA), and American Society of Clinical Oncology (ASCO) held a meeting with the House Energy and Commerce Committee, which shares jurisdiction over the PAMA statute. They met with James Paluskiewicz, Chairman Greg Walden's committee staffer, and Una Lee, Ranking Member Frank Pallone's committee staffer. They are the top committee staffers on this issue and have direct lines to the chair and ranking member.

On September 7, 2017, this same coalition met with Senate Finance Majority and Minority staffers Brett Baker and Beth Vrabel.

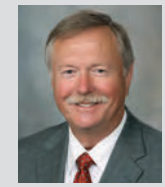
The main "ask" was to require CMS to issue an Interim Final Rule with a comment period. CMS should, in a transparent manner, be compelled to validate the reported data results and adjust the CLFS before implementation. Concern was expressed that the CMS data collected was inaccurate, skewed, and incomplete and did not meet the intent of the statute, which was to have a market-based approach. Additionally it was pointed out that a drastic cut in testing reimbursement would ultimately reduce access for Medicare beneficiaries and others requiring laboratory services. As the economies of testing shift, the numbers of laboratories would decrease and testing laboratory menus would be reduced locally, which would result in delayed testing and subsequent treatment and ultimately cost the Medicare program and other insurers more due to the need of treating a higher acuity patient.

Additional observations

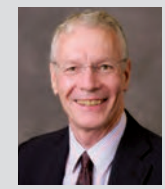
Implementation of the PAMA section 216 will take place and the new CLFS will go into effect in January 2018. Clearly, this price fixing by CMS will fall below cost in many circumstances and precipitate a feeding frenzy by other payors. That will result in even lower medians for the next iteration of reporting and fee setting. Publicly owned laboratories have sustained a major slump in stock prices as an initial reaction. Community laboratories will be required to maintain service levels near to their patients with less revenue from all sources.

The Medicare Access and CHIP Reauthorization Act of 2015 (MACRA) introduced two Quality Payment Program (QPP) pathways, a Merit-Based Incentive System (MIPS) and Advanced Alternative Payment Models (AAPMs). The Act also repealed the SGR legislation. Under MACRA a provider's participation in incentive payments will be based on performance in three categories: quality, advancing clinical information, and clinical practice improvement. Labs can play a key role in all of these initiatives.

In an environment where laboratory reimbursements are being eliminated or diminished, it is incumbent on lab leaders to engage with physicians and demonstrate the value of the laboratory in terms of patient care benefits and reduced aggregate cost of care rather than billed procedures.



Rodney W. Forsman, BS, is Assistant Professor Emeritus of Laboratory Medicine and Pathology, College of Medicine, Mayo Clinic, CLMA Legislative Compliance and Regulatory Committee (LCRC) Member.



Tim Murray, MS, MT(ASCP), serves as CHC National Director of Laboratory Compliance and Corporate Responsibility, LCRC Chair.

Paul Keoppel, MBA, MT(ASCP), serves as LCRC Member, Keoppel Laboratory Consulting, LLC.

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Automated hematology analyzer

Sysmex America, Inc. recently announced the launch of its XN-L automated hematology analyzers in the United States. Now lower volume laboratories can enjoy the same capabilities in CBC testing as larger hospitals and reference labs. The XN-L analyzers will also be the first to feature BeyondCare Quality Monitor program, an innovative, web-based QC and calibration management program.

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Joseph Leonelli, PhD
Vice President of ATCC Microbiology and Government Solutions
American Type Culture Collection (ATCC)

Professional

I joined ATCC in June 2015 as the Vice President of ATCC Federal Solutions. In January 2017, ATCC Federal Solutions and ATCC Microbiology Systems merged to create the Microbiology and Government Solutions Business Unit. Prior to joining ATCC, I held several leadership positions at both non-profit and for-profit companies focused on defense research and product development, including SRI International, Battelle Memorial Institute, General Dynamics, Applied Signal Technology, Raytheon, and Engility.

Education

I earned my PhD in inorganic chemistry from Indiana University in Bloomington. Prior to this, I completed my Bachelor and Master's degrees in chemistry and inorganic chemistry, respectively, from St. Louis University.

Personal

I am on the Board of Directors of Boys Town DC and serve as the Secretary for the Executive Committee. I support several volunteer activities at DC Central Kitchen and the Manassas Family Shelter. I am a major-league baseball enthusiast and actively follow the Washington Nationals and Boston Red Sox.

How are the products and services provided by ATCC of relevance to the clinical lab? ATCC biological materials are often incorporated as standards required by global regulatory agencies and organizations such as the FDA, AOAC International, Clinical Laboratory Standards Institute, U.S. Pharmacopeia, and World Health Organization. The level of authentication and characterization applied to ATCC biomaterials affords them the status of reference materials, which are frequently employed in performance assessments of instrumentation, phenotypic- or molecular-based assays, and traditional microbiological media or test methods used in the clinical lab. The routine use of these standardized reference materials ensures consistent and reliable interpretation of data, resulting in improved clinical outcomes, qualitative and quantitative observation of therapeutic treatment options, and surveillance of emerging drug resistance within microbial communities.

What are your primary responsibilities as Vice President of ATCC Microbiology and Government Solutions? I drive the growth strategy for the business unit and the component business areas of government solutions and commercial products. I work closely with my Government Program Managers, R&D team, and customer-facing personnel to win new government programs and develop new products that expand our catalog offering and sales.

How does ATCC serve federal agencies like CDC, NCI, and NIAID? ATCC has supported the federal government for over 50 years with biological products and innovative solutions, exercising our extensive expertise in global health and infectious diseases, biodefense, noncommunicable diseases, clinical study support, global logistics, and biomaterial management capabilities. The NIAID Microbiology and Infectious Diseases Biological Resource Repository (MID-BRR), managed through BEI Resources, is among the largest of the federal contracts managed by ATCC. BEI Resources provides a combination of reagents, tools, and information for studying Category A, B, and C priority pathogens and emerging infectious diseases relevant to human health. Other competitive government contracts awarded to ATCC include the CDC International Reagent Resource (IRR), which supports influenza research, surveillance, and response; the NCI Human Cancer Models Initiative (HCMI); and management of the NCI Central Repository on subcontract from Leidos Biomedical Research, Inc.

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- Emerging pathogens, including Bourbon virus, Zika virus, recent Influenza A and B outbreak strains, and vaccine-escape strains, such as *Bordetella pertussis*.

How will developments in these areas help clinical laboratories in their testing processes? Rapid advancements in technology, regulatory guidelines, and the emergence of novel or evolved infectious diseases all contribute to the changing needs of the clinical laboratory-testing environment. While PCR- and antibody-based testing have been around for several years, requiring the use of biological controls to measure accuracy and precision under strict adherence to CLIA and FDA Guidance for Industry, newer technologies such as NGS, disease modeling, and bioinformatics are on the horizon. ATCC stands at the forefront of this changing environment, developing new standardized reference materials and resources used to measure the efficiency and validity of these changes.

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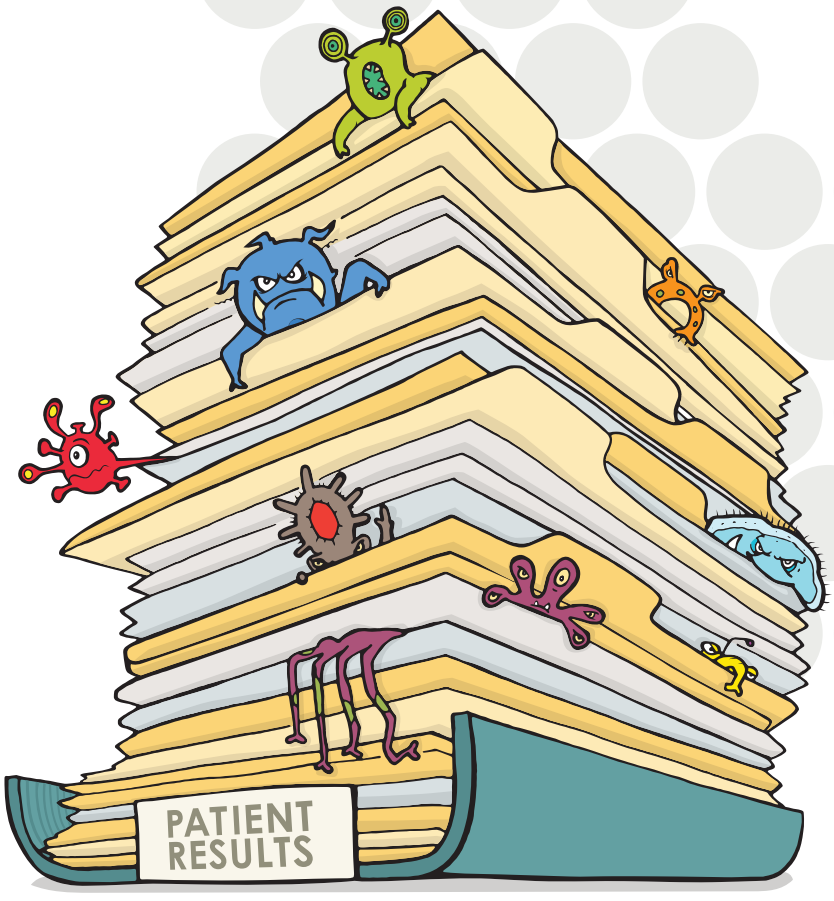

BioFire's syndromic testing allows you to quickly identify infectious agents that produce similar symptoms in patients. BioFire's innovative PCR technology provides hospitals, clinics, physicians and patients with the results they need in just one hour using any of the FilmArray® Panels: respiratory, blood culture identification, gastrointestinal and meningitis/encephalitis.

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- Easy. With just two minutes of hands-on time, the FilmArray® System is easily used by any tech, on any shift and at any size institution.
- Comprehensive. The FilmArray® Panels test for a comprehensive grouping of viruses, bacteria, parasites, yeast and antimicrobial resistance genes associated with a particular syndrome.

To learn how syndromic testing from BioFire can help make YOUR lab more efficient, visit **biofiredx.com**

Data on file at BioFire Diagnostics.

BIOFIRE®

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Syndromic Testing: The Right Test, The First Time.

Respiratory • Blood Culture Identification • Gastrointestinal • Meningitis/Encephalitis