March 2020 | Volume 6

CLINICAL LM Lab Manager

TRANSITIONING TO Digital Pathology

7 Steps to Implementing Digital Pathology in the Lab

THE HUMAN DIMENSION OF LABORATORY AUTOMATION

TIME TO TRANSITION TO CLINICAL LAB 2.0

PRENATAL DIAGNOSIS OF TORCH PATHOGENS

CLINICAL LM Lab Manager

March 2020

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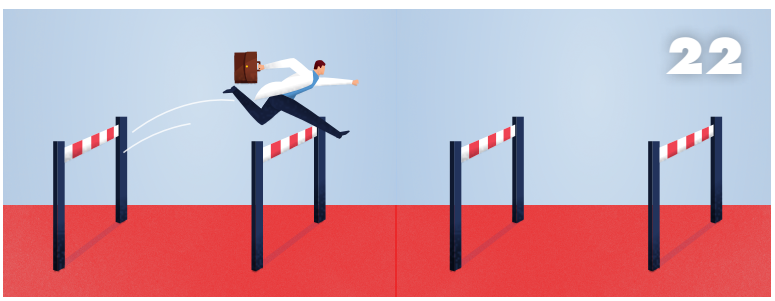

a game changer

Digital pathology has been referred to as revolutionary, a game changer, and a leading trend in pathology practice. Given its potential to transform how many clinical laboratories function, we decided to devote a big chunk of this issue to discussions around digital pathology.

"Clinical Pathology Goes Digital" (page 8) explores the many benefits of digital pathology in a clinical setting, and also takes a hard look at some of the barriers to its adoption. For labs ready to implement digital pathology and looking for some guidance, "Seven Steps to Implementing Digital Pathology in the Lab" (page 11) is required reading. Although digital pathology comes with a clear set of technical challenges, the legal and regulatory concerns associated with implementing it in a clinical setting are arguably harder to wrap one's head around; our feature "Legal and Regulatory Hurdles in Digital Pathology and Telepathology" (page 22) delves head-first into some of those issues.

Staff and people management remain top concerns for laboratory leaders. Discussions around laboratory automation inevitably bring up questions about how it might impact staff, which is the focus of our feature article, "The Human Dimension of Laboratory Automation" (page 14). Ensuring that staff are competent to perform their duties is another struggle laboratory managers face. In "Meeting the Competency Challenge" (page 12), readers will learn about common competency pitfalls in clinical labs and how to avoid them.

This month, I had the chance to sit down with Khosrow Shotorbani, the president and executive director of the Project Santa Fe Foundation and CEO and founder of Lab 2.0 Strategic Services, LLC, to discuss the emerging Clinical Lab 2.0 business model (page 34). The importance of this movement to laboratory leaders and the health care industry as a whole has prompted us at CLM to continue to focus on Clinical Lab 2.0 in our upcoming April webinar and digital Trends series.

There's plenty more to read in the March issue. On page 26, learn about methods available for prenatal testing of TORCH pathogens. Flip to page 16 to read about applications of immunohistochemistry in the fields of oncology and neurology. Find out how laboratories are positioned to improve the lives of HIV patients on page 38 and gain insights into how the lack of critical reagents is hampering neglected tropical disease research on page 39.

In other news, CLM is looking forward to attending Clinical Laboratory Management Association's (CLMA) KnowledgeLab 2020 in Louisville, Kentucky at the end of March—we hope to see you there! We're also thrilled to be partnering with CLMA this year to bring additional original content to our website.

After you finish the March issue, be sure to stop by ClinicalLabManager.com for more insightful editorial and to sign up to receive our weekly newsletters.

Enjoy!

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Our top picks from the literature

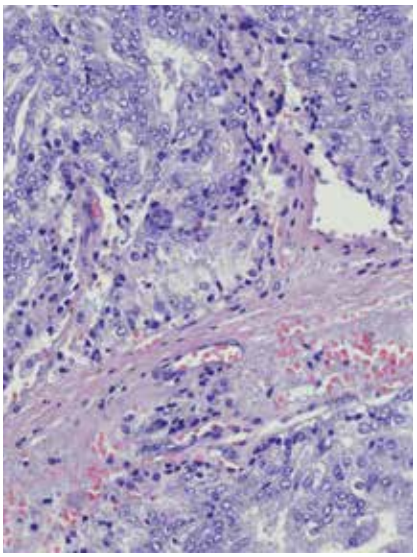
Lung Microbiome Predicts ICU Outcomes

A new study demonstrates for the first time that variation in the lung microbiota of clinically ill patients can predict clinical outcomes. Researchers sampled the lung microbiota of 91 critically ill patients receiving mechanical ventilation within 24 hours of their admittance to the intensive care unit (ICU). Using digital droplet PCR and bacterial 16S rRNA gene sequencing, they analyzed bacterial burden, community diversity, and community composition of the lung microbiota in each sample. After 28 days, the researchers assessed the number of ventilator-free days for each patient. Even after controlling for pneumonia and illness severity, patients with increased bacterial lung burden at the start of the study experienced significantly fewer ventilator-free days, the researchers reported in January 2020 in the *American Journal of Respiratory and Clinical Care Medicine*. They also determined that the presence of the gut-associated family of bacteria *Lachnospiraceae* in patient samples predicted worse ICU outcomes. The presence of another gut-associated bacterial family, *Enterobacteriaceae*, was significantly associated with the onset of acute respiratory distress syndrome. This study points to the lung microbiome as a major source of heterogeneity among critically ill patients that has been understudied. The researchers say the results could provide a novel target for the prevention and treatment of acute respiratory failure.

Dickson, Robert P., et al. "Lung microbiota predict clinical outcomes in critically ill patients." *American Journal of Respiratory and Critical Care Medicine* (2020).

AI in Prostate Cancer Diagnosis

Researchers have developed a deep-learning system to grade aggressiveness of prostate cancer based on biopsies following the Gleason grading standard. According to



a study published in January 2020 in *The Lancet Oncology*, the new system achieved a level of performance similar to that of pathologists, making it potentially useful in prostate cancer diagnosis. To train the system, the researchers exposed it to hundreds of biopsy images already classified by expert urological pathologists. They then collected 5,759 biopsies from 1,243 patients in the Netherlands and compared the performance of the deep-learning system to that of a panel of 15 pathologists from different countries and with varying levels of experience. The system outperformed 10 of the 15 pathologists, with its ability to grade biopsies comparable to highly experienced pathologists, the researchers report. They conclude that this deep-learning system could be implemented to assist pathologists by screening biopsies, providing second opinions on grade group, and measuring volume percentages.

Bulten, Wouter, et al. "Automated deep-learning system for Gleason grading of prostate cancer using biopsies: a diagnostic study." *The Lancet Oncology* (2020).

Simple Cardiac Blood Test Before Surgery

A common cardiac blood test performed before surgery can predict who will experience adverse outcomes after most types of surgery, according to a study published in January 2020 in the *Annals of Internal Medicine*. Globally, of the 200 million adults who undergo major surgery, 18 percent will experience serious cardiac and vascular complications including death within 30 days following their intervention. The new study examines whether levels of a cardiac blood test,

NT-proBNP, measured before surgery can predict cardiac and vascular complications. The study included 10,402 patients aged 45 years or older having non-cardiac surgery with an overnight stay from 16 hospitals in nine countries. The researchers found that higher levels of NT-proBNP, which can be caused by various anomalies in the cardiac muscle, such as stress, inflammation, or overstretch, can help identify which patients are at greatest risk of cardiac complications after surgery. Results of this simple blood test, which is cheaper than more time-consuming tests such as cardiac stress tests and diagnostic imaging, will help doctors predict who is at greater risk of heart attacks and other negative vascular events after surgery. It could also reduce the need for pre-surgical medical consultations for patients that show no risk for cardiac complications.

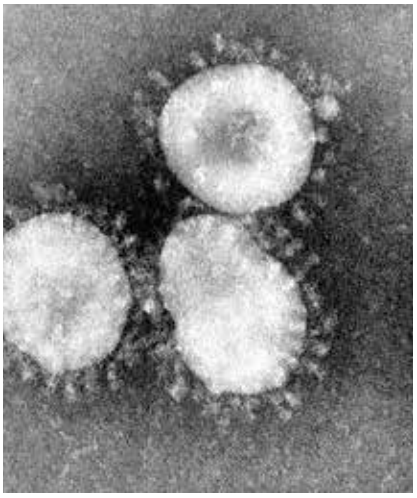
Duceppe, Emmanuelle, et al. "Preoperative N-terminal pro–B-type natriuretic peptide and cardiovascular events after noncardiac surgery: A cohort study." *Annals of Internal Medicine* (2019).

Clinical Trials Slow to Report Results

January 2020 was the third anniversary of the implementation of the US regulations that require clinical trials to report results within one year of completion (Final Rule of the FDA Amendments Act)—but compliance remains poor, and is not improving, with US Government sponsored trials most likely to breach. Less than half (41 percent) of clinical trial results are reported promptly onto the US trial registry, and one in three trials remain unreported, according to the first comprehensive study of compliance since new US regulations came into effect. The findings, published in January 2020 in *The Lancet*, indicate that trials with non-industry sponsors such as universities, hospitals, and governments are far more likely to breach the rules than trials sponsored by industry. US government sponsored trials are the least likely to post results on time at the world's largest clinical trial

registry, ClinicalTrials.gov, the study finds. The authors say that the high rates of non-compliance found in the new study likely reflect the lack of enforcement by regulators, and they call for trial sponsors to be held to account by the FDA.

DeVito, Nicholas J., Seb Bacon, and Ben Goldacre. "Compliance with legal requirement to report clinical trial results on ClinicalTrials.gov: a cohort study." *The Lancet* (2020).



Coronavirus Receptor Recognition

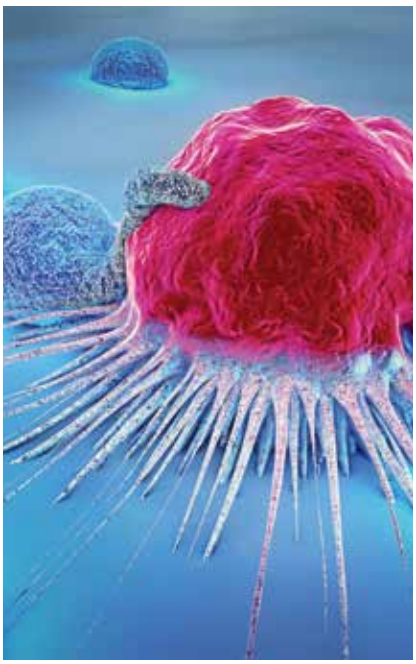
Studies conducted over the past decade have demonstrated how the SARS virus (SARS-CoV) infects animal and human host cells. Now, a study published in January 2020 in the *Journal of Virology* finds that the Wuhan coronavirus (2019-nCoV) uses the same receptors as the SARS CoV to gain entry into cells. The researchers compared previous data on the SARS-CoV spike protein receptor-binding domain 30 (RBD), which recognizes angiotensin-converting enzyme-2 (ACE2) host receptors, with the newly released sequence of the Wuhan coranovirus. They found that the 2019-nCoV RBD sequence is similar to that of SARS-CoV, suggesting that 2019-nCoV also uses ACE2 as its host receptor. Several critical residues in the 2019-nCoV RBM provide favorable interactions with human ACE2, consistent with 2019-nCoV's capacity for human cell infection, the researchers report. Other critical residues in 2019-nCoV RBM are compatible with, but not ideal for, binding human ACE2, suggesting that 2019-nCoV has acquired some capacity for human-to-human transmission. While their phylogenetic analyses indicate a bat origin of 2019-nCoV, the researchers write that the virus also potentially recognizes ACE2 from a variety of animal species including pigs, ferrets, cats, and non-human primates, any of which could potentially serve as intermediate hosts for 2019-nCoV infections. Notably, the study also finds that a single mutation could significantly enhance the Wuhan coronavirus's ability to bind with human ACE2. Therefore, the authors recommend that viral evolution in patients be closely monitored for the emergence of novel mutations, in order to predict the possibility of a more serious outbreak.

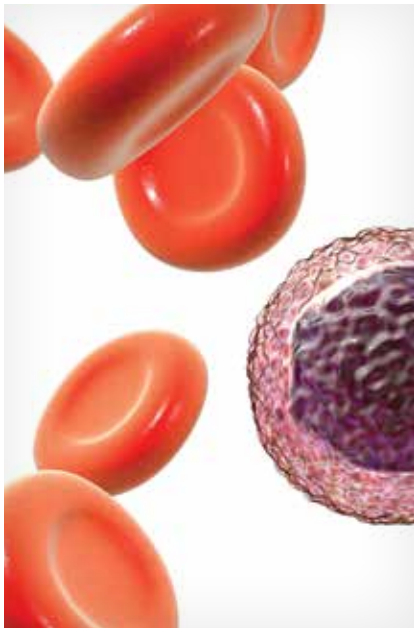
Wan, Yushan, et al. "Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS." *Journal of Virology* (2020).

Lymphopenia and Mortality

Low lymphocyte counts—a condition called lymphopenia—are associated with a 1.6-fold increase in risk of death from any cause, according to a study published in January 2020 in the *Canadian Medical Association Journal*. Lymphopenia is often detected during routine blood tests, but until now, its ability to predict future health was unknown. In this prospective cohort study, researchers examined and followed 108,135 participants enrolled in the Copenhagen General Population Study with a median age of 68 over 12 years. They found that all-cause mortality in participants with lymphopenia occurred at a 60 percent higher rate than in participants with lymphocytes within the reference range. The researchers say the association between lymphopenia and all-cause mortality may be due to reduced immune capacity, which increases patient vulnerability to potentially lethal diseases. Lymphopenia could also serve as a marker of general frailty, which confers high risk of illness and death. Predictors of mortality, such as lymphopenia, are highly valued in clinical practice because they help identify patients who may benefit from additional medical attention.

Warny, Marie, et al. "Incidental lymphopenia and mortality: a prospective cohort study." *CMAJ* (2020).





Predicting Response to Immunotherapies

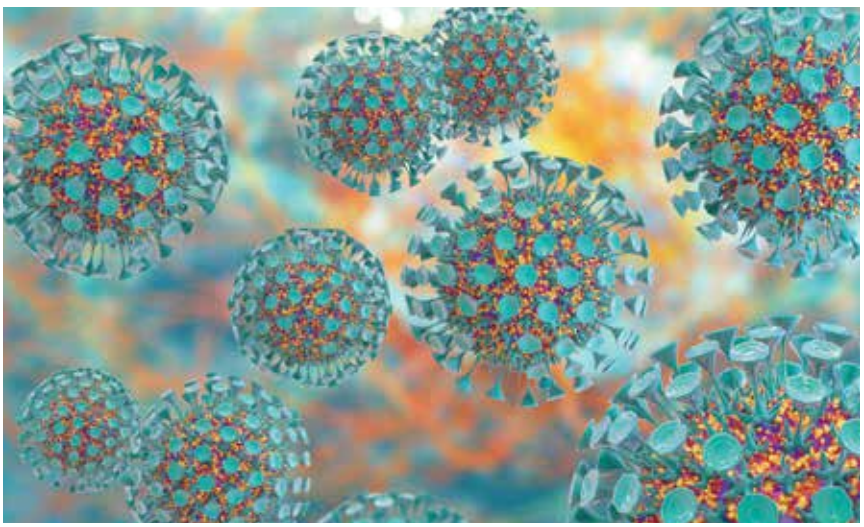
Researchers have identified a role for tertiary lymphoid structures (TLS) in maintaining an immune-responsive tumor microenvironment in patients with melanoma. The study was published in January 2020 in *Nature*. Tertiary lymphoid structures are lymphoid organs that form in non-lymphoid areas experiencing chronic inflammation, including tumors. Researchers examined 177 retrospective tissue samples from patients with melanoma and determined that the presence of TLS was associated with increased patient survival. They also showed that T cells in tumors without TLS had dysfunctional molecular phenotypes that included increased expression of the immune checkpoint inhibitors TIM3 and PD-1 and decreased expression of the cell death regulator BCL-2. These changes in gene expression impair the ability of T cells to identify and destroy tumor cells. The researchers also identified a gene signature associated with the presence of TLS in melanoma tumors that was also associated with overall patient survival. Further, the signature was able to predict patient response to immunotherapy treatment; those with melanoma tumors expressing high levels of the TLS genes were significantly more likely to survive following treatment. Based on these findings, the TLS gene signature could be a valuable tool for predicting response to immunotherapy. The study results may also lead to the development of new therapeutic strategies aimed at enhancing the formation and function of TLS.

Cabrita, Rita, et al. "Tertiary lymphoid structures improve immunotherapy and survival in melanoma." *Nature* (2020).

How HIV Develops Drug Resistance

Researchers have uncovered a mechanism by which HIV can develop resistance to a widely prescribed group of drugs. Integrase strand transfer inhibitors (INSTIs), which include raltegravir, elvitegravir, dolutegravir, and bictegravir, help to control HIV infection by binding HIV's integrase enzyme to prevent it from inserting the virus' genetic information into DNA of human cells. While initially highly effective, over time HIV can develop resistance to these drugs. Using single-particle cryo-electron microscopy to visualize the mode of action of INSTIs at near atomic resolution, the new study, published in January 2020 in *Science*, describes how HIV weakens the drugs' bonds with integrase over time. INSTIs work by interacting with metal ions, which normally allows them to form strong bonds to the viral enzyme's active site. However, the researchers found that HIV can subtly alter the chemical environment of the metals to reduce the strength of drug binding. This new understanding of the mechanism by which drug binding weakens, coupled with the study's visualizations of the viral enzyme's active site, will help to inform the design of more effective integrase inhibitors, the researchers say.

Cook, Nicola, et al. "Structural basis of second-generation HIV integrase inhibitor action and viral resistance." *Science* (2020).



Clinical Pathology Goes Digital

DIGITAL PATHOLOGY IS BECOMING A USEFUL CLINICAL DIAGNOSTIC TOOL—ONE THAT WILL CHANGE THE ROLE OF THE CLINICAL PATHOLOGIST **by Isis Ricaño-Ponce, PhD**

The microscope has always been a critical instrument for pathologists. Every specimen arriving at the pathology department has to be fixed, sliced into very thin layers, and placed on a glass slide. Indeed, examination of glass slides under the microscope remains the gold standard for primary diagnosis.

However, the growing sub-field of digital pathology does not rely on conventional microscopes. Rather, digital pathology makes use of digital technology to speed up and enhance workflows in a pathology lab. Although examination of glass slides under the microscope remains the gold standard for primary diagnosis to this day, digital pathology is making inroads to becoming accepted as equally good or better than regular microscopy for diagnostic purposes in terms of accuracy and efficiency.[1]

What is digital pathology?

Digital pathology employs whole slide imaging (WSI), in which slides are prepared and stained in the same way as in conventional microscopy, but instead of examining the slide with a microscope, the slide is scanned and visualized on a computer screen. The user can navigate the tissue and annotate any findings using software. The digitization of pathology slides through WSI represents a major step toward quantitative assessment in pathology that avoids human bias and enables the precise and reproducible extraction of data from slides. But digital pathology is more than simply scanning glass slides; it refers to the whole workflow from obtaining the slides to scanning them, managing, storing, and sharing data, and interpreting results.

What are the advantages of digital pathology?

One of the main advantages of digital pathology is that it saves time. Digital pathology reduces the need to manually perform certain tedious everyday processes like sifting through boxes for glass slides, setting up the microscope to match previous settings, and searching for a specific spot on the slide. Automated image analysis is especially efficient compared to manual cell counting; one study found that hand counting of tumor cells took an estimated 100 hours per slide compared to three minutes using automated image analysis.[2]

"Digital pathology is more than simply scanning glass slides; it refers to the whole workflow."

Environmental factors can degrade tissue mounted on slides over time. Slides are also prone to breakage, misplacement, or mislabeling, and they take up physical space. Digital slide archives maintain the quality of the slide image over time and provide long-term storage solutions so that only tissue blocks need to be physically stored.

The digitization of histology slides allows them to be accessed anywhere by anyone. Specialists around the world can be sent digital slides in minutes and examine the entire slide instead of relying on the sender to choose a representative section. Access to digitized

FDA APPROVAL OF WHOLE SLIDE IMAGING: A MAJOR STEP FORWARD FOR THE CLINICAL LAB

In 2017, the United States Food and Drug Administration (FDA) approved the first WSI scanner for primary diagnosis in surgical pathology. The scanners are defined as Class III medical devices, and the FDA regulates these instruments to help ensure that images being analyzed for clinical use are safe and effective for their defined purpose.

Before approval was conferred, the whole slide imager was thoroughly validated to show that it produced results comparable to conventional microscopy. Many studies have investigated whether there is a difference in diagnosis when pathologists use conventional microscopy versus WSI. These studies have shown high concordance rates among these two imaging types; however, study participants found that WSI was too slow for routine use when examining slides and that digital images were more difficult to evaluate than were glass slides.

slides also allows for long-term predictive analysis. The ability to compare the same sample with different dyes or at different time points has the potential to revolutionize disease prognosis.

What are the challenges of digital pathology?

Transitioning to a digital pathology system in the clinical lab can be a challenging process in the beginning. Some pathologists are reluctant to make the switch to digital pathology because they feel more confident using light microscopy. Others are resistant to adopting digital pathology due to misinformation about it. For example, although whole slide imaging was initially not as accurate as regular microscopy, studies have shown that it now gives comparable,[3] or even superior,[1] results, yet some pathologists still believe regular microscopy is more accurate. Fortunately, the teaching of digital pathology in medical school is getting new generations of pathologists familiar with its many advantages.

"Some pathologists are reluctant to make the switch to digital pathology because they feel more confident using light microscopy."

Digital pathology might still be more time consuming than light microscopy in specific cases. For instance, different magnifications are needed to detect some microorganisms, and changing magnifications might slow down the process. Moreover, digital pathology can produce vast numbers of images that are time-consuming to analyze. To speed up the process, certain algorithms offer steps to follow to screen for abnormalities.[4]

Digital pathology has shown to be less accurate than regular microscopy in a few circumstances, such as identification and grading of dysplasia, identification of granulocytes, nucleated red blood cells, and amyloid, and locating small diagnostic objects or features such as focal inflammation.[5] In these cases, visual inspection of glass slides should be mandatory.

Additionally, it can be difficult to observe features of three-dimensional cell groups and cells across multiple focal planes via digital pathology. Although improvements have been made in stacking multiple images to create 3D structural images, such images become extremely large, requiring better and cheaper storage options.

What can we expect from digital pathology in the near future?

One of the main promises of digital pathology is computer-aided diagnosis. Virtual microscopy generates hundreds of images from the same tissue that can be analyzed simultaneously. With thousands of annotated images from different samples and tissues, computers can be trained to recognize the regular features in a tissue as well as abnormalities. Although computer-aided diagnosis has become a reality, it is not yet commonly use in clinical labs, but its use will only increase in the next few years. Digital pathology is also a step forward for personalized medicine, as it allows the integration of imaging with radiologic, genomics, and proteomics data for better prognosis and predictive outputs.

It is unlikely that digital pathology will completely replace the full diagnostic capabilities of a pathologist in the short term, but this technology is now a useful clinical diagnostic tool that might lead to a change in the role of the pathologist in the clinical lab, speeding up the pathology department.

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1. Van Es Simone L. "Digital pathology: *semper ad meliora*." *Patología*. 51.1 (2019): 1-10.
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3. Williams, Bethany J., et al. "A systematic analysis of discordant diagnoses in digital pathology compared with light microscopy." *Archives of Pathology & Laboratory Medicine* 141.12 (2017): 1712-1718.
4. Cheng, Chee Leong, et al. "Enabling digital pathology in the diagnostic setting: Navigating through the implementation journey in an academic medical centre." *Journal of Clinical Pathology* 69.9 (2016): 784-792.
5. Qi, Xin, et al. "Content-based histopathology image retrieval using CometCloud." *BMC Bioinformatics* 15.1 (2014): 287.

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SEVEN STEPS TO IMPLEMENTING DIGITAL PATHOLOGY IN THE LAB

Recommendations for a smooth transition to digital pathology

Clinical labs have been slow to adopt digital pathology, mainly due to technical limitations such as low-resolution imaging, high bandwidth requirements, and instability of the operating systems. But now with better computers, faster networks, cheaper storage, and better image resolution, clinical labs can perform digital pathology with fewer roadblocks. Follow these seven steps to make your lab's transition to digital pathology as smooth as possible.[1]

1 GET THE LAB STAFF INTERESTED

The first step of moving to digital pathology is convincing the pathologists and the rest of the lab staff to accept the change. How can you convince staff? With facts. Ask your staff to read digital pathology-related articles, case studies, and best practices. Let them come to understand the benefits of digital pathology based on the evidence.

2 FIND AND INTEGRATE A DIGITAL PATHOLOGY SYSTEM

Find an adequate digital pathology system for the size and requirements of your lab that can be integrated with your laboratory information system. This step is crucial as it will save plenty of time and avoid human errors in the handling and processing of the samples. Do not forget to determine all the infrastructure that you will need to accommodate a digital pathology system, including network, storage, and security.

3 IDENTIFY YOUR AIMS AND HOW TO ACHIEVE THEM

Define the aims of each of your workflow stages, how they will be carried out, and who will carry them out. Establish a check list as an internal quality control for each of the stages.

4 VALIDATE EACH STEP OF THE WORKFLOW

Validate whether the entire workflow is working and improving the efficiency of the lab. The validation can be performed in three steps: First, the technical aspects of the digital pathology system alone must be checked; second, its integration with the laboratory information system (communication and speed) should be examined; third, it is important to determine how well the staff are executing the new system and how the system works in the context of the lab, including extreme scenarios.

5 TRAIN THE STAFF

Provide adequate hands-on training for lab personnel. A week-long intensive training period of 15 hours to cover the key essentials, followed by online access to complementary material and manuals, has been suggested.[1] Remember that there will be a learning curve for staff, resulting in a period during which there might be discrepancies and slower results from digital pathology-based diagnosis compared to glass slides.[2] Full conversion to digital pathology takes time.

6 COMPARE THE NEW AND OLD SYSTEMS

Prior to full adoption, compare the new digital pathology system to the previous system. Once the digital pathology system is in place and each step of the process is working correctly, it is necessary to validate the quality of the scans and ability of the new system to read the whole slide imaging (WSI). In this step, pathologists should review the same slide first using glass slides and again two weeks later using WSI. Concordance between the two methods should be tested, and turnaround times should be compared. Depending on the results, the digital workflow might need to be further refined.

7 EVALUATE THE PROCESS

Designate an individual to continually evaluate the digital pathology system after implementation. This person should keep track of the daily incidences—such as slide preparation problems, laboratory information system communication issues, and problems with the equipment—and document the benefits of the system and staff satisfaction, and provide feedback to the lab manager.

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Meeting the Competency Challenge

VERIFYING THAT LABORATORY TESTING PERSONNEL ARE KNOWLEDGEABLE ABOUT HOW TO PERFORM TESTING IS PARAMOUNT TO ENSURING QUALITY PATIENT CARE

by Darryl Elzie, PsyD, MHA, MT (ASCP), CQA (ASQ)

"Testing personnel competency is the most common deficiency reported by laboratory inspectors across the nation," said Jean Ball, a College of American Pathologists (CAP) Inspection Manager, at the 2019 Clinical Laboratory Quality Regional Meeeting in Newport News, Virginia.

Verifying that laboratory testing personnel are knowledgeable about how to perform patient testing is paramount to ensuring quality patient care. The Clinical Laboratory Improvement Amendments (CLIA) 1988 regulations, the Commission on Office Laboratory Accreditation (COLA), the Joint Commission, and CAP require competency assessments of all personnel performing non-waived laboratory testing. Clinical laboratories are inspected by either Centers for Medicare and Medicaid Services (CMS) or a deemed accrediting body, such as CAP, to verify that testing personnel are qualified and competent to perform patient testing. However, the CAP reports that competency is the most frequently cited deficiency in laboratories across the nation. (The two most frequently cited standards are GEN.55499 Competency Assessment - Waived Testing and GEN.55500 Competency Assessment - Nonwaived Testing.)

Competency pitfalls

There are several assessment pitfalls that laboratory managers should look out for in their efforts to meet competency regulations and standards. Common failures contributing to a laboratory receiving competency-derived deficiencies include missing elements, lack of assessor signatures, unqualified assessors, use of evidence for competency not completed in the assessment year, and not meeting the timeframe for the semiannual (six-month) competencies (required for new employees).

CLIA specifically spells out the elements required for personnel to be assessed for non-waived testing: Direct observation of test performance, monitoring test results, instrument maintenance, proficiency testing or blind samples, and reporting including criticals; review of worksheets, quality control, proficiency testing, and maintenance records; and evaluation of problem-solving skills.

Frequently, laboratories miss documenting a required element such as criticals or fail to use a blind sample when proficiency samples are not available. Missing information or blank fields on competency documentation draws the attention of inspectors and prompts them to ask questions. If an element does not apply to an employee or test system, the inspector will write "N/A" and the lab manager should be prepared to explain why the element is not applicable. (For example, if an employee works a specific shift that does not perform quality control on an analyzer, he or she may receive an "N/A" in the corresponding field. The inspector may then ask to review the staffing schedule to verify the employee has never worked a shift that performs quality control.)

Another error that would lead to a lab receiving a deficiency is competency forms not having an assessor's signature, or the assessor not being qualified to assess the test system due to its complexity. Clinical laboratories have a mixture of moderate-complexity and high-complexity tests (for example, an automated CBC is moderate-complexity, but a manual differential is a high-complexity test).

WHO CAN ASSESS COMPETENCY?

An assessor must have a bachelor's degree in a physical, chemical, biological science, medical laboratory technology, or nursing to assess employee competency of moderate-complexity test systems. This means that medical laboratory technicians with associate degrees are not approved to assess testing personnel competency of moderate-complexity tests. Many clinical labs avoid this problem by only allowing individuals with bachelor's degrees to assess competency.

Timing is important in assessing competency. Annual competencies may be completed throughout the calendar year. However, labs may get confused about the timing of the semi-annual competency for new employees. CLIA and the CAP considers an employee new if he or she has not performed testing under the laboratory's CLIA number. CLIA does not recognize systems; therefore, an employee who may have been working in a laboratory that is part of a larger health care system is considered a new employee when working in another laboratory that is a part of the same health care system but has a different CLIA number. To put it simply, new hires and transfers are both new employees in the eyes of CLIA and must have the training and two semi-annual competencies in the first year of performing patient testing. Labs must train transfers regardless of whether they have been working on the same equipment in the same health care system. The employee's file must contain training for the CLIA number corresponding to where patient testing is being performed.

Laboratory managers should be aware that the hire or transfer date is not the start of the clock for the six-month competency. The clock begins when the employee has been trained on a test system (entering patient test results can be a part of the training period), the trainer signs off, and the employee begins performing patient testing unobserved. The date the employee is placed on the schedule is often used as the six-month competency clock starting date. Managers can notify the employee and supervisors of the two six-month dates on the day the employee is placed on the schedule.

Only two six-month competencies should be in the employee folder. Many laboratories erroneously interpret the standards as requiring two six-month competencies in each discipline. That is incorrect. Only two-six month competencies are required in the first year of patient testing, and they often cover multiple disciplines and test systems depending on training and the laboratory.

Awareness of these common competency pitfalls will help the laboratory avoid having to complete a Phase II write-up (with evidence of corrective actions) to respond to a competency deficiency.

Darryl Elzie *has been an ASCP medical technologist for over 30 years and has been performing CAP inspections for 15+ years. He is also a certified quality auditor (ASQ). He currently works for Sentara Healthcare. Darryl provides laboratory quality oversight for four hospitals, one ambulatory care center, and supports laboratory quality throughout the Sentara system.*

The Human Dimension of Laboratory Automation

WILL AUTOMATION BE A FRIEND OR FOE TO CLINICAL LABORATORY STAFF?

by Todd B. Graham

Laboratory automation has come to full maturity since its introduction in the 1980s. From simply batching samples to run multiple tests to complicated systems that can run multiple modules at once while processing a variety of samples, laboratory automation is coming of age. Automation technologies have been key to reducing costs in the clinical laboratory while expanding the volume of testing that a given facility can perform.

As the technology for laboratory automation has evolved, the skills needed to succeed in a clinical laboratory have evolved with them. Prior to the passing of the Clinical Laboratory Improvement Acts of 1988 (CLIA), clinical laboratory positions commonly went to high school graduates who would learn on the job, rely on their manual or mechanical skills, and slowly develop the expertise required to operate the laboratory. Over time, these individuals were often sent for formal technical training, working their way up the ranks into clinical laboratory leadership positions.

With more formally educated clinical laboratory scientists entering the laboratory, a new standard for know-how is emerging. Certain duties, such as close consultations with health care professionals and deeper analysis of laboratory results, will only increase in importance as clinical laboratories produce ever-greater volumes of information. Laboratory roles and priorities will change as a result.

As laboratory automation gains a greater foothold in the clinical laboratory, major challenges will arise for staff, but with those challenges will come certain opportunities.

HOW STAFF CAN BENEFIT FROM AUTOMATION

+ New roles

Reduction in labor is an unavoidable consequence of lab automation, but it does not always mean job loss. Labs can take automation and the corresponding reduction in manual labor as an opportunity to repurpose staff members for more

challenging roles, which is often a boon to both the business and the staff. Staff may be deployed to help examine quality control and sample trends. They can become more involved in making recommendations for additional testing, reagent changes, and equipment servicing. Staff may find opportunities to develop new tools and assays. Being intimately familiar with the workings of the lab positions staff members to help innovate new workflows for better efficiency.

In some cases, automation affords health care facilities the opportunity to introduce additional departments, such as lab medicine customer service, or new types of labs with services that complement existing labs, opening up additional new roles for laboratory staff to move into.

+ Consultation

As physicians are increasingly presented with greater volumes of testing information from more sources, they require greater and more specific guidance on the higher-level meaning of these tests. Guidance from laboratory professionals with deep experience in the possible interpretations of tests will be needed. Automation gives laboratory professionals greater availability for consultations with health care professionals. The lab professionals may suggest certain factors such as the patient's medical history or complicating factors visible in other lab results that could impact the interpretation of a given test and help evaluate results of a given test in the context of other test results. Lab professionals can also discourage tests that may not be useful and steer health care professionals toward explanations and potential tests that would provide them with the information they need in order to answer key clinical questions.

+ Improved safety

Manual tasks, such as pipetting and plate streaking, can lead to operator fatigue and repetitive stress injuries such as carpal tunnel syndrome. Additionally, laboratory staff frequently expose themselves to biohazards when manually handling potentially infectious samples. A reduction in manual steps and handling inevitably reduces the risks of repetitive injury and exposure to biohazards, thus improving the safety of laboratory staff.

CHALLENGES STAFF WILL ENCOUNTER DUE TO AUTOMATION

- Adjustment of roles

Over the course of their careers, experienced laboratory professionals develop a level of comfort with particular laboratory techniques. They master ways of performing these techniques that are highly efficient. They may also identify strongly with their ability to work with their hands. Some could take the introduction of automation as an affront to their skills. To avoid pushback against the introduction of laboratory automation, efforts must be made to include laboratory professionals as stakeholders in rolling out the technology. Getting their input on equipment purchases, upgrades, and modifications will help staff feel involved in the automation process from the get-go.

- Learning curve

In the 1980s, students in the biological sciences focused on qualitative skills such as manual dexterity and identifying changes in color and clarity. This education has evolved over time to emphasize analytical and quantitative skills such as a deep understanding of statistics and the ability to code basic algorithms, meaning that less experienced professionals are now more likely to come in with the appropriate skills necessary to unleash the full power of laboratory automation. Meanwhile, more experienced professionals may need to brush up on analytical skills they might not have needed in the past.

Encouraging staff to take advantage of learning opportunities—such as classes in statistics and coding to improve their analytical skills—might help ease the shift. Training in statistics is especially powerful because it allows laboratory professionals to use their practical knowledge in new ways. Ensuring that staff are comfortable with new software and giving them the opportunities to make laboratory-specific tweaks is another means of giving laboratory professionals a stake in the process.

Rolling out laboratory automation will pose challenges to laboratory staff. From changes in procedure to emphasis on new skills, figuring out how to adjust to technologies intended to help staff is not always straightforward. However, with some thought and effort on the part of clinical lab managers, laboratory automation can be a boon to staff, unleashing their abilities to improve health and partner with health care professionals.

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Multiplexed Immunohistochemistry: Maximizing the Use of Tissue Sections

A TECHNIQUE AT THE INTERSECTION OF HIGH THROUGHPUT AND HIGH RESOLUTION

by Raeesa Gupte, PhD

Immunohistochemistry (IHC) has long been used in research and diagnostic laboratories to measure protein expression and localization within tissues. It is an important tool for characterizing heterogenous cell types, such as those found in tumors or brain sections. However, traditional IHC cannot reliably detect more than three protein targets at once.

Multiplexed immunohistochemistry (mIHC) can be used to detect anywhere from three to more than 30 protein targets within a tissue section[1]. Thus, the technique allows information on a large number of proteins to be collected from a small tissue sample.

Types of multiplexed IHC

Similar to traditional IHC, mIHC is based on the interaction between an antibody and its target antigen. Primary or secondary antibodies conjugated to a chromogen or fluorescent dye are used for detection.

Chromogenic mIHC

Chromogens are soluble substrates that form colored precipitates in the presence of enzymes. For high abundance molecular targets, chromogenic mIHC may employ primary antibodies that are directly conjugated with different chromogens. Alternatively, the primary antibodies may interact with chromogen-conjugated secondary antibodies to enable signal amplification for low abundance targets. Multiple staining cycles are performed if multiple proteins are to be visualized in a single tissue section.

Fluorescent mIHC

Fluorescent dyes such as FITC, TRITC, Cy3, and Cy5 are used to visualize protein targets. Conventionally, each fluorophore is imaged separately and the individual images are then merged together to evaluate localization of multiple proteins. Alternatively, multispectral analysis can be performed, wherein representative emission spectra of individual fluorophores are saved. The intensity of fluorescent targets is then compared against this multispectral library during quantification. To avoid multiple rounds of antibody staining, imaging, and fluorophore bleaching or antibody removal, mass spectrometry-based approaches have been optimized. These employ rare earth metal-conjugated antibodies for immunostaining, followed by high-frequency laser ablation and mass spectrometry for increased subcellular resolution[2].

Tyramide signal amplification (TSA)

TSA is an enzyme-mediated detection method that uses the catalytic activity of peroxidase enzyme to enhance fluorescence labeling of target proteins. The enzyme catalyzes the binding of tyramide-labeled fluorophores to tyrosine residues on the target protein. With fluorophores deposited on several tyrosine residues around the antibody complex, an amplified signal is produced. This technique allows the use of multiple antibodies raised in the same host species without risk of cross reactivity. Although TSA can be applied to both chromogenic and fluorescent mIHC, the latter is preferred because the fluorescent spectrum allows more dyes to be introduced.

Applications of multiplexed IHC

mIHC is an effective way to extract maximum data from tissues with limited availability. This makes it an ideal tool for studying cellular heterogeneity in oncology and neurology.

Oncology

Failure of cancer treatments is widely attributed to tumor heterogeneity. mIHC on biopsied tissues can aid the rapid classification of tumor subtypes, without using up too much sample. For instance, breast cancer is classified based on the presence or absence of estrogen, progesterone, and Her2 receptors. In one study on breast cancer samples, 32-plex mIHC combined with mass cytometry showed inter-patient variability as well as differences in protein expression within the same tumor[2]. Such classification can facilitate tailored therapy and disease prognosis.

"mIHC is an effective way to extract maximum data from tissues with limited availability. This makes it an ideal tool for studying cellular heterogeneity in oncology and neurology."

Immune cells in the tumor microenvironment also affect response to immunotherapy. Using mIHC, researchers labeled head and neck cancer biopsy samples with 12 antibody panels to measure lymphoid and myeloid phenotype in leukocytes[3]. Myeloid-enriched tumors in the study were associated with lower survival and had poor therapeutic response to the GVAX cancer vaccine. Therefore, mIHC may be used to gain insights into the tumor microenvironment and quantify predictive biomarkers to stratify patients based on their therapeutic response.

Neurology

We are yet to fully unravel the mysteries of the brain. A deeper understanding of neuroanatomy is the first step towards this goal. mIHC can be used to label brain cells, neurotransmitters, the blood-brain barrier, and peripheral players such as immune cells. For instance, mIHC with TSA has been used to detect trace amounts of the neurotransmitter dopamine and choline acetyltransferase in postmortem human brains[4].

mIHC can also be used to identify and monitor biomarkers of neurodegenerative disease progression. One study used mIHC to assess neuroinflammation and neural tissue damage in a rat model of traumatic brain injury[5]. Ten antibodies were used to evaluate changes in the location and functional states of immune cells and characterize neural repair and regeneration over time. Recently, an mIHC protocol was developed to detect signs of Alzheimer's disease in human postmortem brain tissue[6]. An Alzheimer's disease biomarker was co-stained with neuronal and astrocyte markers to develop a protocol that can also be implemented by other laboratories studying neurodegenerative diseases. Fluorescent mIHC combined with mass spectrometry was used to characterize glial phenotypes in multiple sclerosis lesions at various stages of the disease[7].

"Similar to other molecular biology techniques like gene expression profiling and flow cytometry, mIHC provides information on multiple cellular targets in sparse samples."

Advantages of multiplexed IHC

Similar to other molecular biology techniques like gene expression profiling and flow cytometry, mIHC provides information on multiple cellular targets in sparse samples. However, mIHC offers the added advantage of preserving tissue architecture, providing a spatial overview of protein co-localization and interactions, often at subcellular resolution.

Chromogenic mIHC enables detection of low abundance proteins with high sensitivity. The colored precipitates formed produce more durable staining, allowing long-term storage without signal degradation. Fluorescent mIHC combined with tyramide signal amplification, enables detection of a large array of proteins, even those with low levels of expression. It is also an ideal tool for measuring protein co-localization.

Limitations of multiplexed IHC

Chromogenic mIHC has limited multiplexing capacity due to spectral overlap and cross-reactivity between available antibodies. Also, it is not ideal for studying protein co-localizations. Fluorescent mIHC requires specialized equipment such as microscopes fitted with appropriate wavelength filters. The fluorophores may also undergo photobleaching over time, limiting long-term storage of fluorescently-labeled tissue sections.

Compared to other techniques, mIHC may require multiple cycles that make the protocols complex, difficult to optimize, and affect antibody binding. Furthermore, image analysis may be subjective, time-consuming, or require expert input. However, these limitations can be overcome by digital image analysis and artificial intelligence-assisted technologies.

Conclusion

Multiplexed IHC is a powerful tool for investigating the biology of complex disease, especially when there is a paucity of clinical samples. A workflow that seamlessly integrates automated staining, whole-slide imaging, and validated image analysis is crucial for developing clinically relevant *in vitro* diagnostics.

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New General Chemistry Linearities for Ortho Vitros

Linearity FD General Chemistry Panel 1 for Ortho Vitros

Order Number: K900M-5 **Package Size:** 10 x 5 mL
Open Vial: 5 days when stored at 2-8°C
Analytes: Albumin, Alkaline Phosphatase, ALT, Amylase, AST, BUN, Calcium, Chloride, CO2, Creatinine, GGT, Glucose, Iron, Lactate, LDH, Lipase, Magnesium, Phosphorus, Potassium, Sodium, Total Protein, Uric Acid

Linearity FD General Chemistry Panel 2 for Ortho Vitros

Order Number: K901M-5
Package Size: 5 x 1 mL
Open Vial: 5 days when stored at 2-8°C
Analytes: Creatine Kinase (CK)

Linearity FD General Chemistry Panel 3 for Ortho Vitros

Order Number: K902M-5
Package Size: 5 x 1 mL
Open Vial: 2 days when stored at 2-8°C
Analytes: Total Bilirubin, Conjugated Bilirubin, Unconjugated Bilirubin

Linearity FD General Chemistry Panel 4 for Ortho Vitros

Order Number: K903M-5
Package Size: 10 x 2 mL
Open Vial: 5 days when stored at 2-8°C
Analytes: ApoA, ApoB, Cholesterol, HDL, LDL, Triglycerides

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MULTIPLEXING

A BETTER APPROACH TO POINT-OF-CARE DIAGNOSTICS

Diagnostic assays are essential to confirm clinical findings. However, they may be time-consuming and necessitate well-equipped laboratories and trained personnel. Point-of-care (POC) assay technologies have emerged to address these challenges, and offer rapid turnaround times, are simple to perform, and require minimal sample volumes. Despite these benefits, singleplex POC assays only indicate infection by a single pathogen, and a symptomatic patient may obtain a negative test result. Multiplex POC assays enable testing for multiple different pathogens simultaneously, and can be used to distinguish between multiple diseases that present with similar symptoms. Multiplex POC assays are also useful in challenging environments including:

AT HOME

IN AN AMBULANCE

REMOTE SETTINGS

WHEN TRAINED PHYSICIANS OR PERSONNEL ARE UNAVAILABLE

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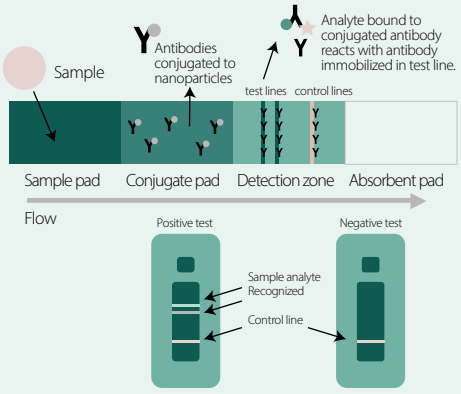
AVAILABLE MULTIPLEX POC TECHNOLOGIES

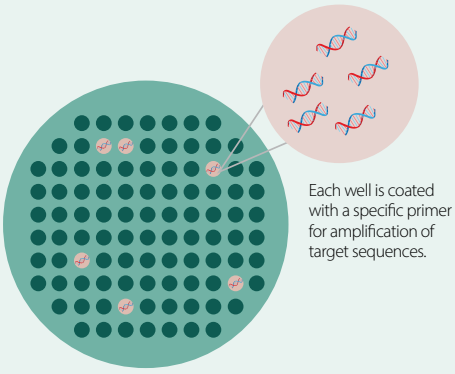
LATERAL FLOW

Multiplexed lateral flow immunoassays (LFIAs) rely on antibodies as a recognition element. The sample travels through the test strip by capillary action, and hydrates the conjugate release pad containing antibodies conjugated to nanoparticles, such as gold, or magnetic or polystyrene beads. The conjugated antibody bound to the target analyte continues to flow through the membrane to the detection zone, where it is immobilized on test lines. Binding the test line indicates a positive result, a lack of binding indicates a negative result, and the control line indicates the test was performed correctly.



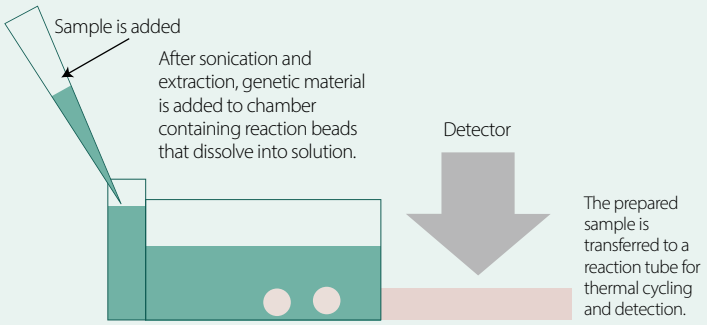
ARRAY-BASED ASSAYS

Multiplex array-based POC devices enable identification of pathogens based on the location of target DNA on the array. Achieving nucleic acid amplification at a constant temperature has been critical for obtaining rapid results in POC testing. The sample is added to the device and cells and viruses are lysed to release nucleic acids. The sample is labeled and aliquoted into individual wells where nucleic acids hybridize with gene-specific targets attached to the array. Fluorescent dye may be used to monitor the reaction, and pathogens may be identified based on positive wells within the array.



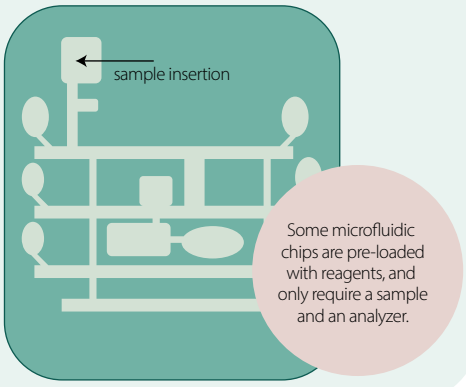
BEAD-BASED ASSAYS

Bead-based real-time PCR systems have enabled rapid, multianalyte, POC diagnostic tests for specific pathogens. The sample is added to the device and a DNA extraction protocol begins. The released genetic material is mixed with reaction beads that dissolve into the solution, and a thermocycling protocol begins PCR amplification. Some devices use an LED light source to illuminate the solution for color detection.



MICROFLUIDIC ASSAYS

Multiplex microfluidic systems, also referred to as lab-on-a-chip technologies, are designed with spatially distinct sections of microchannel networks that direct fluids using various inlets and valves for controlled flow, distinguishing them from lateral flow assays. Protein biomarker detection is relatively simple and fast, with multiplex target detection based on microfluidic immunoassay. Protein immobilization may be achieved with antibody-conjugated magnetic or non-magnetic beads, or via surface-based immobilization using a membrane. Nucleic acid detection is also possible using microfluidics, however, it requires the integration of multiple modules for cell lysis, nucleic acid purification, and DNA amplification into a single chip.



Legal and Regulatory Hurdles in Digital Pathology and Telepathology

PATHOLOGISTS MUST CONSIDER LICENSING REQUIREMENTS, DATA PRIVACY, CONSENT, AND QUALITY CONTROL **by Kimberly Scott**

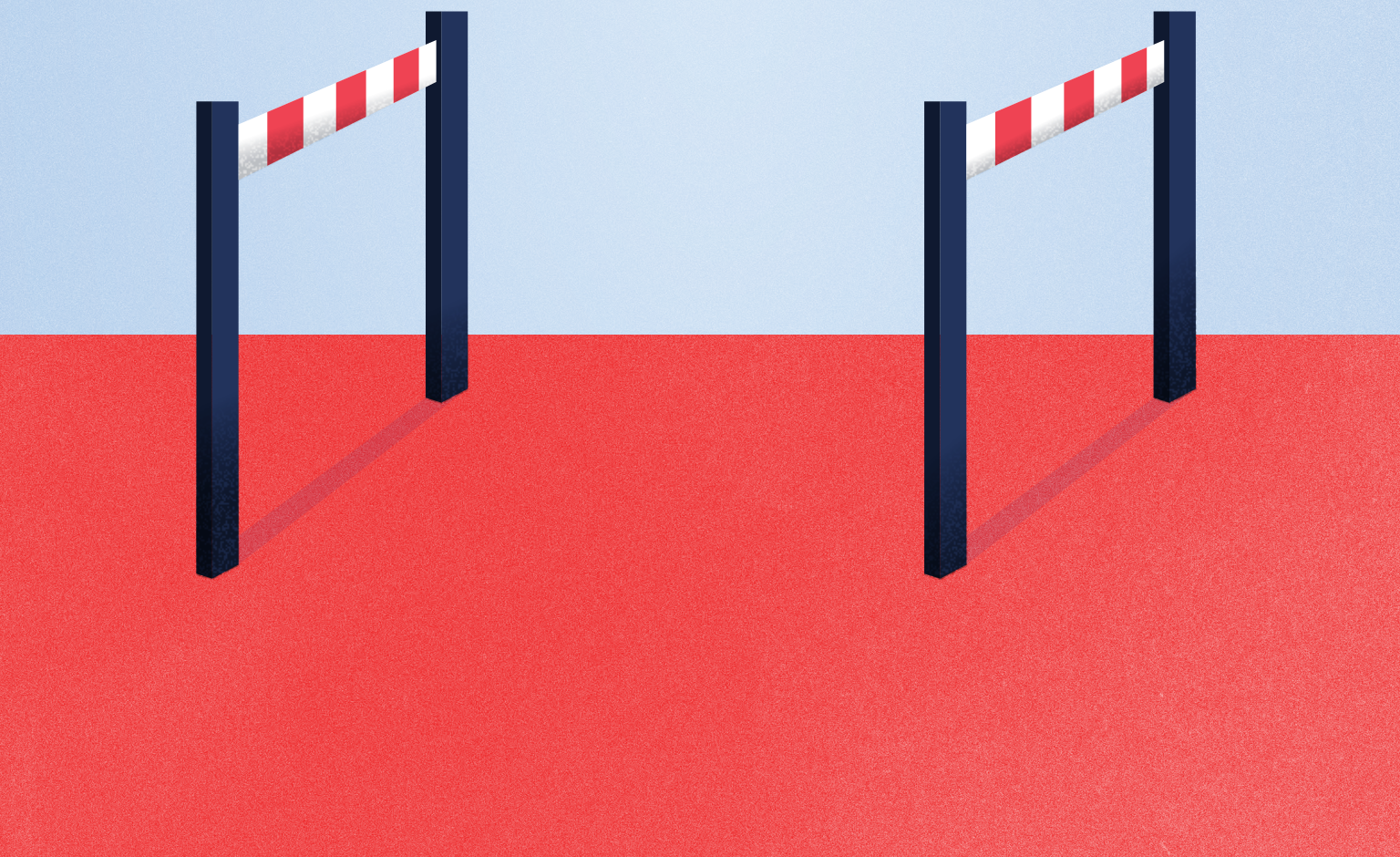
The Food and Drug Administration's (FDA) approval of two whole slide imaging (WSI) systems for primary diagnosis in the last couple of years has helped spur adoption of digital pathology by laboratories, but there still remain a number of regulatory and legal concerns surrounding digital pathology and telepathology, including licensing requirements, data privacy, consent, and quality control.

Philips' IntelliSite Pathology Solution for primary diagnosis in surgical pathology received FDA approval in April 2017, followed by approval of Leica Biosystems' Aperio AT2 DX System in May 2019. While digital pathology systems have been in use in the United States for years, overall adoption of this technology has lagged behind adoption by laboratories in Europe, Canada, and Singapore, according to an article in the *Archines of Pathology & Laboratory Medicine*.[1] However, early experience from these laboratories indicates that at least 95 percent of cases can be reported digitally without the need to defer to glass slides.

Whole slide imaging (WSI) technology provides pathologists with an ability to archive, review, analyze and share their digital slides. This gives pathologists and laboratories the opportunity to engage in telepathology, digitize slides for quality purposes, education, and documentation, says Anil Parwani, MD, PhD, president-elect of the Digital Pathology Association and director of anatomic pathology at The Ohio State University. Parwani is also director of pathology informatics and director of the digital pathology shared resource at The James Cancer Hospital at Ohio State.

Digital pathology is useful not only for pathologists but also for many patients who have an opportunity to have their cases reviewed by recognized experts, which can improve diagnosis and potentially affect outcomes. Marilyn Bui, MD, PhD, scientific director of analytical microscopy care at Moffitt Cancer Center in Tampa, immediate past president of the Digital Pathology Association and vice chair of the College of American Pathologists (CAP) Digital Pathology Committee, says that digital pathology that gives rise to computational pathology can allow pathologists to generate more data from the patients' tissue and cells and provide more actionable diagnostic, prognostic, and predictive information to guide effective and quality patient care.

"With computer algorithms and training, digital pathology lets us see things we cannot see with the naked eye," she says. "The combination of the naked eye and artificial intelligence can be very powerful in improving diagnostics."



Even so, fewer than 20 percent of laboratories in the United States are using digital pathology for secondary diagnosis while less than one percent of labs use it for primary diagnosis, estimates Parwani. Among the barriers to adoption often cited are cost, data storage requirements, change in workflow, and fear of pathologists in using new technology.

Still, the number of laboratories expected to adopt digital pathology and venture into telepathology is expected to grow significantly in the next decade, which means that pathologists will need to consider telemedicine-specific regulations, state licensure requirements, and privacy and data concerns when determining whether to practice across state lines.

Licensing

For an American Board of Pathology certified pathologist, no additional certificate is needed to read slides digitally. For the state the pathologist practices in, medical licensing is typically handled by state medical boards. While a pathologist does not need to be licensed in a particular state to consult or render a secondary diagnosis, the pathologist does need to be licensed in the same state where a primary diagnosis is rendered in a CLIA-certified laboratory setting.

"Fewer than 20 percent of laboratories in the United States are using digital pathology for secondary diagnosis while less than one percent of labs use it for primary diagnosis."

The Interstate Medical Licensure Compact, which encompasses 29 states, the District of Columbia, and the Territory of Guam, offers an expedited pathway to licensure for qualified physicians seeking to practice in multiple states, according to Amy Lerman, an attorney with Epstein, Becker and Green in Washington, DC. Under this agreement, licensed physicians can qualify to practice medicine across the state lines within the compact if they meet the agreed upon eligibility requirements. The application process is expedited by leveraging the physicians' existing information previously submitted in their state of principal license. Once qualified, the physician may select any number of compact states in which they wish to practice.

"The compact makes it much easier to get licensed in another state that is covered by the agreement," explains Lerman. "But pathologists would still need to go through the full licensing process in states where the compact is not recognized."

Data security and privacy

In addition to being licensed in other states where the primary diagnosis is rendered, pathologists practicing telepathology must also follow both federal and state privacy and security laws, including the Health Insurance Portability and Accountability Act (HIPAA), says Lerman. "We advise providers to have privacy policies that are very specific," she notes. "With more data flowing, it's more important than ever to protect patient privacy."

According to the American Telehealth Association (ATA), which has published clinical guidelines for telepathology, all data transmission used in telepathology should be secured through the use of encryption that meets recognized standards.[2] The ATA also recommends that protected health information and other confidential data only be backed up to or stored on secure data storage locations. Cloud services unable to achieve compliance should not be used for personal health information or confidential data.

Other potential solutions for confidentiality include anonymization of data by removing patient identifiers or limiting user access to stored patient information. Platforms that support secure data transfer and data encryption are an essential element of a successful telepathology program.

If WSI is used for diagnostic or other related clinical purposes, procedures must be in place that ensure that sites using WSI provide reasonable and expected confidentiality and data security in both data storage and data transmission. The security and privacy requirements of HIPAA apply, as they would for any other potential use of protected health information (PHI). Procedures might include message security, system and user authentication, activity logs, and access restrictions.

With respect to patient identification, as is the case for any laboratory analysis, processes, procedures, and training must be in place to ensure that patient identification linked to glass and digital slides is accurate, maintained, and secure. There are multiple ways to ensure positive patient identification, including use of verbal communication, barcodes, or images of slide labels.

Consent

Individual states may also have varying requirements regarding patient consent, which is another area for pathologists practicing telepathology to consider. "The standard is no different just because you're doing this via telehealth," says Lerman. "We advise providers to be very clear about what patients are consenting to."

While CMS does not require that an informed consent be obtained from a patient prior to a telehealth-delivered service taking place, a majority of states either require informed consent be obtained within their Medicaid program or in their statute or rules regulating health care professionals, according to the Center for Connected Health Policy.

Quality control

While the FDA regulates the companies that make digital pathology systems, pathologists themselves—whether or not they practice telemedicine—are regulated by the Centers for Medicare and Medicaid Services under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and must meet CLIA quality requirements. Quality control portions of CLIA apply to digital pathology, including the analytic phase of testing, which requires monitoring the testing of personnel, the test system and the laboratory environment.

CAP has developed proficiency testing in evaluating the quality of whole-slide images through its Histo-QIP program. Additionally, CAP's whole-slide imaging validation guideline, the HER2 immunohistochemistry quantitative image analysis guidelines,[3] and the digital pathology committee are the go-to resources for quality improvement in digital pathology, notes Bui.

Whole slide imaging systems must go through independent validation studies by laboratories if they are to be used for primary clinical diagnostic purposes, according to the CAP guideline.[4] The validation study should closely emulate the real-world clinical environment in which the technology will be used, should encompass the entire WSI system, and should be revalidated whenever a significant change is made to any component of the WSI system.

Uses of digital pathology that would not be considered a primary diagnostic use include: digital imaging studies for biomarker testing; interpreting digital slides with immunohistochemical or *in situ* hybridization tests that augment or refine the primary diagnosis; frozen sections using digital imaging, where a glass slide is subsequently reviewed to provide a final diagnosis; and second opinion consultation.

Digital pathology outlook

The verdict is still out on whether digital pathology will replace traditional microscopy any time soon in the United States. "Country-wide adoption of digital pathology is slow, which does not meet the needs of the quality and efficient care that patients deserve," says Bui. "There are more regulatory hurdles here that they don't have in Europe." Even so, Bui believes digital pathology has great potential for improving the efficiency and accuracy of pathology diagnosis through the use of artificial intelligence.

"Country-wide adoption of digital pathology is slow, which does not meet the needs of the quality and efficient care that patients deserve."

Despite the regulatory and cost hurdles, Parwani does foresee a time when all pathology diagnoses will be made digitally. "It's going to take some time, maybe 10 years," he says. "But I do think we'll start to see barriers fall."

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Prenatal Diagnosis of **TORCH** Pathogens

DETECTION CAN INVOLVE COMBINATIONS OF ULTRASOUND EXAMINATION, MATERNAL BLOOD SEROLOGY, AMNIOCENTESIS, AND PCR ANALYSIS

by Suzanne Leech, PhD

During pregnancy, numerous pathogens present significant risks to the fetus, the number and severity of which vary depending on geographical location. In middle to lower income countries, 50 percent of the deaths of children under one year old are due to infection[1].

The core set of pathogens known as TORCH present the most significant risks to the unborn child. TORCH comprises *Toxoplasma gondii*, others (including syphilis, *Treponema pallidum*, listeria, varicella, HIV, and parvovirus B19), rubella virus, cytomegalovirus (CMV), and herpes simplex virus (HSV). These pathogens can cause miscarriage, premature labor, retarded growth, and various other developmental abnormalities. The risks are amplified by the fact that pregnant mothers and their unborn children have weakened and undeveloped immune systems, respectively, increasing their vulnerability to infection. To minimize harm to the fetus, infections should be diagnosed as early as possible, preferably in the first trimester.

TORCH Infections

- **T** oxoplasmosis
- **O** thers
- **R** ubella
- **C** ytomegalovirus
- **H** erpes simplex virus

Broad-spectrum TORCH screening

TORCH pathogens include a variety of viruses, bacteria, and protozoal parasites, and an "others" category comprising a set of ill-defined pathogens that tend to vary over time and between countries. Many

of the pathogens present as only mild symptoms in the mother; therefore, awareness of the risks and the ability to recognize the symptoms are important aspects of prenatal care. Most TORCH infections can be screened by detecting pathogen-specific antibodies, typically in the maternal blood. In addition, there are research projects developing simple microfluidic tests that cover the TRCH pathogens in one serological test.[2] However, these do not cover the myriad of pathogens included in the "others" category. The value of general TORCH screening has been called into question by physicians who say that only individual tests need be applied when there is reason to suspect infection.[3]

"TORCH pathogens include a variety of viruses, bacteria, and protozoal parasites, and an 'others' category comprising a set of ill-defined pathogens that tend to vary over time and between countries."

Ultrasound screening

In some cases, ultrasound investigation will reveal signs of a prenatal infection, such as plural effusions. For example, many of the fetal abnormalities associated with syphilis can be identified with high-resolution ultrasound. However, any abnormalities must be verified as being due to infection using more definitive serological or amniotic fluid tests (amniocentesis).

Immunological tests

TORCH antibody serological assays are available for the detection of pathogen IgG and IgM antibodies for *Toxoplasma gondii*, rubella, CMV, and HSV, which can be used in a variety of assay formats including ELISA, rapid assays, and bead-based assays. Reactivity for the IgM, but not IgG, usually indicates a current infection, while IgG without IgM suggests a past infection. However, for some pathogens, including toxoplasmosis and CMV, IgG can indicate a primary infection and the use of IgM is not considered reliable. Paired-serological tests are probably the most useful technique for analyzing the mother's blood; a blood sample is taken during symptoms of the illness and the test is repeated four weeks later to determine any changes in the IgM/IgG antibody titers and avidities, which are used for diagnosis. In rapid IgM capture assays, which facilitate sensitive diagnosis of early-stage infections, an anti-IgM antibody fixed to a solid phase is used to capture pathogen IgM in patient samples. Immunofluorescent antibody assays (IFA) can also be used to definitively diagnose certain TORCH agents and may be necessary in some cases to distinguish between strains, e.g., HSV-1 and HSV-2. Immunological serological tests can provide information on the mother's likelihood of infection; however, the pathogens may not cross to the fetus. To confirm congenital infection, molecular analysis of amniotic fluid is usually recommended.

Molecular tests

Real time PCR kits are available for the diagnosis of most TORCH pathogens, and PCR analysis of amniotic fluid samples is considered the gold standard for many congenital infections. A meta-review concluded that PCR for toxoplasmosis performed on amniotic fluid sampled up to five weeks after maternal diagnosis has a sensitivity of 87 percent and specificity of 99 percent.[4] However, there was considerable heterogeneity between studies because of the lack of test standardization. The reviewers expressed hope that use of quantitative PCR could lead to better test standardization. In addition, false negatives may occur because of the small amount of pathogen DNA in the amniotic fluid, particularly in early stages of infection.[5] Current research has shown that multiplex nested PCR could provide the simultaneous and highly sensitive testing of seven pathogens.[1] The nested PCR technique can be used to amplify very low copy number sequences, decreasing the incidence of false negative results.

Examples of prenatal infection diagnosis

Toxoplasmosis

The protozoan *Toxoplasma gondii* is a prolific parasite of humans, infecting approximately 30 percent of the global population. Severe infection can lead to fetal death and miscarriage, pre-term birth, and neurological or ocular abnormalities. In the US, pregnant women

are only tested for toxoplasmosis if there are abnormal signs on ultrasound; in suspected cases, the maternal blood is checked using IgM and IgG serology, and congenital infection is confirmed with PCR analysis of amniotic fluid.

HIV/AIDS

An HIV-positive pregnant woman will pass the virus to her unborn child in around one out of three cases if treatment is not provided.[6] Along with hepatitis B and syphilis, the HIV test is part of the standard prenatal screening recommended by the CDC. The American College of Obstetricians and Gynecologists recommends antibody–antigen combination screening tests for HIV as early as possible in pregnancy.[7] Early use of combined anti-retroviral treatment can reduce the risk of vertical transmission to one to two percent.

Varicella-zoster virus

Commonly known as chickenpox or shingles virus, the varicella-zoster virus (VZV) can cause a particularly severe form of pneumonia in pregnant women. Furthermore, VZV infection of the fetus can result in serious abnormalities and even mortality. Because of extensive vaccination programs, the virus is not a common problem in places like Europe and the US; however, the vaccination rates are much lower in less developed countries. The virus may be diagnosed using PCR for viral DNA in amniotic fluid, at least one month after maternal infection to minimize false negatives. However, there is evidence that viral DNA in amniotic fluid, without infectious virus, leads to false positive results. In addition, serology has been shown to have poor sensitivity and specificity, even for umbilical cord and fetal blood samples. Therefore, there is no gold standard test for this pathogen,[8] although molecular methods are considered to be the most reliable of the available tests.

Conclusion

Advances in vaccination, prenatal care, clinical hygiene, anti-infective and anti-viral drugs, prophylaxis, and birthing methods have greatly decreased the risk and severity of many pathogens *in utero*. However, obstetricians are still commonly challenged with unknown or potentially serious cases of prenatal infection. In most cases, early diagnosis is the key to minimizing the risk to the unborn child. A combination of ultrasound examination, maternal blood serology, amniocentesis, and PCR analysis is usually the most effective strategy for the diagnosis of congenital infections.

"In most cases, early diagnosis is the key to minimizing the risk to the unborn child."

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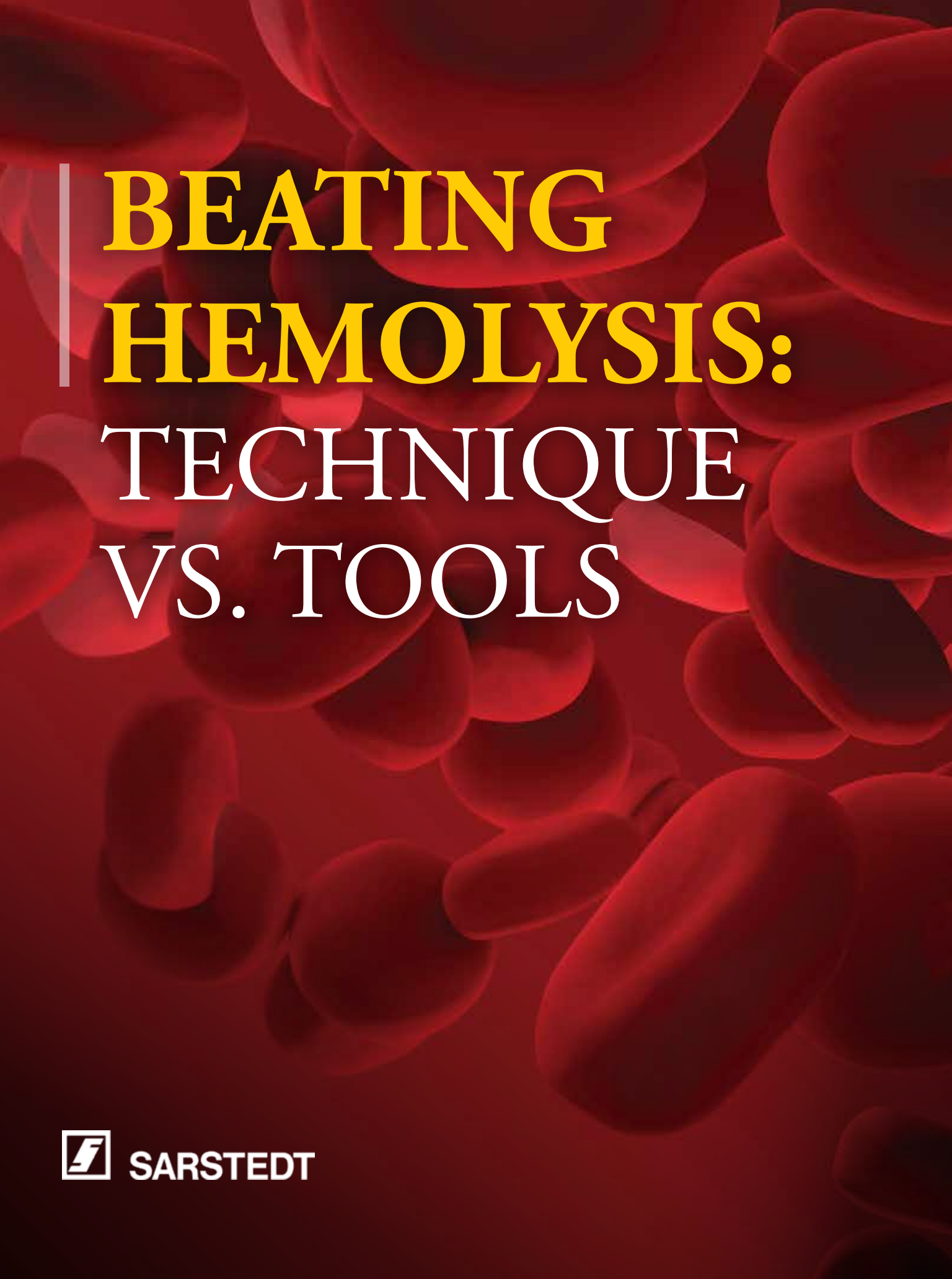
BEATING HEMOLYSIS: TECHNIQUE VS. TOOLS



SARSTEDT

BEATING HEMOLYSIS: TECHNIQUE VS. TOOLS

Training will only take you so far

"Houston, we have a (preanalytical) problem".

That bold statement was made last year in the *Journal of Laboratory and Precision Medicine.*[1] Although the authors noted that certain other diagnostic areas are even more error-prone than laboratory medicine, their key message was that the preanalytical phase is the most vulnerable part of the testing process because it is complex and manually intensive. It has been reported that preanalytical errors constitute between 25 and 50 percent of all errors in the clinical laboratory.[2] The most common preanalytical error—one with which clinical lab managers are all too familiar—is hemolysis. A staggering 10 to 30 percent of all blood samples sent from the emergency department (ED) to the central laboratory for testing are hemolyzed.[3]

The fact that most hemolyzed specimens come from the ED is ironic, given that it is the department in which timing is most critical.[4] Time delays are inevitable when it comes to hemolyzed specimens because blood must be redrawn and retested which, according to one study, results in delays of 65 minutes on average.[5] In practice, that means a patient in an emergency situation may wind up being treated based on symptoms alone because their blood work results haven't arrived in time. Another potential consequence is hospital overcrowding due to the increased length of stay of patients in the ED.

Not all hemolyzed specimens get rejected. If the specimen is borderline, it could fly under the radar. In such cases, the laboratory might unknowingly report values that have been impacted by hemolysis, potentially leading to troubling scenarios in which a normal value is reported when the specimen is in fact abnormal, or the reverse, where hemolysis pushes what should read as a normal value out of the normal range and subsequent therapeutic decisions are made based on these inaccurate results.

When hemolysis occurs, patients and health care facilities lose, and labs inevitably get blamed. To curb high rates

Between 10 and 30 percent of all blood samples sent from the emergency department to the central laboratory for testing are hemolyzed.

of hemolysis, staff tasked with blood draws are often put through rigorous training and retraining regimes to improve their technique. Such training typically covers proper catheter placement, tube filling, and how much force to use when aspirating blood, all of which can lead to preanalytical errors if done incorrectly.

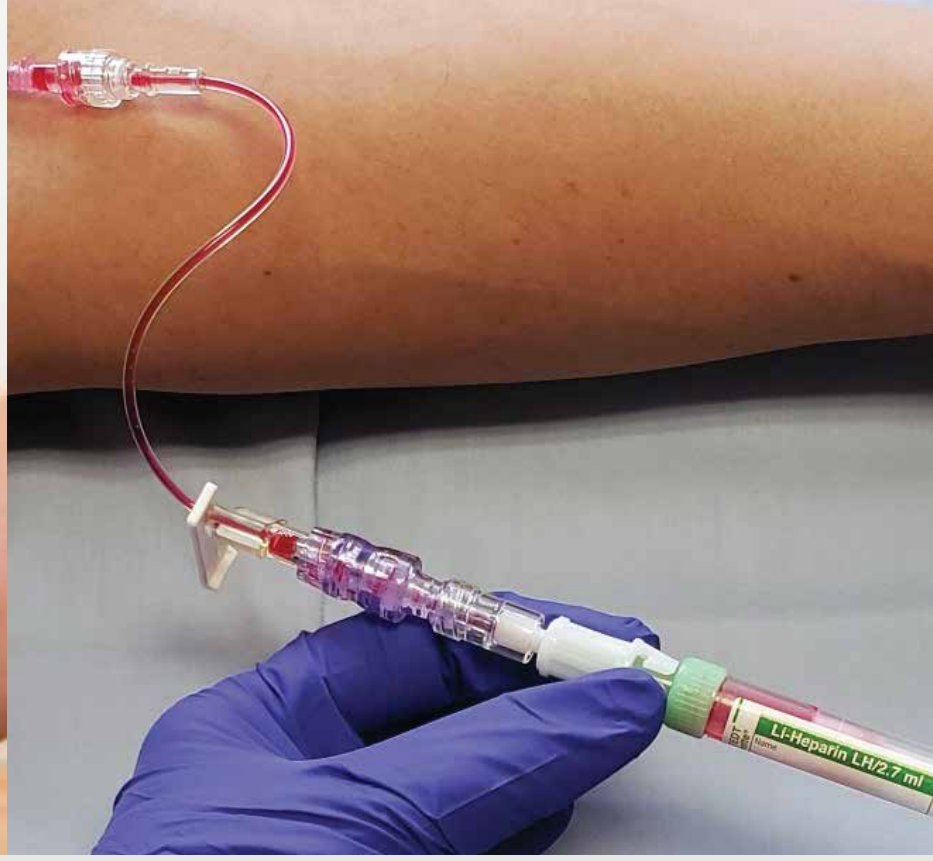
Although proper technique is critical to obtaining blood samples suitable for testing, there's another important yet often-overlooked source of preanalytical errors, and hemolysis in particular—the tools.

Use of IV catheters for blood draws is practically unavoidable in EDs and critical care areas, despite the fact that venipuncture is considered the standard of care.[6] The trouble with IV catheters comes down to physics: drawing blood through a long, tubular catheter leads to turbulence and shear forces, which aggravate red blood cells and, in many cases, result in hemolysis.

One proposed solution is collection of blood through a syringe and subsequent transfer to a blood tube. However, while the transfer step provides an opportunity for clotting, it also introduces an opportunity for hemolysis to occur. To eliminate the transfer step, one can place an evacuated blood tube directly on the end of the line, the downside being that the forceful draw causes more turbulence and shear forces, ultimately leading to higher rates of hemolysis. The ideal solution is a gentle, slow syringe draw without the transfer step.

Enter the Sarstedt S-Monovette®—a syringe that converts to a test tube. The system looks like a syringe and contains an adaptor that allows the user to connect the collection tube directly to the line. Blood can be aspirated by gently withdrawing the plunger until the tube is filled. Once aspiration is complete, the plunger locks into the base of the tube and snaps off. The primary tube is used for processing, eliminating the need for transfer devices. Research has shown that the S-Monovette® drastically reduces the occurrence of hemolysis when drawing blood from IV catheters.[3]

The preanalytical stage of testing is rife with opportunities for error. To improve the situation, health care facilities often look first and foremost at ways to improve upon technique. However, they should also consider the extent to which the wrong tools can contribute to the problem, and consequently, how the right tools, like the S-Monovette®, can become part of the solution.



While proper technique is needed to obtain blood samples suitable for testing, it is also critical to use the right tools.

Sarstedt's S-Monovette® drastically reduces the occurrence of hemolysis when drawing blood from IV catheters.

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Khosrow R. Shotorbani, MBA, MT (ASCP)

ASK THE EXPERT

Time to Transition to Clinical Lab 2.0

by Erica Tennenhouse, PhD

Khosrow Shotorbani is the president and executive director of the Project Santa Fe Foundation and CEO and founder of Lab 2.0 Strategic Services, LLC. Mr. Shotorbani was instrumental in the creation of Project Santa Fe—an initiative launched in 2016 with like-minded executives that helps to forge new frontiers that will define future economic valuation and placement of diagnostic services.

Formerly, as president and chief executive officer at TriCore Reference Laboratories, Mr. Shotorbani oversaw the corporate direction and strategy of TriCore, focusing on leadership and innovation, as well as operations, growth, and the financial health of the company. He led TriCore's initiatives based on the premise to improve health outcomes and lower costs by utilization of laboratory data. Before joining TriCore in 2014, Mr. Shotorbani served as senior vice president and director of business development/business innovations at ARUP. He joined ARUP in 1984 as a medical technologist and while at ARUP advanced to positions with progressive responsibility over his 30-year tenure.

Q: Can you briefly explain what Clinical Lab 2.0 is?

A: Clinical Lab 2.0 is a movement that provokes the lab industry to view itself through a new value lens aligned with the forces driving the future of value-based health care. Naturally, the primary purpose is to improve health care, and that requires a necessary emphasis on improved clinical outcomes, population health, and reduction of the overall cost of care. We intend to elevate the quantitative value of the clinical lab and align with systems enterprise goals and objectives in health care delivery.

Clinical Lab 2.0 is not about testing, although it's based on appropriate utilization, because if we're not appropriately utilizing clinical tests then our analyses of those data points is flawed. Clinical Lab 2.0 is about post-diagnostic computation of aggregate, patient–centric, longitudinal data with the goal of producing actionable insight addressing health care drivers, those being clinical intervention and clinical prevention, leading to cost avoidance in the space of value-based care. The focus is really on improved outcome and adjustment of financial risk. Clinical Lab 2.0 argues that the lab is the "first responder" and can be a catalyst for managing population health and value-based care.

Q: In what ways can clinical labs offer value?

A: Value is an interesting concept; I personally believe the current definition of value, quality over cost, is somewhat subjective. Clinical Lab 2.0 defines value differently—to us, value becomes outcome, both clinical and financial, and outcome is based on measurable, timebound actions.

If the focus of value is to move from sick care to wellcare, and the business model of health care is going to be based on keeping individuals out of the hospital, we argue that the clinical lab is a great asset to help this happen. Given the fact that lab data is actionable and has zero latency, it is the perfect first responder in population health. It really starts with developing an access-agnostic, longitudinal patient-centric view, tying together outpatient to inpatient to urgent care and emergency—so we can actually detect analytical trends and measure change. This gives us foundational Clinical Lab 2.0 attributes, which include the ability to:

1. Risk stratify the population against the known prevalence of chronic conditions. Think diabetes, chronic kidney disease, Hepatitis C, and even prenatal care.
2. Identify care gaps, which is critical for population health.
3. Identify high-risk patients early, before they are admitted into emergency room or hospital.
4. Facilitate intervention between a care provider and the person needing care early in disease stage.

These attributes are new frontiers for clinical labs and may require a new business model and alternative payment models.

Our value cannot be subjective; it must be absolutely quantifiably relevant and aligned with the drivers of health care.

Q: What factors might motivate labs to pursue version 2.0?

A: We argue that the business model of Lab 1.0 is no longer sustainable and has reached a strategic inflection point. But let me be very clear that Lab 1.0 is and will remain an essential part of medicine. We still need to help physicians choose the right test at the right time for the right patient—that's not going to go away. Lab 1.0 focuses on sick care and de-escalation, while 2.0 focuses on early detection and early escalation. It's a symbiotic relationship. From a business model standpoint, however, 1.0 focuses on volume and cost per unit, while 2.0 focuses on value and total cost of care delivery. This is ultimately why a lab might pursue a 2.0 vision.

I can offer three reasons why the current model is not sustainable:

1. There have been massive changes in reimbursement, the most prominent example being PAMA, a Centers for Medicare & Medicaid Services (CMS) reimbursement policy that impacts profit and loss. Above and beyond the reimbursement changes, there's this notion of patient-centric bundle payment, meaning that for the first time there is no independent economic value to an independent test.
2. The commercial market of labs is shrinking due to the physician employment model.
3. Massive consolidation on the payer side puts the current business model at serious risk.

For the first time, increase in volume no longer masks the business problems at hand; it may actually exacerbate the problem. Those labs who elect to do nothing about it may face significant subsidization, and when an organization or department becomes subsidized, it becomes the target of outsourcing. Project Santa Fe argues that when a system outsources part of the lab or the entire lab, it seriously limits their ability to transition to value-based care.

Q: To make the transition to Clinical Lab 2.0, what steps must labs take?

A: I will offer four steps:

1. We must get out of the four walls of the lab and align our value proposition at the enterprise level, with an eye on health care trends in population health, value-based care, and financial risk.
2. We must have a seat at the table to help design future delivery models based on the predictive value of clinical lab data.
3. We must be able to demonstrate to our C-Suites the importance of preserving and protecting the clinical lab assets, instead of making them the targets of outsourcing for short-term capitalization.
4. We must mobilize and create patient-centric longitudinal data that will allow us to begin to risk stratify the population, identify the care gaps, and identify the high-risk patients early.

Our role will become what I call the Uber of medicine, connecting the care managers to the most critical patients at much earlier stages. Longitudinal lab data coupled with domain knowledge of pathology will become the holy grail of lab medicine.

Q: How do you see health care evolving in the coming decades?

A: We all know that current health care delivery is unsustainable. Given the growth in chronic diseases, it is just impossible to manage that increase with the current health care model. So, prevention, intervention, and early detection, all proactively leading to improved outcomes and cost avoidance, are going to be significant. But I don't believe we can solve this just between health plans and health providers. I believe that patients and/or consumers have to be an active part of the health team managing their health affairs. If we're not able to engage the patients or consumers, our ability to solve these gigantic problems in health care is going to be limited. We have to embrace the whole notion of consumerism in health care. Technology may help in this process.

I also believe that we must advocate to change current policies and/or coverage. One of the challenges that Clinical Lab 2.0 faces is underutilization. For example, reimbursement of screening is still not part of the current CMS policies. We need to change some of those policies in order to align incentives, otherwise we'll see limited success in that transition. I remain hopeful that this will happen.

Q: What advice can you offer to clinical lab leaders interested in undertaking Clinical Lab 2.0?

A: My advice to my peers, clinical lab leaders, is we don't have to boil the ocean to add new value. I would say we need to think big but act small. We can start by mobilizing clinical data in an aggregation that makes clinical sense. We can add basic delta checks on some critical assays and actually start reporting that change to our clinical colleagues and seek input. But we can't do this in a vacuum. Getting out of the lab and telling a different story is critical to our mission.

Another piece of advice I can offer to my colleagues is to avoid retrofitting the Clinical Lab 2.0 new way of valuation with old ways of doing business. This may require different strategic planning, and subsequently, different operational planning that demonstrates the tangible value of the clinical lab for customers we have not served before, customers who are on the hook for improved outcome and financial risk and associated penalties. Otherwise we're going to be at the mercy of inadequate reimbursement models.

I will close with a quote that I like very much from the chair of our board, Dr. James Crawford: "There has never been a better time to demonstrate the value of laboratory medicine and pathology in the delivery of health care, but it must be quantitatively proven and attributable to the lab's ability to support such value."

Disclaimer: This interview is based on Mr. Shotorbani's tenure as CEO of TriCore and as president and executive director of the Project Santa Fe Foundation, and on a collective body of knowledge of all the members that are involved in Project Santa Fe and the Clinical Lab 2.0 movement.

***Erica Tennenhouse**, PhD, is the managing editor of* Clinical Lab Manager.

solutions FOR THE CLINICAL LAB

From assays to analyzers, these are some of the latest and greatest products for use in clinical research and diagnostic labs

THERMO FISHER SCIENTIFIC ION TORRENT GENEXUS SYSTEM

Thermo Fisher Scientific has launched its Ion Torrent Genexus System, the first fully integrated, next-generation sequencing (NGS) platform featuring an automated specimen-to-report workflow that delivers results economically in a single day. This turnkey solution redefines the genomic profiling paradigm and enables a future in which local hospitals can adopt NGS testing. The Genexus System changes the current paradigm. Its unprecedented turnaround time and fully automated workflow minimize user intervention and the potential for human error. The system also requires minimal amounts of tissue sample and can run small batches cost-effectively to deliver a comprehensive report in one day. Laboratories can scale their sequencing runs with an innovative sequencing chip design that will enable in-house sequencing facilities to cost-effectively process small batches of samples as they arrive at the lab. The Genexus System includes the Genexus Integrated Sequencer, the Genexus Purification System and an onboard reporting software. Thermo Fisher plans to seek FDA approval of the Genexus System to accelerate future development of a broad menu of diagnostic assays in oncology and other clinical applications.

OXFORD GENE TECHNOLOGY SURESEQ™ CLL + CNV PANEL

Oxford Gene Technology (OGT), A Sysmex Group Company, launched its SureSeq CLL + CNV Panel—the company's latest high-quality, next-generation sequencing (NGS) offering for research into chronic lymphocytic leukemia (CLL). The SureSeq CLL + CNV Panel fulfils the desire for reliable copy number variation (CNV) detection by NGS, including trisomy 12 and loss of heterozygosity, as well as somatic variants, even at low allele frequency. The panel, which has been tested to show excellent concordance with array data, can detect both small and large CNVs at 10 percent minor allele frequency (MAF), SNVs, and indels down to 1 percent MAF and LOH at 5-10Mb. The comprehensive panel covers all the most up-to-date, evidence-based genes and genomic aberrations for CLL and will enable laboratories to simplify their laboratory workflow by replacing multiple assays with a single one. Reliable data analyses can be carried out with OGT's Interpret software, a complementary software solution for accurate identification and visualization of all variants including CNVs.

THE NATIVE ANTIGEN COMPANY ANTIGEN AND ANTIBODY CONJUGATION KITS

The Native Antigen Company has announced the commercial release of its liquid format conjugation kits. The kits utilize a novel chemistry to generate highly reproducible antigen and antibody conjugates with a range of different labels, including enzymes, fluorochromes, and biotin. The Native Antigen Company's conjugation kits are multi-use, licence-free, and fully scalable. These kits sit alongside the company's existing portfolio of products to provide researchers with the opportunity to prepare stable conjugates, either from The Native Antigen Company's extensive selection of viral and bacterial reagents or from their own in-house reagents. These easy-to-use kits are available with a range of different labels including alkaline phosphatase, horseradish peroxidase, fluorescein isothiocyanate, r-phycoerythrin, and biotin, and include all of the reagents needed to conjugate proteins at optimal ratios. The liquid format requires no reconstitution and the kits are fully scalable from 0.01mg, to gram scale, meaning small scale trial conjugations can be rapidly developed into large scale conjugations.

PERKINELMER PG-SEQ™ RAPID NON-INVASIVE PREIMPLANTATION GENETIC TESTING KIT

PerkinElmer, Inc. has introduced its PG-Seq™ Rapid Non-Invasive Preimplantation Genetic Testing for Aneuploidy (PGT-A) kit. This solution tests spent embryo culture media for chromosomal abnormalities during *in vitro* fertilization (IVF) treatment. PGT-A is used to identify viable embryos, so the transfer or storage of embryos with an incorrect number of chromosomes can be avoided, as those typically lead to failed IVF cycles. Traditionally, PGT-A requires a biopsy of a developing embryo by creating an opening in the outer coating prior to removal and testing of a few cells. However, recent studies have shown that an embryo releases small amounts of DNA into the culture media in which it is growing, allowing the surrounding fluid to be genetically tested instead. PerkinElmer's PG-Seq Rapid Non-Invasive PGT-A kit is specifically designed for this type of sample, which enables embryos to remain fully intact. Leveraging the science behind PerkinElmer's biopsy-based PG-Seq kit 2.0, the new non-invasive kit tests the spent embryo culture media to accurately detect aneuploidies, as well as structural rearrangements, including unbalanced translocations, and segmental errors.

SIEMENS HEALTHINEERS ARK™ FENTANYL ASSAY

Siemens Healthineers is now distributing the ARK™ Fentanyl Assay, providing laboratories with a greater window to screen for fentanyl to more effectively triage patients who have been exposed to the dangerous opioid. The ARK Fentanyl Assay extends the detection window to provide the information clinicians need to triage their patients more appropriately. This is important because fentanyl absorption occurs at different rates depending upon the type of exposure, and during this time a patient will typically face suppressed respiratory function and should be closely monitored. When consumed, fentanyl is metabolized to norfentanyl and other metabolites. About 90 percent of the dose is excreted in urine as norfentanyl, while parent fentanyl accounts for less than seven percent. Because fentanyl metabolizes quickly, assays measuring solely the parent fentanyl typically have a shorter window for detection. The ARK Fentanyl Assay from ARK Diagnostics, Inc. can detect both norfentanyl and the parent fentanyl, enabling laboratories to identify more true positives. The ARK Fentanyl Assay is 510(k) cleared by the FDA and is CE Marked. It is available on automated clinical chemistry analyzers offered by Siemens Healthineers including on the Atellica® Solution, Viva Systems, Dimension, and Dimension Vista Systems.

GOLDEN HELIX VSCLINICAL

The clinical interpretation of variants in next-gen sequencing is a quickly evolving field. While the body of knowledge is growing exponentially, experts have to derive sound clinical decisions leveraging an ever-expanding set of specialty databases, clinical publications, and algorithms that are designed to predict the impact of specific variants in the resulting protein. Golden Helix's solution, VSClinical, guides clinicians through the clinical assessment of germline and somatic variants via workflows modeled off the guidelines issued by the American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP). The ACMG workflow focuses on germline diseases, and the AMP Guideline add-on expands the tool to interpret somatic mutations. It also applies AMP Tiers to the available clinical evidence for drug sensitivity, drug response, and prognostics and diagnostics, supports small mutations (SNPs, InDels) along with CNVs, fusions, and wild-types as relevant biomarkers for the reporting of clinical evidence, and develops a lab-specific knowledgebase of interpretations that allows maximum reuse of interpretations and descriptions from one patient to the next. Used in conjunction with Golden Helix's full clinical stack, hospitals and testing labs are able to conduct consistent, high-quality interpretations of NGS data, to increase lab throughput, and to provide a framework for newer, less experienced clinicians resulting in high-quality clinical reports.

Big Ideas About the Clinical Industry

Why the Laboratory Is at the Forefront of the Global Fight against HIV

by Fernando Chaves, MD

As the laboratory assumes an ever-more relevant role in health care it finds itself on the front lines in the global fight against HIV. With effective drug regimens well established, the biggest challenge these days is getting those infected with HIV diagnosed as early and accurately as possible.

The CDC estimates that in the US, more than 1.1 million people are infected, and that 162,500 of them—15 percent—are undiagnosed and unaware of their status. This is a critical problem given that diagnosed patients now have options available that would allow them to enjoy a quality and duration of life not much different from that of noninfected individuals.

Undiagnosed HIV patients represent a serious challenge. From a public health perspective, nearly 40 percent of all new infections are transmitted by people who do not know they have the virus. From a clinical perspective, patients diagnosed early have favorable long-term outcomes, potentially staying healthy for years to come and lowering their chances of HIV-related illnesses.

By addressing this challenge, clinical laboratory managers have the power to save lives and reduce health care expenses. HIV tests vary greatly in performance characteristics, so clinical laboratory managers often have a critical choice to make.

To ensure that as few patients as possible go undiagnosed, laboratorians must select assays with high sensitivity and low risk of erroneous results caused by interference. This means not only using the CDC's recommended assays and its algorithm for HIV testing, but also carefully reviewing individual assay performance.

While all fourth-generation assays allow detection of infections earlier, there are key differences even among those. Lab leaders should ask these questions:

- What is the analytical sensitivity of this assay?
- In seroconversion panels from recently infected patients, how many days did it take for this assay to become positive?
- How many different HIV genotypes is this assay capable of detecting?

- What safety mechanisms does the analyzer offer to detect common interferences such as hemolysis and lipemia?

These questions are becoming increasingly critical in the choice of the best HIV test. Why do they matter?

Use of pre-exposure prophylaxis is gaining wider adoption among high-risk populations, so infected patients may exhibit nearly undetectable levels of virus. In this situation, small differences in the limit of detection is no longer a technicality, but rather can mean the difference between life and death—impacting dozens of individuals who can potentially become infected by a person who receives a false negative result.

Additionally, international travel is increasing exponentially, meaning rare HIV genotypes currently undetectable by some existing tests may become more prevalent. Again, in this scenario, the ability of an assay to detect more genotypes can impact many lives.

Finally, HIV patients often have co-infections. A complete panel incorporating HCV, HBV, and STDs provides a one-stop testing solution for this high-risk population. Access to more complete infectious disease testing has immediate logistical benefit, helping laboratories increase operational efficiency. Through these more comprehensive panels, labs can reduce the number of analyzers needed, allowing managers to focus their resources where they matter the most—giving them added flexibility.

It all adds up to this: Advances in technology combined with changes in the challenges posed by the HIV epidemic have created a new reality in which the major opportunity medicine has to improve patients' lives is now in the hands of laboratories and those who manage them.

***Fernando Chaves**, MD, serves as the global head of medical, clinical, and scientific affairs for Ortho Clinical Diagnostics. Dr. Chaves is a board-certified anatomical and clinical pathologist, with further certification in hematopathology.*

Better Access to Reagents Is Needed for Neglected Tropical Disease Research

by Andy Lane, PhD

Neglected tropical diseases (NTDs) are a diverse group of infectious diseases that almost exclusively affect low-income populations in settings that have inadequate sanitation and limited access to health care. Found in tropical and subtropical climates, these diseases affect billions of people across 149 countries and are responsible for over half a million deaths every year. Moreover, unlike many of the acute infectious diseases in the developed world, NTDs tend to be chronic, disabling, and disfiguring, leading to social marginalization and stigmatization. As a result, NTDs also prevent people from working or receiving an education, which further perpetuates the socioeconomic conditions in which these diseases thrive.

Significant resource commitments are made to combat the emerging and endemic diseases that affect the developed world. In contrast, NTDs remain sidelined in public health priorities, which is illustrated by the fact that only three to four percent of drugs approved between 1975 and 2011 were indicated for NTDs. As a result, a profound gap persists between the global burden of these diseases and the availability of clinical solutions to treat them. The constellation of NTDs has continued to grow since the term was coined in 2003—and none of these diseases have permanently lost their NTD status.

Diagnostics and vaccines

The failure to control NTDs is in part due to a failure to diagnose them. The symptoms of tropical diseases are often insidious and unspecific, meaning that diagnosis is typically not made until the late stages of a disease's progression. Moreover, diagnostic laboratories are often poorly resourced and struggle to support remote areas, so improved diagnostics are essential to better guide treatment, interrupt transmission, carry out basic surveillance, and eliminate disease. But despite increased interest, few such tests have yet to reach the market and existing devices often lack the required accuracy, specificity, and robustness, or are unable to differentiate between past and latent infections.

The best prospect for sustainably controlling NTDs is with safe and effective vaccines. But like diagnostics, vaccines for NTDs have lagged behind, with much of the available funding going to malaria and HIV. Compounding poor financial incentives, formidable scientific hurdles—such as complex genomes, lack of *in vitro* systems to maintain laboratory pathogens, few suitable animal models, and poor correlates of vaccine protection—have also impeded vaccine development.

Access to reagents

The foundations of any effective vaccine or diagnostic are the critical reagents. From small chemicals to large, complex proteins, these components drive the fundamental reactions of medical technologies and ultimately determine their clinical utility. Consequently, access to such reagents is crucial in stimulating research and development. However, with an absence of commercial markets, product pipelines and their basic precursors have dried up in recent years. This is compounded by the fact that reagent sharing in academia is often restricted by material transfer agreements and regulations that impede collaboration with industry. A simple Google search for NTD reagents reveals a lack of proteins, nucleotides, conjugates, and cell lines available for research use. This dearth of reagents creates barriers to research, requiring greater resources and investment in product R&D, and contributes to longer development cycles. Alternatively, if a broad range of high-quality reagents were available, researchers might be more inclined to embark on research projects—especially if materials are validated and well-characterized, and offer a means of reducing experimental error.

While commonly identified barriers to NTD product development include access to finance or manufacturing capacity, a lack of critical reagents is rarely accounted for. Making these precursors openly available is key to developing effective solutions for NTDs, and their scarcity will only further impede progress in this area. As such, funding for open-access reagents, improved resource repositories, and increased collaboration with industry will be crucial in stimulating future product development.

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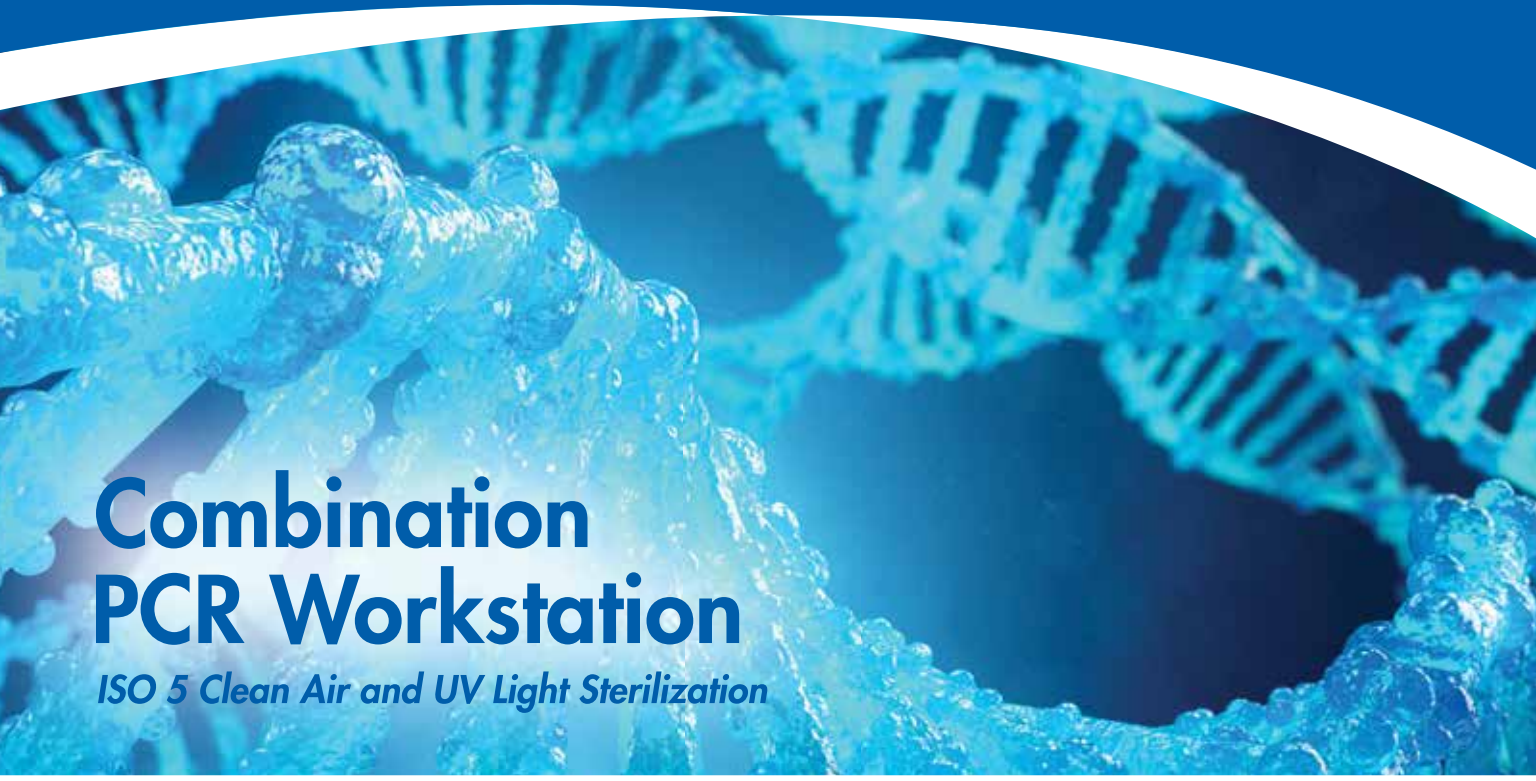

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