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MEDICAL LABORATORY OBSERVER

DECEMBER 2019 ■ Vol 51 ■ No 12

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CE

The evolution of AUTOMATION

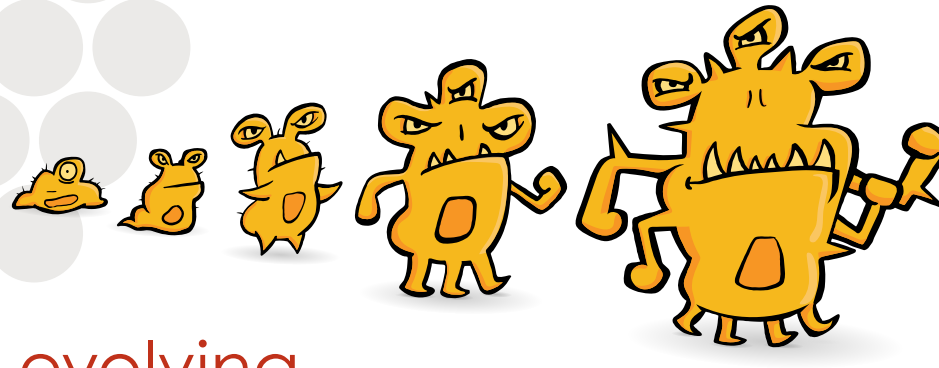
A history of HPV

Biomarkers for neurodegeneration

The prevalence, pathogenesis and diagnostics of celiac disease

EXECUTIVE SNAPSHOT

Dan Scungio
MT(ASCP) SLS, CQA(ASQ)
"Dan the Lab Safety Man"



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  - *Proteus*
  - *Salmonella*
  - *Serratia marcescens*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Stenotrophomonas maltophilia*

GRAM-POSITIVE BACTERIA
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- *Enterococcus faecium*
- *Listeria monocytogenes*
- *Staphylococcus*
  - *Staphylococcus aureus*
  - *Staphylococcus epidermidis*
  - *Staphylococcus lugdunensis*
- *Streptococcus*
  - *Streptococcus agalactiae*
  - *Streptococcus pneumoniae*
  - *Streptococcus pyogenes*

YEAST
- *Candida albicans*
- *Candida auris*
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Cryptococcus neoformans/gattii*

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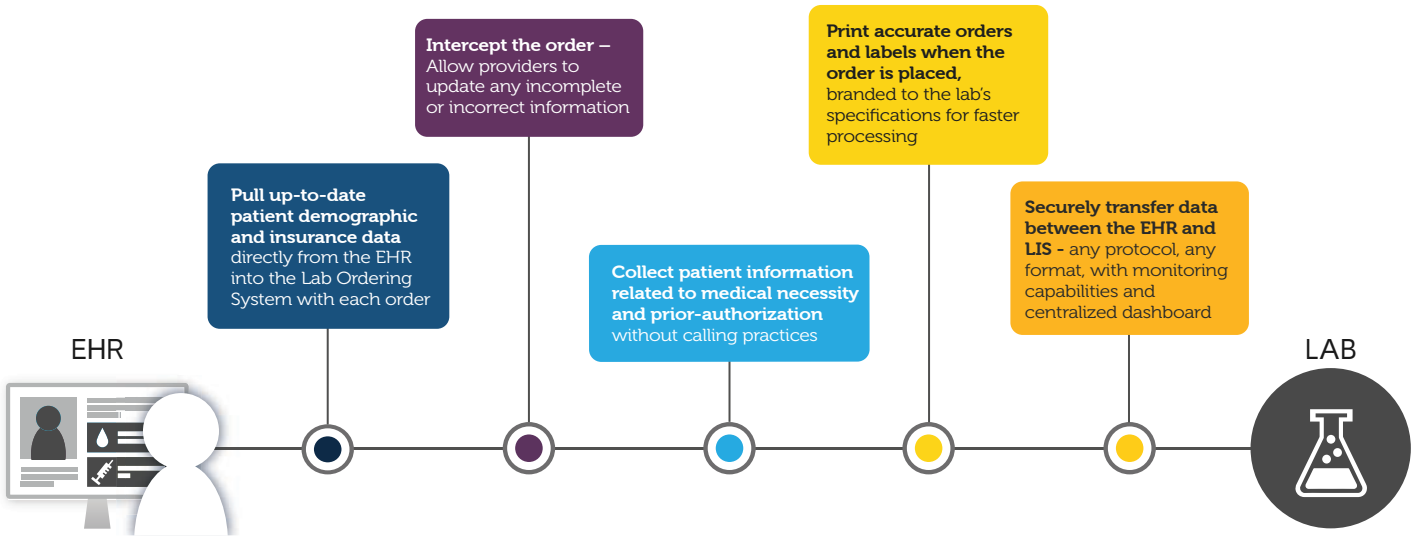

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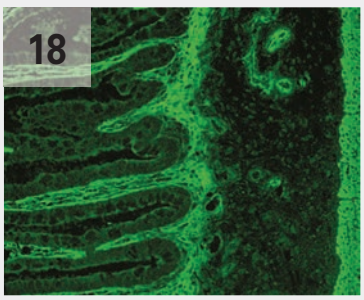

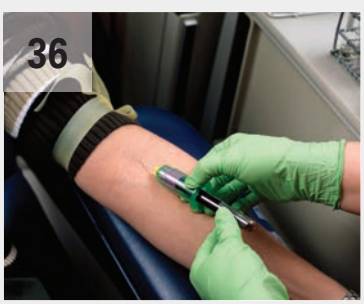

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Rethinking laboratory waste

By Lisa Moynihan
Managing Editor

When my college-aged stepdaughter announced she was majoring in "sustainability" my inital reaction was...surprise! How was this young adult, who had a history of not recycling food containers, going to excel in a program that catered to saving the planet? My concern dissipated when I learned sustainability consists of scientists, engineers, educators, social activists and business professionals who promote global development in ways that respect the natural environment.

Her recent declaration has had me thinking about adults (including a friend who recently ostracized me when I offered her a beverage with a plastic straw) and sustainability; more specifically, clinical laboratorians. In fact, did you know laboratories use 10 times more energy than offices, more than four times more water, and generate billions of pounds of waste every year, nearly all of it considered hazardous?[1]

Disappointingly, only myself and three other attendees sat in Grenova's AACC press conference this past summer. They discussed how their technology provides a smarter, cleaner and greener way to process samples using washed and reused consumable liquid handling tips. CEO and founder, Ali Safavi, stated the industry's tremendous waste is contributing to both land and air pollution. He reminded us that every patient sample uses multiple tips to get test results, resulting in millions of samples daily, and that over 40 million pounds of pipette tips annually end up in landfills.[2] Grenova's mission is to reduce lab consumable waste through innovative devices, aka reusable pipette tips. To learn more, visit https://www.grenovasolutions.com

I was also intrigued with the folks at Envetec. This company, a product of Technopath Clinical Diagnostics, is tackling healthcare's greatest environmental challenge: treatment and disposal solutions for Regulated Medical Waste (RMW). Their 4,000-pound, on-site, non-thermal technology challenges the status quo. Did I mention it's safe, quiet, clean, simple to operate and EPA-approved, too? Learn more at https://www.envetec.com

Research by the University of Exeter in the U.K. estimates that labs worldwide generate 5.5 million tons of plastic waste each year.[3] Perhaps inspiration can be gained from Francis Crick Institute (FCI), a biomedical research facility based in London. They have "sustainability reps," who, in addition to being scientists, also advocate for reducing plastic in their labs. FCI offers both sustainability workshops and waste training to employees, however, they know behavior change is only the beginning. An eco-friendly waste-management company is also an important ally.

Just recently, I reached out to a local hospital laboratory to see what they were doing recycle-wise. I was told they do recycle, however, it is mainly due to their hospital environmental services department. The lab use recycling bins for cardboard, styrofoam and plastics. Sounds pretty standard, right?

But is it enough?

What is your lab doing to embrace and/or support sustainability? What happens to your lab-generated waste? I want to hear from you. In the meantime, I'll be sourcing paper straws.

Please visit mlo-online.com for references.

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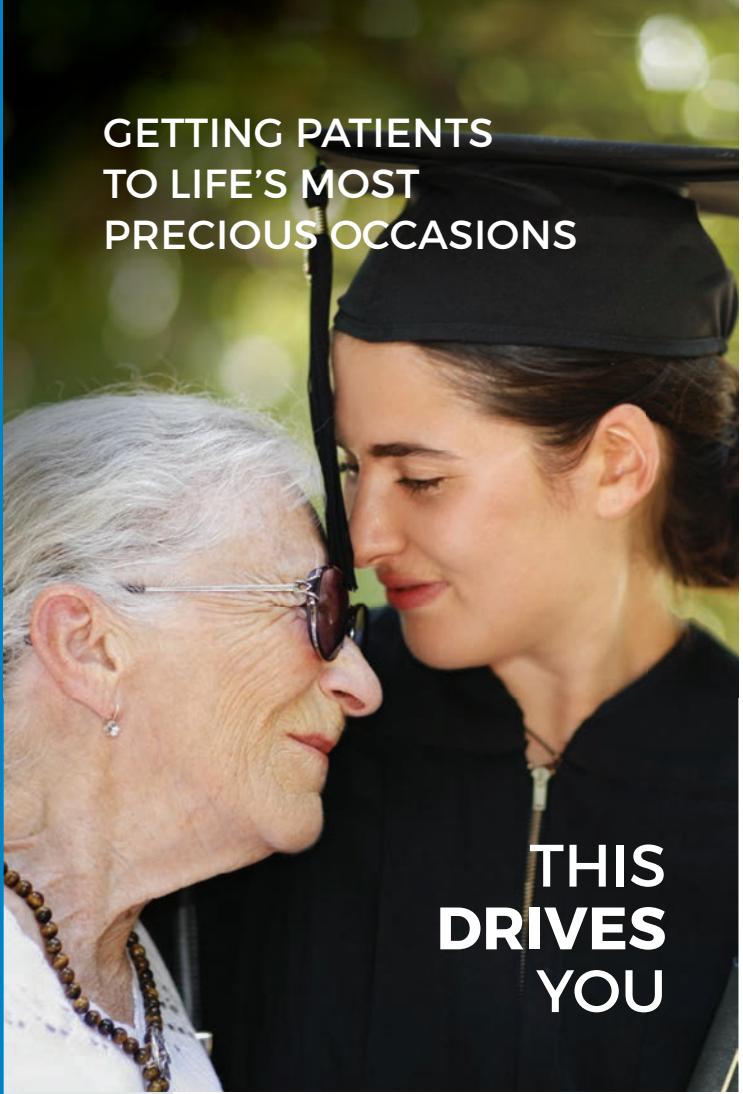

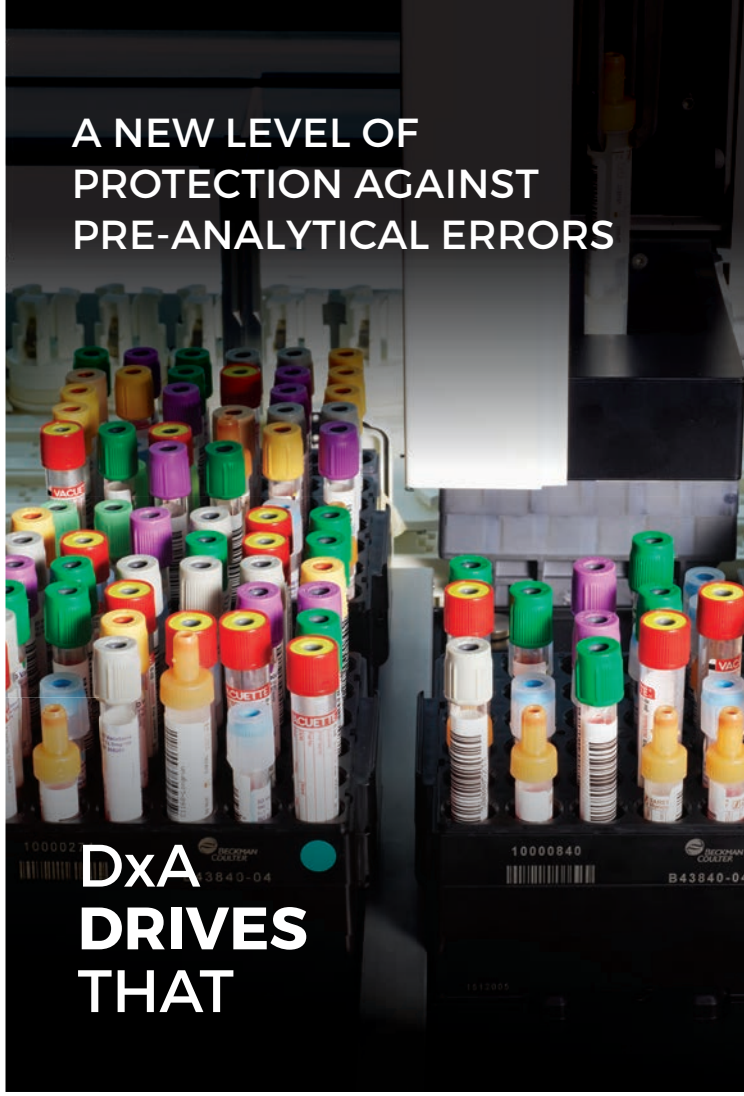

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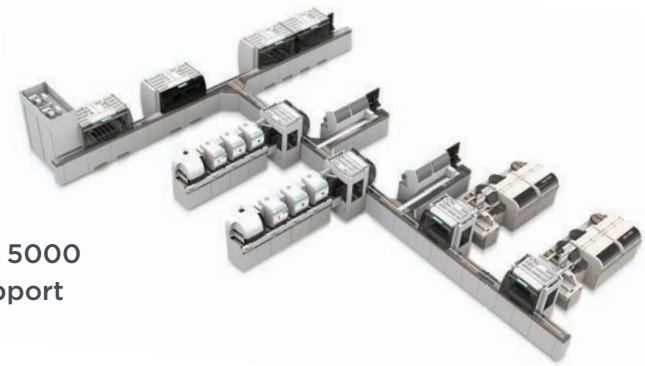

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FAST FACTS
Blood Supply

1 pint
is the amount of blood given during donation.

10 pints
is the amount of blood adults have in their bodies.

100 pints
is the average amount of blood a single car accident victim requires.

1 blood donation
can potentially save up to three lives.

Every 2 seconds
someone in the U.S. needs blood.

< than 38 percent
of the population is eligible to donate blood/platelets.

56 days
is the amount of time required between blood donations.

7 percent
is the number of people in the U.S. who have universal blood type "O."

1940
is the year Red Cross began collecting donations for patients.

500
is the number of blood drives Red Cross has every day.

• **Source:** https://www.redcrossblood.org/donate-blood/how-to-donate/how-blood-donations-help/blood-needs-blood-supply.html

Blood transfusions

Further restricting blood transfusion guidelines could save lives and money. Blood transfusion guidelines may require further evaluation with an eye to reducing transfusions for patients with stable blood pressure, according to Divyajot Sadana, MD, from the Cleveland Clinic, who presented the study findings at the CHEST Annual Meeting 2019 in New Orleans, LA.

The Joint Commission on Accreditation of Healthcare Organizations has identified blood transfusion as an overused procedure, and the American Association of Blood Banks (AABB) advises limiting transfusion to patients who are at risk of shock, cardiovascular failure, circulatory collapse and decreased blood flow, or who have a hemoglobin value of less than seven g/dL.

The researchers evaluated the necessity and outcome of transfusions by reviewing charts of the 888 patients who accounted for the 1,009 admissions to the medical intensive care unit (MICU) and required at least one blood transfusion from January 2015 to December 2015.

Although the Cleveland Clinic MICU closely adheres to blood conservation, a proportion of patients with stable blood pressures who received blood transfusions might have benefited from closer scrutiny. The researchers found that 13 percent of transfusions were prescribed in deference to transfusion guidelines and could have been potentially avoided.

"We identified a specific group of patients for whom blood transfusion can be safely avoided," explained Sadana. "Closer inspection, greater scrutiny and a vigorous investment in a restrictive transfusion practice could have significant implications on both financial and patient outcomes."

Michelle Cao, MD, member of the American College of Chest Physicians Scientific Presentations and Awards Committee and Clinical Associate Professor at Stanford University, CA, commented on the study: "Clinical practice guidelines are developed after an extensive review of the evidence in order to optimize clinical care and to standardize medical care. This study, although a retrospective outlook, shines light into the transfusion practices of intensive care providers in a typical tertiary hospital. Beyond the investigators' findings, it is worth taking a deeper dive into the rationale or conditions that contributed to medical professionals not adhering to recommended practice guidelines. Their decision-making has prognostic implications on morbidity, mortality and economic burden."

Blood collection safety

Final guidance document on bacterial safety standards for platelet collection and transfusion. Cerus Corporation applauds the publication of the FDA's final guidance document titled, "Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion; Guidance for Industry." The FDA has formally recognized the risk of transfusion-transmitted bacterial infections and the need for additional safety measures, including the use of pathogen-reduction technology, to protect patients.

"This is an important day for patients that require platelet transfusions," said Dr. Richard Benjamin, Cerus' chief medical officer. "It is estimated that approximately one in every 1,500 platelet units contain bacteria despite current testing protocols, thereby putting these often immunocompromised patients at risk of sepsis, including the possibility of death. The publication of the final FDA guidance document and the implementation of its recommendations will enhance the safety for platelet transfusions."

The FDA's guidance document outlines approaches that blood centers and hospitals will need to implement over a period of 18 months to reduce the risk of transfusion-transmitted bacterial sepsis. One approach that offers comprehensive protection against not only bacteria, but also emerging viruses like Zika, is pathogen reduction. Cerus' INTERCEPT Blood System is currently the only FDA-approved pathogen-reduction technology

to treat platelet components. The INTERCEPT system uses amotosalen, a well characterized photochemical compound that specifically binds to DNA and RNA of bacteria, viruses and other pathogens, rendering them inactive. Many of the nation's largest blood centers and academic hospitals, including the American Red Cross, have deployed the INTERCEPT system in advance of this final FDA guidance document.

Blood infections

Young infants with fever may be more likely to develop infections. Infants with a high fever may be at increased risk for infections, according to research from Penn State College of Medicine, PA.

In a new study, researchers determined that for an infant less than eight weeks old, having a fever was associated with a doubled risk for developing serious bacterial infections, including meningitis and infections of the blood or urinary tract. Based on the findings, the researchers encourage parents of infants younger than eight weeks old to seek immediate care if their child develops a fever (a temperature higher than 100.4 degrees Fahrenheit).

Dr. Joshua Davis, an emergency medicine resident at Penn State Health Milton S. Hershey Medical Center, said that identifying criteria that can increase an infant's risk for infection is crucial.

According to Davis, the study findings, published in The Journal of Emergency Medicine, are an exception to the traditional teaching in medical education that the height of a fever doesn't necessarily correspond to the likelihood of infection. This exception to the rule only applies to infants younger than two months old.

In working with his colleague Erik Lehman, a statistician at the Milton S. Hershey Medical Center, Davis discovered that as a temperature rises in infants younger than 60 days old, their risk for serious bacterial infection increases.

For the study, Davis used data from the Pediatric Emergency Care Applied Research Network study on infants with fevers. He studied 4,821 eligible infants with a single, recorded fever who had at least one blood culture completed. The infants included had no record of prematurity, sepsis, or recent antibiotic use.

The researchers evaluated three characteristics of the fevers—height, duration and location of where the temperature was taken—to see if any were associated with increased risk for infants developing a serious infection. Approximately 70 percent of the temperatures collected were taken at a health care facility instead of at home.

Duration and location were not associated with risk for infection, but there was a statistically significant relationship between height of the fever and risk of infection. According to Davis, serious bacterial infections occurred in 20.4 percent of infants with a fever, compared to 9.1 percent of infants with a lower temperature.

According to Davis, understanding the criteria that put infants at risk for severe infections might someday reduce the extensive panel of tests that infants with a fever undergo when visiting an emergency room. Spinal taps and bloodwork are just a few of the procedures that are done to assess for bacterial infection, and these may be painful or invasive to small infants.

Chemicals in blood

Hygiene products are associated with the presence of chemicals in women's blood. Women who use a vaginal douche could be at a higher risk of exposure to potentially dangerous chemicals, according to a University of Michigan (U-M) study that looked at the correlation between the use of female hygiene products and the levels of volatile organic compounds in women's blood.

The study found a significant association between vaginal douching and higher blood concentrations of 1,4-dichlorobenzene (DCB), a volatile organic compound (VOC). Because black women in the study reported significantly more use of vaginal douching, researchers believe they could be at higher risk of exposure to the chemicals and their negative effects.

According to the study, women who used a vaginal douche two or more times per month had concentrations 81 percent higher than those that never used a douche. Women who used douches occasionally (once a month) had 18 percent higher concentrations of the chemical.

VOCs are chemicals that are used in a wide range of products including deodorants, nail polish and paints. Some of these chemicals have been associated with respiratory symptoms, cancers and neurological disorders, as well as adverse effects in reproductive systems.

While additional studies are needed, women would be better off heeding the recommendation from the American Society for Obstetricians and Gynecologists (ACOG) not to use certain products, said Ning Ding, a doctoral candidate in epidemiology at U-M's School of Public Health and lead author of the study.

"While they are more concerned about disrupting the balance of bacteria in the genital area or interrupt the pH level, they have not focused on the toxicity of those endocrine-disrupting chemicals, which is really important and needs to be highlighted," said Ding, pointing out that 20-40 percent of women use this kind of product in the U.S. "I would recommend women not douche."

The study, published online in the *Journal of Women's Health*, uses data from a representative sample of 2,432 women aged 20-49 from the National Health and Nutrition Examination Survey 2001-2004. Participants were asked about their use of feminine products including tampons, sanitary napkins (pads), vaginal douches, sprays, powders and wipes/towelettes.

Researchers used regression models to estimate percentage changes in concentration of VOCs in blood to establish whether a dose-response relationship existed. Among the chemicals analyzed were seven VOCs: bromoform, bromodichloromethane, benzene, chloroform, dibromochloromethane, DCB and ethylbenzene.

In addition to the relationship between douching and DCB levels, researchers found that the use of feminine powder in the past month was significantly associated with higher concentrations of ethylbenzene.

A journey from tube- to patient-focused solutions

The evolution of laboratory automation

By Anthony M. Barresi

Today's clinical laboratory is evolving to keep pace with the changing reality of global healthcare. Laboratorians are seeing their roles shift from being technologists to first responders in the patient care pathway. This shift is being enabled, in part, with laboratory automation which is increasingly being used to reduce manual work, improve turnaround time, enhance quality and increase throughput. Depending upon the size of the laboratory, its needs and abilities, automation solutions can take many forms. Regardless of the specific role it plays, however, laboratory automation is beginning to emerge as a necessity for diagnostic laboratories seeking to dramatically improve performance. Despite this trend, currently, the number of laboratories around the world that have been able to embrace automation is still small.[3]

Defining automation

In 1998, automation was loosely defined as "any device, software or process that improves the efficiency of the laboratory."[1] In 2012, Kevin Olsen, author of, The First 110 Years of Laboratory Automation: Technologies, Applications and the Creative Scientist, offered a more specific description, "a complex integration of robotics, computers, liquid handling and numerous other technologies."[2]

Earning CEUs

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LEARNING OBJECTIVES

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

1. Recall the history of operating laboratories' shift from manual to automated.
2. Describe modular-based automation systems and the problems that arise by their design type.
3. Discuss pre-analytical, analytical and post-analytical errors and how automated solutions resolve these errors.
4. Discuss cloud-based automation and its benefits in the modern day laboratory.

Most important, while definitions and technologies may have changed over time, the reason organizations choose automation solutions has remained unchanged for a century; that is, "to save time and to improve performance."[2]

The most popular form of automation, in the traditional sense of the word, is total laboratory automation, which is primarily used in large core or reference laboratories. Medium-sized laboratories, which are faced with challenges similar to their larger colleagues, are also trying to harness the power of automation. However, due to lack of space, low volume and economic pressures, these labs tend to use the most basic kind of automation—an integrated workcell. This certainly represents an improvement over laboratories that are manual, but it's still a limited solution that does not allow laboratories to fully exploit the benefits of automation and thrive in today's value-based healthcare environment.

A short history of laboratory operations

The first automated laboratory system was introduced into the chemistry laboratory by Leonard Skeggs, an American biochemist best known for inventing the AutoAnalyzer in 1956.[5] It offered automatic analysis of blood from start to finish without the need for much manual intervention.[6]

Since that time, traditional automation has been seen as technology that helps move tubes through parts of the laboratory workflow. In 1981, Japan was first to implement total laboratory automation in its clinical laboratories.[7] This solution relied on robotics and conveyer mechanisms and was referred to as systemized automation.[7] Sixteen years later, in 1997, the first total laboratory automation solution was implemented in North America.[1]

By 1998, it was estimated that only eight percent of laboratories could afford the expense of total automation, but that 100 percent of laboratories could benefit from implementing at least some automated systems, even if on a smaller scale.[4] The industry's answer to this disparity was to offer a modular solution in the way of "consolidated analyzers, integrated analyzers, modular workcells and pre- and post-analytical automation."[4]

Workstations, the "most basic unit of automation," feature automated clinical analyzers for general chemistry or immunoassay testing, or both.[4] Workcells offer integrated clusters of analyzers that perform like a single system, and can be managed by one technologist.[4] Physical workcells are typically connected via small tracks with one inlet/outlet module used for loading and unloading samples, while virtual workcells are groups of systems linked digitally, by middleware.[4]

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The problem with a modular approach

Most laboratories consider automation using an either/or approach; choosing between total laboratory automation or modular workcells, with system costs and space constraints remaining the primary drivers in the decision. For many facilities—especially small- and medium-sized laboratories—total laboratory automation remains cost- and space-prohibitive. However, workcell solutions can leave gaps that expose vulnerabilities or represent lost opportunities for offsetting cost pressures and labor shortages. For example, integrated chemistry-immunoassay workcells may handle various tasks, such as routing samples between analyzers, performing analyses and conducting results reviews, but this does nothing to address the dozens of other time-consuming steps in a laboratory's workflow. As a result, laboratories remain overexposed to possible human error, and potential productivity gains are left unexploited. Safety isn't improved either, with laboratory personnel left unnecessarily vulnerable to repetitive motion injuries and biohazardous substance exposures.

Single vs multiple points of sample entry

Modular automated solutions often bundle testing, and use a single point of entry for all samples. While a single point of entry may appear to help simplify operation, disruptions can occur when the sample introduction module becomes unavailable for any reason. Recognizing this design vulnerability, innovative companies have begun offering integrated solutions that feature multiple points of sample entry, helping laboratories preserve uptime with sample introduction redundancy.

Notwithstanding such design improvements, workflow slowdowns are still a risk inherent to bundled chemistry and immunoassay systems. Immunoassay preparation typically takes time. In fact, immunoassay testing can take two to five times longer than chemistry testing. Managing both immunoassay and chemistry samples with one automated track means that chemistry tubes are not released until immunoassay tests complete their cycles. In effect, access to the chemistry-only tubes is blocked by the longer cycles of tubes requiring immunoassay testing.

Insufficiencies like these highlight the need for laboratories to evaluate factors beyond the workcell when considering automation. They also emphasize the need for scalable automated solutions that are

capable of optimizing workflow outside the realm of traditional automation systems. For example, independent analyzers connected by multi-lane tracks and intelligent routing enable chemistry and immunoassay tubes to be released as soon as their respective testing is completed, ensuring efficient workflow and timely results delivery.

To achieve breakthrough performance improvement and cost reduction, innovative automation solutions are needed; solutions that leverage not only robots, tracks or workcells to move tubes, but also algorithms, analytics and the cloud to move data.

Automating for improved patient care

The pre-analytical phase of testing is actually the most labor-intensive and error-prone, typically consuming 60 percent of a laboratory's labor hours and accounting for up to 75 percent of its errors.[8] Moreover, approximately 13 percent of errors may have an effect on patient health.[8] Accordingly, automation innovators have come to view automation of pre-analytical activities as an effective means of significantly improving laboratory operations and, thus, patient care.

In an article written over a decade ago, Errors in Laboratory Medicine, Mario Plebani, professor of clinical biochemistry and molecular biology at the University of Padova School of Medicine and department chief of Laboratory Medicine at the University Hospital of Padova, Italy, addressed the issue of lab safety. He noted, "technological solutions for pre-analytical processes, such as order entry, barcoding identification of patient and related samples and information sharing, had the potential to make the laboratory safer."[9]

With this in mind, new pre-analytical automated systems are being designed to perform comprehensive specimen inspections in just seconds, helping laboratories prevent potentially wasteful pre-analytical errors (e.g., mislabeled tubes, insufficient sample quantities and wrong tube types) from entering their analytical workflow. The most sophisticated of these emerging capabilities enable laboratories to automate identification of tube and cap color, sample volume checks, patient identification verification, spin status detection and sample tube weight, while also capturing an image of the tube. Protections for both technologists and patients are offered with these advancements. In addition, they give technologists more time to focus on high-impact patient-care activities.

Meaningfully harnessing the power of massive data in laboratories is another way laboratories can improve patient care. The most cutting-edge clinical informatics technology has begun to merge seamlessly with mechanical automation systems. As Plebani stated, "more effective integration between

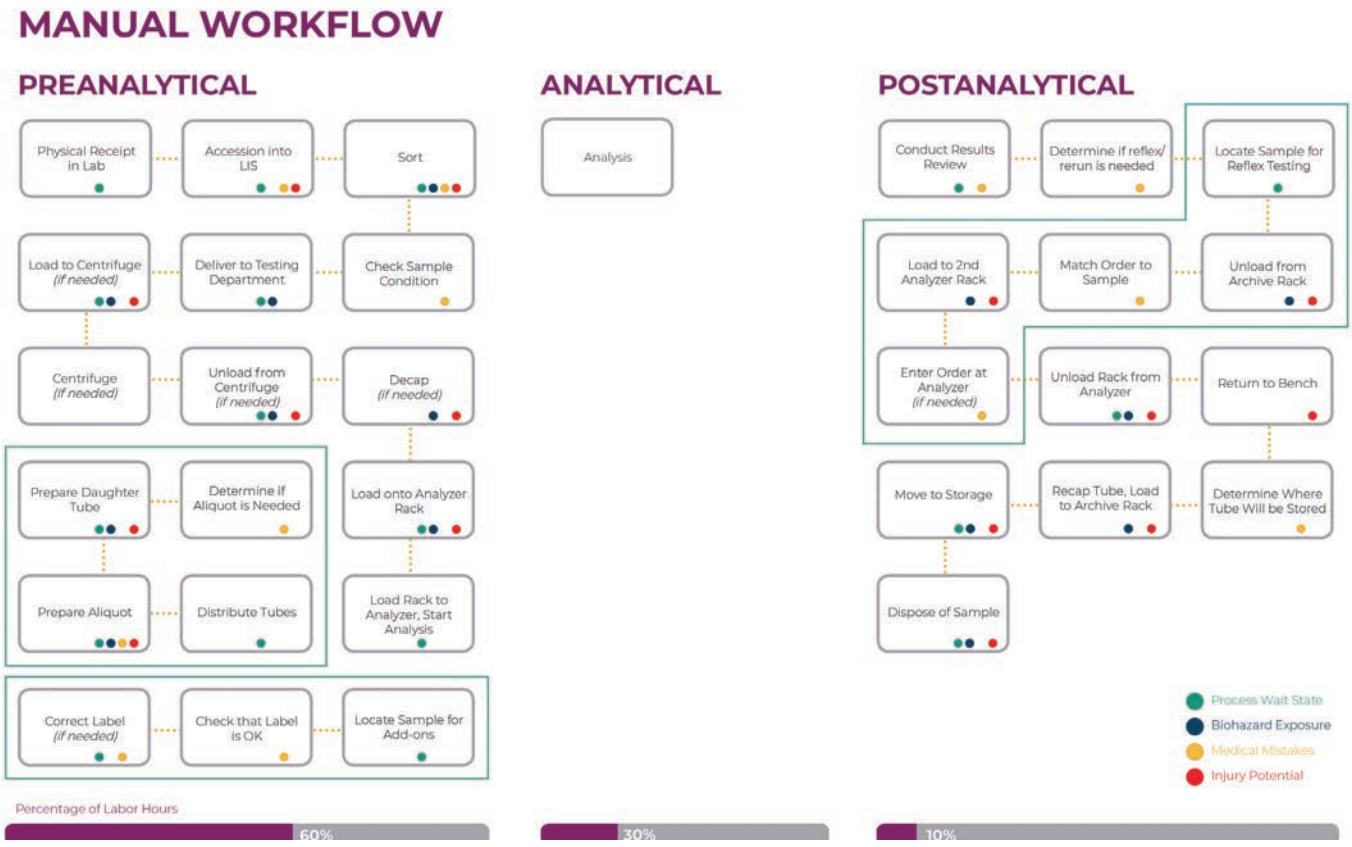

Figure 1. Typical manual workflow in a clinical laboratory.

New FDA Regulations, Hospital Glucose Meters

FDA Product Code PZI, 2019:
"Blood Glucose Meter for Near-Patient Testing"①

FDA Product Code NBW, 2016:
"Blood Glucose Test System, Over the Counter."② **"These device types are not intended for use in healthcare or assisted-use settings such as hospitals, physician offices, or long-term care facilities because they have not been evaluated for use in these professional healthcare settings."**③

Use of a meter cleared by the FDA as NBW is considered "OFF LABEL" when used anywhere in a hospital. Is your hospital glucose meter cleared as FDA Product Code PZI for hospital use or NBW cleared and off label for hospital use?

Stat Strip® GLUCOSE

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Intended for use in near-patient testing.

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① U.S. Food and Drug Administration. Product classification [Product Code PZI]. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/classification.cfm?id=678

② U.S. Food and Drug Administration. Product classification [Product Code NBW]. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/classification.cfm?id=631

③ U.S. Food and Drug Administration. Self-monitoring blood glucose test systems for over-the-counter use. Draft guidance for industry and Food and Drug Administration staff. Silver Spring, MD: 2018.https://www.fda.gov/media/119828/download

automation and information management is crucial for assuring process controls that allow [laboratories] to identify and improve on the critical steps in pre-, intra- and post-analytical phases."[9]

Furthermore, recent advancements in cloud-based technology have greatly helped laboratories in their pursuit to optimize all operations that impact their workflows. For example, a time-consuming task for any laboratory is inventory management. When there are inefficiencies in managing inventory, operations are affected, workflow is disrupted, the ability to produce results is jeopardized and unplanned costs are incurred. By automating inventory management through cloud-based applications, however, laboratories are now able to mitigate these effects, obviate administrative tasks and devote more time to patient care.

Automation for all

In the past, some laboratories, especially smaller ones, have lacked the space and budget required for systems that automate laboratory data flow. But with industry forces making access to automation increasingly critical to successful performance, subscription-based laboratory management systems that use cloud technology to save space and reduce cost have begun to grow in popularity. These new systems bring the benefits of automation to laboratories of any size—including labs that are smaller—and offer a user-friendly, reliable solution for managing and accessing data from clinical laboratory instruments. They provide the same benefits as traditional automation, such as improved turnaround times, reduced errors and improved efficiencies—but they achieve these results by automating data flow. They have either a small or non-existent footprint, low upfront cost and require less or no involvement from IT departments.

Automating for the future—from today to tomorrow

The healthcare paradigm is shifting from one that is procedure-based, to one that is value-based—this is changing how clinical laboratories work. Laboratories are finding that to adapt to this change in the healthcare environment, they must adopt a broader, future-focused approach that enables them to achieve greater time and cost savings. This allows staff members to focus on priorities that directly contribute to improved patient care.

The focus on value-based care is not a surprise as scientists and leaders have been predicting it for years. Dr. Peter Wilding, an award-winning pathologist, declared over a quarter of a century ago in his clinical chemistry article, The Changing Role of the Clinical Laboratory Scientist: Coming Out of the Basement. "In the future, laboratory scientists must align their expectations to the demands for new technologies, medical practices and healthcare systems that will require justification for all activities, expense and personnel."[10]

Today, healthcare entities have shifted their focus from products and procedures to therapeutic and value-focused solutions, and the role of laboratorians has transitioned from that of service providers to problem solvers. However, the number of problem solvers is

Case Study I

Result: Reclaiming one day a month by automating inventory management: a time decrease from seven hours to five minutes.

Mason District Hospital in Havana, IL, is a 25-bed critical-access facility, processing up to 200 billables per day with a staff of four technicians. There, lab staff members were spending an average of seven hours a month on inventory management tasks. Despite this, products were often out of stock or expired, which resulted in the amassing of unplanned expediting and repurchasing costs. By automating inventory management processes, the lab has not only reduced its inventory-related tasks to just five minutes a month, it has done so while keeping stock reliably on hand, saving costs and providing consistent, quality patient care.

Case Study II

Result: Achieving an 80 percent auto-verification rate with cloud-based middleware.

Faced with significant resource challenges, Jefferson Memorial Hospital, a 37-bed acute-care facility processing 50,000 samples per year in Louisville, GA, sought to improve turnaround times (TAT) and lab efficiency. By implementing a cloud-based middleware system, the lab was quickly able to achieve an 80 percent auto-validation rate. With eight out of 10 results going to the LIS without staff intervention, the lab was able to alleviate pressure from staffing shortages. In addition, with technologists only needing to manage results by exception, the lab realized a 27 percent improvement in TAT shortly after installing the system. Excitingly, these already-impressive, auto-validation and TAT results have continued to improve as the staff has continued to tailor the system to its specific needs.

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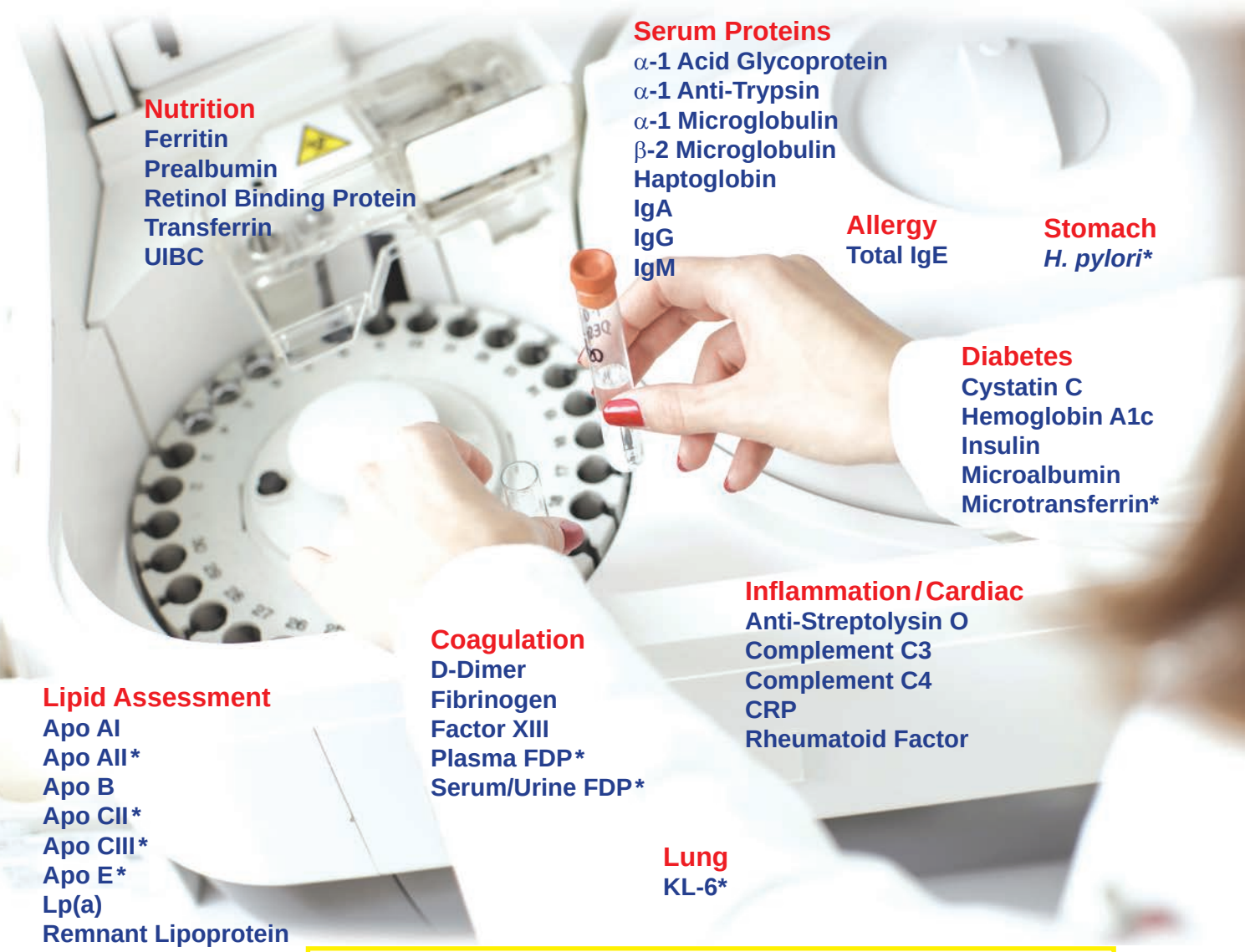

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diminishing. In 2016, the need for new medical laboratory staff workers was forecasted to be 12,000 per year, with only an estimated 5,000 new laboratory technology students graduating annually.[11]

The resulting labor shortage, coupled with the pressure of value-based affordable healthcare and the increasing demand for testing, is leading laboratorians to rethink how they work.

While laboratory processes can differ, a typical pre-analytical, analytical and post-analytical workflow has more than 31 distinct steps **(Figure 1)**. In most labs—particularly in small- and medium-sized facilities—many of these steps are still performed manually. This presents a problem. Simply put, if highly trained medical technologists are occupied with executing repetitive, labor-intensive and error-prone tasks within the workflow, they will not have time to collaborate with clinicians and directly contribute to improving patient care.

Conclusion

When looking at automation solutions, laboratories of all sizes should consider future capabilities and ensure platforms are designed to accommodate growing test volumes, expanding menus and static or diminishing resources. To improve performance across *all* aspects of their operations, laboratories should seek automation solutions that comprise the integration of reliable instruments, intelligent track systems and cloud-based clinical informatics portfolios. Rethinking automation in this way will enable technologists to take a more influential role as patient-care team members and empower laboratories to expand into new disciplines as the needs of patients and healthcare organizations change.

Automation is about more than reducing the number of process steps and expediting the physical movement of tubes. It is about improving the ability of today's laboratories to meet increasing demands by focusing on work that yields the greatest benefits for patients and healthcare organizations. What is most beneficial to tackling today's laboratory challenges is a total workflow-optimization solution—a multifunctional approach that integrates instruments, tube movement and data management to automate pre-, intra- and post-analytical processes, as well as the work necessary to support them. By adopting this approach to automation, laboratories may be able to overcome resource constraints, manage increasing workloads, deliver accurate results and partner with clinicians to ensure patients receive the best healthcare possible.

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TEST QUESTIONS

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1. **The most common form of automation in clinical laboratories is being used to**
   - a. enhance quality, increase throughput and improve turnaround times.
   - b. reduce manual work, enhance quality and increase throughput.
   - c. reduce manual work, increase throughput and improve turnaround times.
   - d. reduce manual work, enhance quality, increase throughput and improve turnaround times.

2. **Total laboratory automation is used for**
   - a. small laboratories.
   - b. medium-sized laboratories.
   - c. large core or reference laboratories.
   - d. none of the above

3. **Medium-sized laboratories typically still use manual laboratory processes.**
   - a. True
   - b. False

4. **In the late 1990's, the automation industry responded to the cost burden of hospital labs by offering modular solutions which consist of**
   - a. consolidated analyzers.
   - b. integrated analyzers.
   - c. physical/virtual workcells.
   - d. all of the above

5. **Clusters of analyzers that are linked by middleware describes**
   - a. physical workcells.
   - b. virtual workcells.
   - c. cloud-based automation.
   - d. all of the above.

6. **The first automated lab was introduced to the _____ department in _____.**
   - a. microbiology, 1956
   - b. chemistry, 1997
   - c. hematology, 1997
   - d. chemistry, 1956

7. **North America implemented total automation in its clinical laboratories in**
   - a. 1956.
   - b. 1976.
   - c. 1981.
   - d. 1997.

8. **Automated system costs and space constraints are the main concern when determining between total laboratory automation or modular workcells.**
   - a. True
   - b. False

9. **The main shortcoming(s) of modular workcells is/are**
   - a. exposure to biohazardous substances.
   - b. exposure to increased human error.
   - c. lack of productivity gains.
   - d. all of the above

10. **Workflow slowdowns pose a major risk to**
    - a. hematology analyzers.
    - b. chemistry/immunoassays analyzers.
    - c. coagulation analyzers.
    - d. microbiology analyzers.

11. **Improvements in analyzer performance and cost reduction need to include algorithms, analytics and cloud systems to move data.**
    - a. True
    - b. False

12. **Which phase of testing is the most labor-intensive and error-prone?**
    - a. pre-analytical
    - b. analytical
    - c. post-analytical
    - d. all of the above

13. **Pre-analytical testing accounts for _____% of its errors.**
    - a. 10
    - b. 25
    - c. 55
    - d. 75

14. **Preanalytical errors include all but**
    - a. mislabeled tubes.
    - b. wrong tube type.
    - c. failure to post results.
    - d. insufficient sample quantities.

15. **Sophisticated automated capabilities that assess pre-analytical error include the following variables**
    - a. patient identification verification, spin status detection, sample tube weight and image capture of the tube.
    - b. identification of tube and cap color, sample tube weight and image capture of the tube.
    - c. identification of tube and cap color, sample volume checks, patient identification verification and spin status detection.
    - d. identification of tube and cap color, sample volume checks, patient identification verification, spin status detection, sample tube weight and image capture of the tube.

16. **In the case study, cloud-based technology has offered the ability to reduce administrative inventory tasks by**
    - a. > 1 day.
    - b. > 12 hours.
    - c. > 6 hours.
    - d. none of the above

17. **In the case study involving the implementation of auto-verification, the hospital found**
    - a. 80% auto-validation rate and 27% improvement of turnaround time.
    - b. 80% auto-validation rate and 72% improvement of turnaround time.
    - c. 27% auto-validation rate and 80% improvement of turnaround time.
    - d. 27% auto-validation rate and 72% improvement of turnaround time.

18. **In small- and medium-sized laboratories, there are over _____ steps in pre-analytical, analytical and post-analytical tasks that are performed manually.**
    - a. 10
    - b. 20
    - c. 30
    - d. 40

19. **The shortage of medical laboratory scientists and the need of value-based affordable healthcare are the most concerning factors in implementing sophisticated automation technologies into the current laboratory settings.**
    - a. True
    - b. False

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Flexible testing can help navigate the latest IDSA guidelines for C. diff infections

By Christina Olivas, BSc and Brian Harrel, MS

While scientific developments and perceived clinical significance are what fundamentally drive breakthroughs in the healthcare world, it is widely accepted that reimbursement is a key factor in determining which products make it to market, as well as how accessible those products are to both healthcare providers and the patients who need them.[1] Additionally, investment in and development of healthcare drugs, technologies and medical devices often hinge on uncertain circumstances, such as the future need for a product, and how actions by health coverage programs will drive future reimbursement for a technology. Taken together, these factors create a cycle in which reimbursement policies compete with clinical need to determine which healthcare technologies or pharmaceuticals are invested in and developed.

Social health coverage programs and private health insurance plans—or payers—such as the Centers for Medicare and Medicaid Services (CMS) are the key drivers for establishing reimbursement policies. They define what therapeutics and technologies will be covered as part of beneficiary services, as well as what terms must be met in order to receive that coverage.[1] Once CMS establishes these terms, private payers may incorporate publicly available policy information to determine the scope of their healthcare coverage, adding an additional loop to the healthcare cycle.

Flexible testing is one of the latest solutions for navigating reimbursement challenges, dwindling resources and time-consuming diagnostic procedures. As it becomes more widely available, flexible testing will allow clinicians to order one diagnostic panel, while paying for only the targets they suspect, which will provide improved patient care and a higher likelihood for reimbursement.

Who decides what is best for patients?

To enable clinicians to deliver the best patient care possible, the Infectious Diseases Society of America (IDSA) publishes guidelines on infectious disease testing and treatment practices, and serves as the biggest advocate for individual patient needs. The IDSA is composed of thousands of physicians, scientists and public health experts who use empirical data to establish these guidelines, influencing the management and prevention of infectious diseases. Additionally, the IDSA works with federal agencies, Congress and labs across the country to help "protect diagnostic innovation and patient access to care."[2] These guidelines, in conjunction with CMS-issued policies, determine which diagnostic tests and healthcare technologies are considered medically necessary and reasonable, assisting in compliance with reimbursement policies.

Even with the IDSA's recommendations, healthcare providers still face several challenges when navigating the gray area between providing treatment and ensuring financial solvency. This is especially true when molecular diagnostics are used for gastrointestinal (GI) illnesses. Because of the numerous sources for contracting a GI infection and the similarity of symptoms across multiple illnesses, the diagnostic method for GI infections is often a process of elimination. Although many GI illnesses can resolve without treatment, some pathogens, like *Clostridium—or Clostridioides—difficile (C. diff)*, can lead to serious complications if left untreated. Further, because gastrointestinal diagnostics have historically required multiple testing methods, the clinical need for a customizable assay has become increasingly apparent.

The role of molecular diagnostics in healthcare

In the last decade, clinical microbiology has significantly evolved, and the diagnostic arsenal now includes molecular syndromic panels that can simultaneously detect and identify multiple pathogens associated with bloodstream, gastrointestinal and respiratory infections. These tests have enabled better patient outcomes due to faster turnaround times (TAT) and improved sensitivity,[3] which affects everything from the decision to admit a patient to hospital infection control strategies, the recommendation for a specific antibiotic therapy or the avoidance of antibiotics altogether, and how long it takes to get a patient on the most appropriate treatment regimen.

These developments have helped propel a shift away from traditional diagnostic testing methods such as culture or microscopy, because long TAT, low specificity and sensitivity, as well as laborious workflows, tend to increase long-term costs. This is often the case if the first diagnostic test does not yield a positive result and additional testing is required. While syndromic panels can cost more up front than their predecessors, they lead to downstream savings due to a simplified workflow, increased diagnostic yield, improved specificity and a higher likelihood of getting an actionable diagnosis—faster.[3] Because of these benefits, molecular syndromic testing is expected to become increasingly common in the coming years.[4]

Prior to 2016, there were no official policies for how syndromic assays should be used. Since then, policy has evolved to recommend testing based on whether a given test will positively affect patient care. To demonstrate some of the potential circumstances clinicians may face when considering how to test for a suspected case of infectious gastroenteritis, we will discuss three scenarios where a patient's symptoms and epidemiology drive the choice for testing, as well as the application of flexible testing as a solution to navigate some of these scenarios.

Scenario 1: When a full panel is the right call

There are some cases when reimbursement for a complete panel is clearly justified. For example, an immunocompromised patient who presents with nausea, frequent diarrhea and fever would likely be screened for the entire molecular panel up front, due to an increased clinical need to manage their symptoms as quickly as possible. This testing might include the most common pathogens linked with diarrheal symptoms, such as *C. diff, norovirus* and *Giardia*, as well as some of the less common viral, bacterial and parasitic targets.

In this way, reimbursement for diagnostics is linked to the clinical needs of the patient and the likelihood that a positive diagnosis can change the patient's outcome. However, in general, there has been a shift away from paying for the full panel of testing when a diagnosis won't necessarily change the course of action for the patient.

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Scenario II: Making a run for better diagnostics

A patient who has recently undergone shoulder surgery and was given perioperative antibiotics presents with persistent diarrhea and a fever. According to the 2017 IDSA guidelines, considering the patient's recent antibiotic treatment, the most likely pathogens responsible for the patient's symptoms are *C. diff* or *Salmonella*. *C. diff* is known to account for 20-30 percent of antibiotic-associated diarrhea,[5] and is the most common cause of infectious diarrhea in healthcare settings.[6] Further, some studies have reported that as many as 96 percent of patients with symptomatic *C. diff* infection had received antibiotics within two weeks before the onset of diarrhea, and all of the patients who had symptoms of *C. diff* infection had received antibiotics within the last three months.[7-9]

Because only two pathogens are suspected, a clinician would be forced to either order separate tests for these targets or order a complete molecular panel. In the event that the molecular panel lacked *C. diff*, this test would also have to be ordered separately. However, this also has the potential for reimbursement challenges. Since reimbursement for the entire panel is not easily justifiable, the patient could be responsible for paying more than they may expect or the clinician could defer to traditional testing methods, which would delay time to therapy up to 92 hours and potentially circumvent critical infection control measures.[10] For this patient, a flexible testing solution would enable the doctor to order a test with the suspected targets only, as opposed to a pre-established panel that has more targets than are clinically necessary, at a much higher cost.

Scenario III: A trip to remember

A patient has recently returned from Thailand and presents with a low-grade fever, stomach pain and has had prolonged diarrhea since the last week of their trip. Traveler's diarrhea is common, with infection rates between 30-70 percent, depending on the destination and the time of year.[11] Because of their travel history, this patient may be infected with several different pathogens. In this case, stool testing would be the primary method for detection to narrow down the list.

The prescribing doctor would initially order a screening panel for common causes of traveler's diarrhea, including *E. coli, Salmonella, Campylobacter, Shigella* and *Vibrio*. If the panel came back negative for all the selected pathogens, the doctor would then need to expand the search. For this patient, a flexible testing solution could permit the doctor to simply reveal additional targets instead of having to retest the original specimen, saving valuable time and resources.

Flexible testing - a gateway towards the future

The application of flexible testing in syndromic panels is a recent innovation that is gaining momentum. It was developed to address the clinical need for customizable testing when a patient's symptoms could be due to several different pathogens. A flexible testing option would enable technicians to perform one assay and then pay to reveal additional results. For example, a physician may order a targeted molecular panel that returns all negative results. Instead of performing an additional test, the physician would request to reveal additional targets on the panel that was already performed, which would be reimbursed, provided a positive result would change the patient's treatment strategy.

In conclusion

In concert with the latest IDSA guidelines for GI illnesses, flexible testing can help clinicians or physicians navigate the different scenarios that are encountered when screening for infectious diseases, and the examples highlighted here address the need for different levels of diagnostic flexibility. As molecular diagnostics and policies concerning reimbursement for these tests continue to evolve, it will be imperative that the future of healthcare has flexible solutions to navigate the unique needs of every patient. Customizable testing, based on an individual's symptoms, will lead to improved patient outcomes by reducing time spent re-running tests, decreasing the time to therapy, improving infection control management and increasing operational efficiency at healthcare facilities and diagnostic labs. Faster time to result translates into better patient care, including improved treatment strategies, in a clinically actionable timeframe. Flexible testing synergizes with the latest IDSA GI testing guidelines, as well as current guidance for reimbursement. Because of the potential for downstream savings, it's likely that tests with flexible target selection will lead to improved long-term clinical, economical and operational benefits for the entire healthcare system.

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The prevalence, pathogenesis and diagnostics of celiac disease

By Yara Burmeister

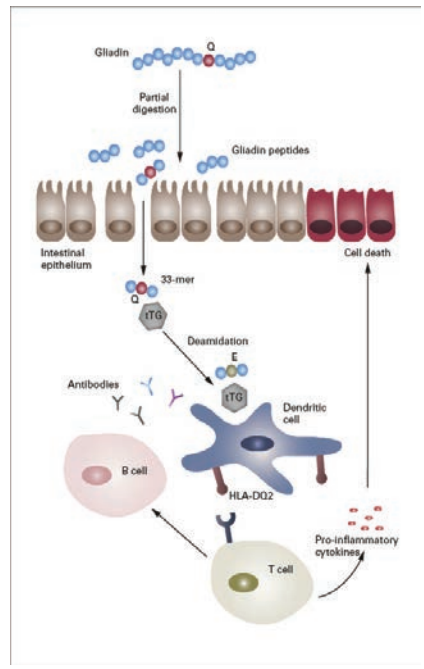
While biopsies for celiac disease (CD) remain the standard screening practice in the United States, European gastroenterologists and laboratories are steadily moving away from this invasive procedure, except to clarify particularly difficult cases. One of the key reasons for this change in diagnostic approach is the multitude of different serological assay methods now available to labs. Many of these blood-based assays have been developed with greater specificity and sensitivity than ever before, enabling standard diagnostic criteria to be based on detecting CD-specific antibodies. Specifically, those against tissue transglutaminase (tTG-IgA) and deamidated (modified) gliadin peptides (DGP), immunoglobulin (Ig)A and IgG.

Celiac disease

CD is a lifelong, gluten-sensitive autoimmune disease deriving from environmental (gluten) and genetic factors (human leukocyte antigen [HLA] and non-HLA genes). CD is becoming more prevalent and common in developed regions of the world, such as the U.S. and Europe, as increased awareness and detection of the disease continues to grow. The disease presents with gastrointestinal symptoms, non-gastrointestinal symptoms or no symptoms at all. It usually manifests with severe gastrointestinal problems such as diarrhea, vomiting, abdominal pains and cramps. However, about half of CD patients present with non-gastrointestinal symptoms, which can include anemia, osteoporosis, skin conditions and weight loss.

CD was originally thought to affect European populations exclusively but is currently prevalent around the world. Areas such as South America, Asia, the Middle East and Africa, previously thought to be unaffected by CD, are now believed to have been underdiagnosed, contributing to the notion that CD is becoming one of the most common autoimmune and genetic diseases.

It is thought that CD generally followed humans' evolutionary migratory and dietary flows—from once feeding primarily on meat, fruits and vegetables, to the development and prominence of farming, when gluten-containing cereals such as wheat, barley and rye became an integral part of a diet. As human expansion continued, farming became more of a staple way of life and agricultural practices, as well as the gradual replacement of indigenous inhabitants, introduced genetic anomalies into the population leading to increased gluten sensitivity.



Pathogenesis of celiac disease.

Disease pathogenesis

CD is typically activated by a combination of environmental and genetic causes. The main genetic factor derives from the HLA-DQ genes, DQ2 and DQ8. In genetically pre-disposed individuals, disease of the intestinal tract (enteropathy) is triggered by the immune system's over-response to the prolamins within gluten, particularly gliadin. Gliadin peptides are not fully digested in the small intestine, enabling the gliadin remnants to be taken up into the intestinal wall and the surrounding intestinal connective tissue. At this point, the enzyme tTG deamidates (modifies) the gliadin by converting the amino acid glutamine into glutamate. The DGP affect intestinal permeability causing an immune reaction in CD patients because of their resistance to gastrointestinal enzymes. This process results in the production of antibodies against DGP and the bodies' own tTG, and the secretion of inflammatory cytokines. The inflammation of the small-intestinal epithelium leads to atrophy of the intestinal villi. This cascade results in swelling of the small intestine and the variety of gastrointestinal symptoms mentioned above.

CD-specific antibodies and diagnosis

In addition to HLA genetic predisposition, tTG autoantigens play a crucial role in CD pathogenesis. Autoantibodies against tTG are a very sensitive and specific marker for CD. They can be detected by monospecific immunoassays, such as antigen-coated ELISA or immunoblot, or indirect immunofluorescence tests (IIFT). tTG mediates deamidation of gliadins, creating an epitope that binds efficiently to DQ2 and is recognized by gut-derived T-cells. Antibodies against these deamidated epitopes of gliadin fragments are specific for CD. Antibodies against native gliadin, however, are less specific and are no longer a determinant as they are frequently also found in healthy individuals. Anti-DGP antibodies can be determined by ELISA, immunoblot or monospecific IIFT assays.

The prolamin trigger

Gluten is a protein found in cereals such as wheat, barley, rye and oat, amalgamating the proteins prolamin and glutelin. Most of the proteins in food that are responsible for immune reactions in CD are the prolamins. Because of their high glutamine content and certain specific sequence patterns, prolamins are resistant to gastrointestinal enzymes that protect the small intestine from autoimmune response. The incomplete

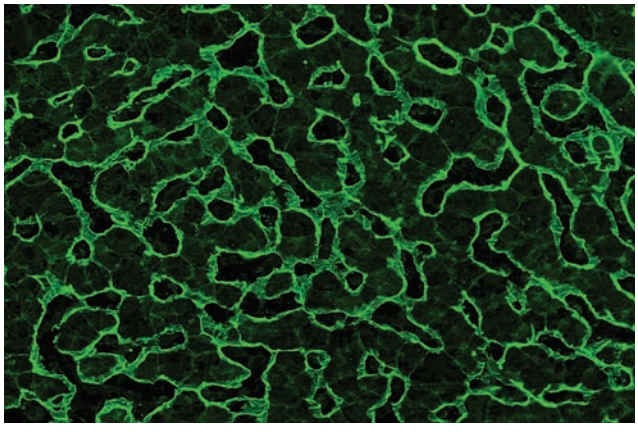
gastrointestinal digestion of the gluten leads to the presence of gluten-derived gliadin peptides. The ingestion of prolamins from cereals causes changes in the small intestine mucous membrane of celiac patients leading to a syndrome of poor absorption, causing an autoimmune response after the ingestion of gluten. When screening for CD, it is mandatory that the individuals are on a regular gluten-containing diet as the CD-specific antibodies disappear when on a gluten-free diet.

New ESPGHAN diagnostic guidelines

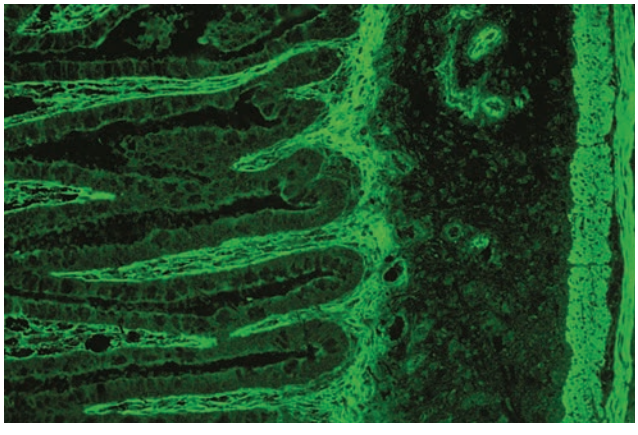
In September 2019, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published updated guidelines for the diagnosis of CD. Since guidelines were last released in 2012, there has been the development of more sensitive and specific assays to detect antibodies leading to a refinement in thinking about diagnostics testing for CD. Among the new guidelines are recommendations that, for initial testing, consider the combination of total IgA and IgA-class antibodies against transglutaminase 2 (TGA-IgA) to be more accurate than other test combinations. If the test is positive, then a confirmatory test is encouraged; if both tests are positive, then a biopsy is no longer recommended.

Regarding pediatrics, a no-biopsy approach for CD diagnosis is safe in children with high TGA-IgA values (≥10 times the upper limit of normal) with appropriate tests and positive endomysial antibodies (EMA-IgA) in a second serum sample. If TGA-IgA is ≥10 times the upper limit of normal (10xULN) and the family agrees, a no-biopsy diagnosis need be applied, provided that endomysial antibodies (EMA-IgA) test positive in a second blood sample. However, children with positive TGA-IgA but lower titers (<10 times upper limit of normal) should undergo biopsies to decrease the risk of false positive diagnosis.

HLA testing and presence of symptoms are not compulsory criteria for a serology-based diagnosis. Presence of HLA-DQ2 and/or DQ8 does not constitute a diagnosis of CD, nor does it mean one will ever develop it. However, carrying HLA-DQ2/DQ8 increases the risk of developing CD from the overall average of one to three percent of the population.



Detection of EmA by IFA on liver tissue.



Detection of EmA by IFA on small intestine tissue.

The current diagnostic landscape

The current environment of laboratory testing can be quite challenging for laboratories in the diagnosis of CD simply because of the recent burgeoning and availability of a variety of methods and approaches. Very different testing systems now exist with diverse diagnostic algorithms to test for the disease, so it becomes increasingly difficult for labs to decide how to implement various modalities into their routines.

For example, the highly specific indirect immunofluorescence test (IIFT) allows for the use of a variety of different tissues, including small intestine and liver. Different tissues may require a different set of experiences and skills in reading the fluorescent patterns from the tissues. In monospecific assays, such as ELISA and immunoblot, there are different parameters including the anti-tTG and anti-DGP testing, as well as different combinations of these parameters. Assays that specifically test for antibodies against deamidated gliadins also represent a major advance in diagnostic testing. These tests are more sensitive and accurate in determining CD in that deamidated gliadin antibodies are more likely found in CD patients than native gliadin antibodies.

One of the biggest challenges gastroenterologists and laboratories still face, however, is diagnosing patients who exhibit all the symptoms of CD yet have no antibodies. At this time, there are no real criteria that would help physicians in diagnosing these types of patients. In response, researchers are trying to determine a biomarker, but as of yet none have been found. This situation remains an obstacle.

It is expected that the ESPGHAN guidelines will become more widespread outside of Europe, resulting in fewer biopsies over time—making it easier for both patients and gastroenterologists. With screening of serological markers becoming more sensitive and accurate, these guidelines would inevitably be the standard of diagnosis for the detection of CD, globally.

Yara Burmeister, serves as product manager for **EUROIMMUN**, a PerkinElmer company. EUROIMMUN is a global leader in autoimmune testing and an emerging force in infectious disease, allergy and molecular genetic testing. Its expertise and capabilities extend across immunology, cell biology, histology, biochemistry and molecular biology.

Driving workflow, efficiency and accuracy in laboratories and POCT

By Lisa-Jean Clifford

Technological advances in healthcare have provided clinical laboratory operations the ability to deploy IT infrastructures that support better process and performance. The business focus of the lab has been more prevalent in the past 20 years—from being primarily specimen-focused diagnosis to data- and pathologist-driven, individualized medicine. When technology advances in ways that enable the business to continue to grow and evolve, it creates a perfect alignment of knowledge and technical capability to deliver meaningful, advanced outcomes for patients, while simultaneously delivering improved dividends.

No time has been better for determining which technology is the best one for any given lab and their various business models. Whether the facility is small and privately owned or a large, multinational, multi-site reference lab, Laboratory Information Management Systems (LIS's) can be on-site (turnkey), web-based or a hybrid. Patient data should be transparent to the LIS in its ability to access and incorporate the data. The LIS data should also be distinct in how it is able to follow the chain of command to receive and send information.

Laboratory-data management within a LIS

In every laboratory, the LIS is the source of truth. LIS is the central data hub for every test performed, every case, every visit and every patient seen throughout their history within the healthcare facility. This is a virtual gold mine of information. Most LIS's today are able to store, sort and filter discreet data elements and fields. If your LIS cannot perform these tasks, then your LIS strategy should be revisited. Data management and its tremendous benefits are not possible without discreet data. A LIS should be able to interface seamlessly with all of your healthcare applications, bi-directionally. This enables a data stream to be read, reviewed and accessed in real-time.

Order history, patient information, clinical input and all relevant information (including comments) should be filtered into your lab's LIS whether it is through EMRs, EHRs, HISs, other LIS's or manually. A 360-degree view of the patient enables personalizing medicine during the testing and diagnostic process. All of the information that is analyzed and interpreted on that specific patient should then be stored in your lab's database and sent back to the ordering facility or physician. This too, should be automatic with connectivity.

The real value of data management is its availability for study, analysis and interpretation as both unique and larger sets of data. This information is valuable both internally and externally to continue the treatment of sets, and subsets, of the patient population. The ability to search and find cases by diagnosis, demographic, geographic region, treatment plan, outcome, etc., is what identifies trends. Analysis of data as it relates to a specific set of patients or diagnosis provides the ability to identify best practices based upon specificity.

Point of care (POC) data management

There are many sources of external data that are extremely valuable during the diagnostic process. This information can reside in various other applications and documents. Having a process and the ability to digest this information is beneficial to the full view of the patient. There are a wide variety of applications that can provide this information automatically and electronically. There are various apps that capture health information and personal habits, including the record of weight, exercise, heartrate, BMI, blood pressure, blood glucose levels, etc. All of this information is germane to a 360-degree patient view and should be readily available for interfacing and management.

Think of the value of the above sets of data and how that dovetails more accurately into personalized medicine and its application to a specific patient. Once this information is included, we can begin to identify how these data classifications can be applied to larger data sets to further identify trends, understand demographic predispositions, disease identification and treatment plan outcomes.

This information can also come in the form of previous reports from other labs, additional providers on the continuum of care, reports from pharmacogenomics organizations and other external documents. With the technological capabilities that are available today, all of these documents can be imported into your lab's data center and managed as data assets in conjunction with specific cases for the benefit of that patient—but also in the larger context of data management.

Laboratory management with external data

Optical character recognition (OCR) is the electronic or mechanical conversion of images of typed, handwritten or printed text into machine-encoded text. OCR software enables large amounts of data to be read, sorted, characterized and processed. Software programmers and Artificial Intelligence (AI) algorithms are putting an end to manual data entry using OCR, and are helping organizations to automate the management of both internal data and data from documents, rendering it instantly useful.

Do you consider laboratory information that is captured and processed on the instrumentation to be external data? It isn't! This may surprise you, however,

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even though the information appears to be external to the software applications (LIS), it is internal to the laboratory and specific to the case being processed.

The information on instruments may be minute, though precise and invaluable to the pathologist's diagnosis. Or, the information can include voluminous pages of data yet still very precise as it relates to the findings in the specimen. This type of data is invaluable to the diagnosis of a specific patient. Keep in mind patient data can also be captured and managed within a central data base while still being tied to a specific case.

Clinical laboratory value

The ability to manage patient data is valuable to the clinical laboratory for three reasons:

1. Speed, accuracy and efficiency

When a pathologist or physician has access to all of the information specific to a patient and their history in a single or easily accessible location, they are able to quickly and efficiently assess and diagnose that patient. When this information is automated, the risk of human error is significantly decreased. Therefore, automation ensures a much greater rate of accuracy and safety for the lab, pathologist and patient.

2. Market expansion

Vast sets of patient data are a valuable commodity. The lab has the ability to mine and generate their own information and statistics, such as developing assays and determining new test menus that would benefit their bottom line. There are also other facilities who are willing to purchase datasets. Many vendors, research institutes, clinical trial companies and payers are interested in purchasing information as it relates to de-identified sets of data. This information can be a great source of additional income for the lab. It is often very valuable to other organizations working in areas to advance the management and development of other healthcare technologies, services and diagnostics; thus expanding your lab's market.

3. Business management

The information and data that is gathered and reportable in a laboratory is not just patient specific. Labs that are looking to better manage their businesses through operational efficiencies are able to use this information for a variety of reasons.

Labs can now track where their inventory is going and who is sending them business (ie: ordering physicians and/or other labs). For example, is your laboratory sending a disproportionate amount of supplies to a customer based upon the cases and case types that they are sending you?

Laboratories can also manage staff and productivity in terms of speed and accuracy, turnaround time at each process point, specimen tracking and corrections or amendments by cytotechnologists, pathologists, etc.

Labs are also able to monitor the quality of the specimens sent or gathered by location, loss rates, collections and other customer service metrics, and identify areas and people for retraining. In addition, labs can identify opportunities for new and existing customer engagement that would improve outbound marketing and sales activities.

In conclusion

Data management is a broad term. Discreet data has infinite possibilities. The types of data that are captured include everything from patient specific information to details of the ordering physician and facility. Information can be made more valuable by including treatment plans and outcomes—information not usually captured by the lab but available as part of the patient record. Operational information is captured by reviewing lab staff interactions and workflow to include legally defensible information such as who accessed a file, why and what changes they made.

Data management provides clinical laboratories a myriad of possibilities for use because there are vast amounts and types of information captured. All of this information is able to be analyzed and presented for better management of the patient, the laboratory diagnostic process and the operation of the laboratory business.

Lisa-Jean Clifford serves as COO & CSO, **Gestalt Diagnostics.**

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- *Neisseria gonorrhoeae*
- *Trichomonas vaginalis*

Bacterial Vaginosis
- *Atopobium vaginae*
- *Gardnerella vaginalis*
- *Lactobacillus spp.*
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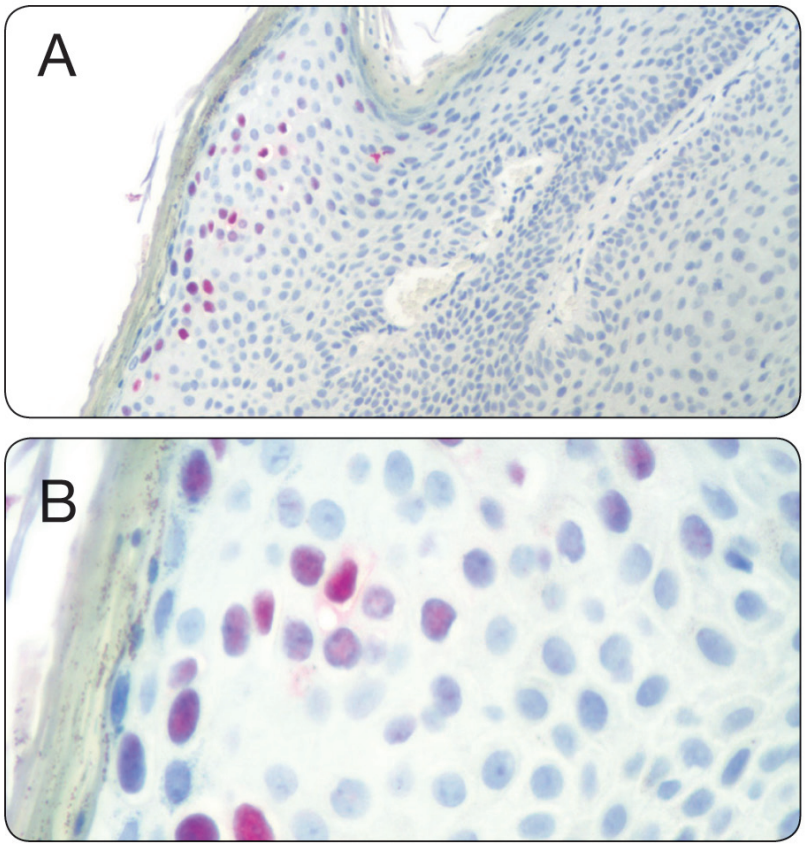
A history of diagnosing HPV

By Kayla M. Hager

Papillomaviruses are a large family of small viruses specific to their hosts[1] that are detectable in a plethora of animal species, including humans.[2] In the case of humans, more than 200 types of human papillomavirus (HPV) have been identified and among those, 85 genotypes are characterized.[1] The link between HPV and cervical cancer was discovered in the 1980s by Harald zur Hausen—a discovery that led to the 2008 Nobel Prize for Physiology or Medicine.[3] Over the next decade, more evidence would continue to confirm the link between certain HPV genotypes and cervical cancer. Thus far, 14 genotypes of HPV, known as the high-risk HPVs, are linked to cancer, with HPV 16 and 18 causing 70 percent of cervical cancers and pre-cancerous cervical lesions.[4] Fortunately, infection does not always lead to cancer. Some women are infected with these high-risk strains but remain asymptomatic. Their immune system may even clear itself of infection altogether. However, for other women, the persistent infection does cause medical problems.



DIGX HPV type 6/11 probe was used to assay cervical tissue using a Leica Bond III, showing strong staining and low background. Images were acquired with (A) 10X and (B) 40X objectives.

Worldwide, cervical cancer is the fourth most prevalent cancer in women. In 2018, it was estimated that 570,000 new cases of this disease were diagnosed, and cervical cancer accounts for approximately 7.5 percent of all female cancer deaths.[4] HPV is most often contracted through sexual contact. However, skin-to-skin contact is a well-recognized mode of transmission as well. Not surprisingly then, there is evidence that HPV can also play a role is some other cancers including rectal, vulval, vaginal, penile and oropharyngeal cancers.[4] However, to date, cervical cancer is the most studied cancer linked to this viral infection. This is in part due to the availability of extensive screening methods and the evolution of our understanding of how certain strains of HPV can induce carcinogenesis.

The emergence of cytological evaluation

There is a significant latency period between infection and the development of cervical cancer or pre-cancerous lesions. This led doctors to ask, "Would it be possible to detect infection early and pre-emptively treat the patient to minimize the risk of danger?" Dr. Georgios Papanicolaou found that abnormalities in cellular morphology could be observed when examining cells collected from a women's cervix under the microscope. The first mass screening program was launched in 1952 in Tennessee. Since then, it has been estimated that the Pap smear test has successfully prevented 105,000 to 492,000 cases of cervical cancer in the United States over the past three decades.[5] While cytological examination can allow a pathologist to infer the presence of HPV based on morphological abnormalities, diagnosis relies heavily on molecular biology techniques, allowing identification of the HPV strain(s) and subsequent classification of the infection as high risk or low risk.

Nucleic acid hybridization methods

The emergence of molecular techniques enabled the detection of HPV strains that have a high association with cervical cancer. Original nucleic acid hybridization methods, such as Southern blotting, in situ hybridization (ISH), and dot blot hybridization, used radioactive nucleic acid probes to detect the presence of HPV.[6] Each of these hybridization methods takes advantage of the complementarity of nucleic acid bases. By applying high temperatures

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and detergents, it is possible to denature the DNA inside of a cell, introduce the radiolabeled DNA probes, and with slow cooling, renaturation of the probe to the native DNA strands.[7] However, these protocols were very time-consuming and radioactivity is harmful to human health. Thus, a need for faster, non-radioactive methods arose.

First non-radioactive testing method

The first non-radioactive ISH probe, made available in the 1980s, answered this need. It provided a safe, faster, and highly sensitive and specific method for detecting the presence of HPV DNA in situ.[8] One of the biggest advantages of ISH is that it is easily applied to tissues that have been fixed and processed. It also allows you to simultaneously assess the localization of HPV DNA in the cellular nuclei of your sample while observing the cells for any morphological abnormalities. ISH can be performed using either DNA or RNA oligonucleotide probes. While DNA probes have been the "gold standard" for the last four decades, the use of RNA probes provides several key advantages over DNA. First, RNA probes can be synthesized easily in vitro by transcription, enabling efficient synthesis of large amounts of continuously labeled probes of uniform lengths.[9] These RNA probes form more stable hybrids with the target RNA or DNA, which results in improved sensitivity.

Polymerase chain reaction

In situ PCR is a powerful method whereby low copy amounts of DNA or mRNA can be amplified and measured using polymerase chain reaction (PCR) or reverse transcriptase PCR (RT-PCR), respectively, in frozen or paraffin-embedded tissue sections or cell suspensions. Amplicon detection is then accomplished using labeled probes. There are several PCR-based methods that can be used to identify the genotype of HPV, and thus infer the treatment needs for that patient. These techniques are highly sensitive, specific and commonly used. Conventional PCR assays use consensus primers that enable amplification of many HPV genotypes at once. These primers target the homologously conserved regions of the HPV genome that are similar across most strains. After amplification, the individual genotypes can be ascertained using several different techniques, such as restriction fragment length polymorphism (RFLP) analysis, linear probe assays, direct sequencing, or genotype-specific primers. However, PCR is not fail-safe and false negative results can occur, particularly with samples that are infected with multiple HPV genotypes and have a low viral copy number.[6]

In vitro RNA-RNA hybridization

As technology continues to evolve, other methods continue to emerge and improve our ability to reliably diagnose and treat individuals infected with

high-risk strains of HPV. Currently, there is an in vitro DNA-RNA hybridization methodology that is one of the two FDA-approved tests for diagnostic testing in the U.S. This system uses a non-radioactive signal amplification method based on the hybridization of the target HPV-DNA to labeled RNA probes in solution. Specific antibodies attached to the well of a microtiter plate then capture the DNA-RNA hybrids formed in solution. Luminescence is then used to detect the captured hybrids. The strength of the emission can be directly correlated with the concentration of the target DNA present in the specimen. While this assay cannot genotype individual HPV strains, it does differentiate between high-risk and low-risk infections. Furthermore, this assay can be fully automated, reducing the risk of human error in sample processing.[6]

Detection of mRNA expression

HPV RNA has become an area of interest as a target for molecular diagnosis of HPV infection. Rather than testing for the presence of viral genomes, assessing the level of viral mRNA expression measures the viral activity in the infected cells. This can be very useful when trying to assess the severity of the infection, particularly if the HPV strain is one of the 14 cancer-causing strains. PCR-based methods, including RT-PCR, could be used to measure the levels of mRNA expression.[10] Another emerging method uses flow cytometry to detect the presence of HPV mRNA in whole cells. It combines the in situ hybridization technology with flow cytometry analysis, whereby the probe does not emit a fluorescent signal unless it is bound to its target sequence. Using this technique, the overexpression of HPV oncogenes can be assessed, and a prediction can be made on the likelihood of cervical cancer progression.[11]

Beyond cervical cancer

As mentioned earlier, HPV can be linked to several other cancers, including cancers at extragenital sites such as anal/rectal cancers, oropharyngeal cancer and penile cancers. In recent studies, it has been reported that the incidence of sexually transmitted infections (STIs) at extragenital sites, including the oropharynx and rectum, is on the rise.[12] While the discussion has been predominantly centered on extragenital gonorrhea and chlamydia infections, it raises awareness for the need of routine screening at extragenital sites. Like HPV, gonorrhea and chlamydia can often be asymptomatic.

As it stands today, the only approved HPV screening methods center around cervical screening. There are no CDC guidelines for HPV screening at extragenital sites in men and women; or urogenital sites in men.[13] Also there is no approved test currently for HPV detention in the mouth or throat.[13] Establishing testing methods to screen for HPV infections at these extragenital sites will be paramount in our continued battle against HPV infection-induced cancers.

In conclusion

Technology clearly evolves over time, improving our ability to diagnose and treat infections appropriately. Physicians can use the current available testing methods to not only diagnose HPV infection early on, but to predict the likelihood that the infection could one day turn into cancer. However, much work is still needed to improve HPV screening beyond the cervix, so that we can catch infections of the penis or extragenital sites early on, and use the same preemptive strike approach for other HPV-related cancers that physicians have been using to combat cervical cancer for the last six decades.

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The physiological significance of homocysteine in women's health

By Emma Callaghan

Homocysteine is a sulfur-containing amino acid produced by the intracellular demethylation of the essential amino acid, methionine. Homocysteine has three metabolic functions within the human body: first, to be remethylated into methionine; second, to enter the biosynthetic pathway of cysteine; and third, to be released into the extracellular medium (blood and urine).The third metabolic function is the direct cause of elevated homocysteine concentrations in urine and plasma. Hyperhomocysteinemia (elevated levels of homocysteine) has been identified in numerous conditions and disease states including cardiovascular disease (atherosclerosis and thrombosis), pregnancy complications, psoriasis, cognitive impairment in the elderly, mental disorders, neural tube defects and birth defects.[1,2] Homocysteine levels have been proven to be influenced by several factors, including: sex, age, the C677T polymorphism of the MTHFR gene and folate status.[3] While men in general have higher levels of homocysteine compared to women, elevated homocysteine levels have been identified as bearing an increased risk of disease in women compared to men. In this article, several disease states and conditions will be identified whereby homocysteine levels are elevated in women.

Cardiovascular disease (CVD)

Elevated levels of circulating homocysteine correlate with an increased risk of vascular occlusion (blockage of a blood vessel). Hyperhomocysteinemia can cause inflammation of the endothelium (thin layer of cells linking the interior blood vessels). Failure to lower homocysteine levels can cause further inflammation of the arteries, veins, and capillaries causing atherosclerosis. Consequently, blood and oxygen supply to tissues is reduced, increasing the risk of CVD. Hyperhomocysteinemia correlates with higher diastolic and systolic blood pressure, causing hypertension. However, this correlation is stronger in women than in men. Women with elevated levels of homocysteine have a three-fold increased risk of CVD, whereas men have a two-fold increased risk.[4]

Colorectal cancer

Hyperhomocysteinemia correlates with an increased risk of colorectal cancer with elevated homocysteine levels being highly prevalent in patients with inflammatory bowel diseases (IBDs), which is believed to be associated with either an increased or decreased absorption of folate and other B vitamins.[5] Furthermore, it has been established that elevated homocysteine levels are a significant risk factor for colorectal adenoma in women.[6] In addition, more studies are emerging linking hyperhomocysteinemia to a variety of cancers including prostate and breast, however, research is required to determine if homocysteine has any effect on the growth and proliferation of tumor cells.[7]

Pregnancy complications and birth defects

Homocysteine levels should decline during pregnancy, however, in some cases, levels increase. Hyperhomocysteinemia is associated with fetal neural tube defects which causes various conditions, characterized by placental vasculopathy, including preeclampsia, abruption and recurrent pregnancy loss. It has been identified that folate supplementation can reduce the risk of fetal neural tube defects by half.[8] One study found that hyperhomocysteinemia was associated with a two- to three-fold increased risk of abrupyio placentae, pregnancy-induced hypertension and intrauterine growth restriction.[9] Furthermore, hyperhomocysteinemia has been identified as inducing oxidative stress in pregnancy. Oxidative stress in the utero-placental tissues has been recognized in the development of severe uteroplacental disturbance, an uncommon yet serious complication of pregnancy. Moreover, oxidative stress and hyperhomocysteinemia was also observed in women with preterm birth or at risk of abortion.[10]

These complications in women as a direct result of hyperhomocysteinemia indicate the necessity for homocysteine testing.

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How LC-MS technologies are breaking down barriers to adoption in the clinical laboratory

By Debadeep Bhattacharyya, PhD

Advances in mass spectrometry (MS) over the past few decades have opened up a wealth of techincal opportunities for the clinical laboratory. From therapeutic drug monitoring and clinical toxicology assays to the analysis of hormones and steroids, MS has established itself as a versatile tool for an expanding range of clinical applications. However, despite its broad utility, MS is not always the go-to technique when it comes to enabling the development, implementation and optimization of routine tests conducted by laboratories focused on clinical research. In this article, we look at ongoing advances in liquid chromatography–mass spectrometry (LC-MS) technology, and how it is ensuring clinical research laboratories can address their scientific and business goals.

The evolution of MS as a clinical research tool

From its inception at the turn of the 20th century, MS has long been recognized as an important tool for the characterization of critical analytes, enabling the determination of the mass-to-charge ratio (*m/z*) resulting in elucidation of their structure. But, while the origins of MS lie in the pioneering work of physicist Sir Joseph John Thompson and chemist Francis William Aston more than a century ago, its adoption into routine clinical research labs is considerably more recent.[1-4]

Despite some research into the use of MS for respiratory gas analysis in the late 1950s,[5] many of the earliest applications date from the 1970s, with the coupling of MS and gas chromatography (GC) for the determination of various biological analytes in urine and other biological fluids.[6,7] However, as many biologically active molecules are thermolabile, polar and insufficiently volatile, GC-MS approaches typically required complex extraction and derivatization steps. As such, a lot of routine MS applications were predominantly limited to the field of forensic toxicology.

Driven by the need for improved accuracy, speed and cost-efficiency in routine clinical research laboratories, the last few decades have seen a considerable emphasis on the development of robust, reliable, accurate and sensitive LC-MS-based assays. LC is much more amenable to the separation of thermolabile and biologically active molecules than GC, and it gives analytical scientists the flexibility to analyze a diverse range of biological matrices, including blood, plasma, serum, oral fluid, urine, etc. Recent years have witnessed significant improvements in the separation resolution offered by modern high-performance liquid chromatography (HPLC) and ultra-HPLC (UHPLC) techniques, while the latest advances in column technologies, such as new biphenyl reversed phase chemistries, provide enhanced selectivity for more challenging analytes.

The demands for MS to provide accurate data for identification, confirmation of molecular identity and structure has been explored quite extensively leveraging high-resolution accurate mass (HRAM) systems. However, for routine quantitation of analytes in biological, recent advances in MS are enabling clinical laboratories to develop novel, reproducible and sensitive assays.

Two of the most widely used MS technologies that are efficiently leveraged for quantitation are triple quadrupole (QqQ) and HRAM systems. QqQ instruments, known for their sensitivity and selectivity, as well as their operational robustness and throughput, comprise three sets of quadrupole rods (referred to as Q1, q2 and Q3) that filter analytes based on the way their ions fragment. In typical selected reaction monitoring (SRM) experiments (**Figure 1**), molecular ions entering the instrument are filtered by the first quadrupole (Q1), and only those with a specific *m/z* may be analyzed further. A second quadrupole (q2), which acts as a collision cell, subsequently fragments the selected precursor ions to generate daughter ions, which are filtered by the third quadrupole (Q3), enabling the detection of specific fragments. Steady improvements in instrument design mean modern QqQ instruments now offer excellent reproducibility for quantitative analysis and can routinely support the sensitive determination of analytes even at picomolar concentrations in complex biological matrices.

The development of HRAM systems based on Orbitrap technology has led to considerable improvements in mass resolution, permitting the detection of incredibly small differences in the mass of analytes of interest, thereby enabling routine quantitation assays.[8] Orbitrap mass analyzers conducting parallel reaction monitoring (PRM) (**Figure 1**) consists of three electrodes: two outer electrodes that form a barrel shape, surrounding a coaxial inner electrode, which trap ions in an orbital motion around a central axis. The *m/z* of the analytes can be determined by measuring the image current generated by the circular motion of individual ions. The exceptional mass resolution provided by these systems enables outstanding spectral clarity, facilitating confident mass analysis.

By combining the sensitivity, selectivity and robustness offered by quadrupoles with the outstanding mass resolution offered by Orbitrap technology ensures HRAM systems deliver impressive quantitative performance. A major difference between QqQs and HRAMs is the fact that HRAMs, in addition to PRM, can also perform full-scan analysis, enabling unknown screening and untargeted analysis of analytes and mixtures. This feature also enables retrospective data analysis, allowing analysts to ask different questions of the same sample without having to re-run tests at a later date.

Realizing the benefits of LC-MS for clinical research

The exceptional performance offered by the latest instruments means modern LC-MS techniques overcome

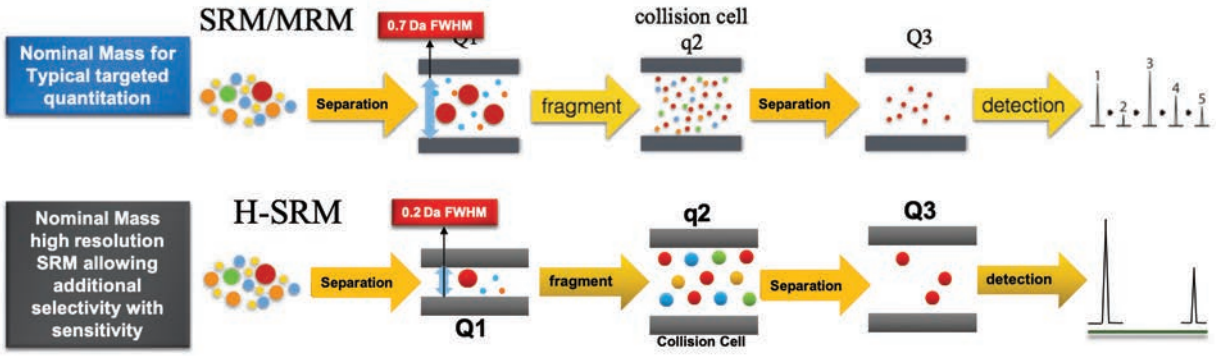

Figure 1. Ion fragmentation schemes illustrating selected reaction monitoring (SRM) using a QqQ instrument and parallel reaction monitoring (PRM) using a hybrid quadrupole-Orbitrap system.

many of the challenges associated with traditional ligand binding assays, which are still widely used by many clinical research laboratories. While these established immunoassay methods continue to be used by clinical research laboratories owing to their familiarity and ease-of-use, they require analytes to bind to specific antigens, which can be impacted by interference from molecules with similar structural characteristics. Consequently, some immunoassays can potentially lead to inaccurate or imprecise results leading to higher false negatives or false positives.

LC-MS technology, whether HRAM or QqQs, however, offers exceptional specificity and selectivity, resulting in multiple approaches to isolate specific analytes of interest from complex background matrices. QqQs in particular also offer a high degree of sensitivity, and their impressive selectivity ensures excellent signal-to-noise ratios and high analytical confidence.

Another challenge associated with immunoassays is the length of time and cost associated with implementing these tests. As immunoassays require the generation of highly selective antibodies to bind to the target analyte, it can be complex and time-consuming to develop immunoassays for some target molecules. Additionally, laboratories that are dependent on immunoassays may find they need to outsource some non-routine tests to external providers if they do not have the necessary analyte-specific reagents in-house. This can be costly and result in extended turnaround times that can ultimately delay decision-making. As LC-MS techniques do not rely on specific antigens or reagents, the methods are typically easily reproduced and replicated, regardless of the location and expertise of the user.

New LC-MS technologies designed specifically for clinical research

Despite the exceptional analytical power of LC-MS and benefits of the technique over immunoassay methods, many clinical research laboratories have yet to embrace this tool for routine analyses. LC-MS has traditionally been perceived as being an operationally complex analytical technique, and this concern is not without some foundation. LC-MS systems were initially developed with a high degree of system flexibility to enable a wide range of experiments. However, this versatility also meant that developing and optimizing routine assays using traditional LC-MS systems requires a high level of specialist technical knowledge in order to exploit its full potential. The challenge facing clinical research laboratories around recruiting and retaining trained personnel with the necessary skills and experience has therefore limited the broader adoption of LC-MS, especially when it comes to clinical research laboratories with fewer resources. Fortunately, ongoing improvements in LC-MS technologies are helping more research labs access this selective and sensitive analytical technique by automating and standardizing LC-MS-based assays.

Conclusion

From its early use for metabolite profiling to the latest clinical research assays, recent advances in LC-MS technology are enabling clinical research laboratories to address a variety of analytes with the development of robust, reliable, sensitive and reproducible assays, regardless of the type of molecules, matrix complexity and user expertise.

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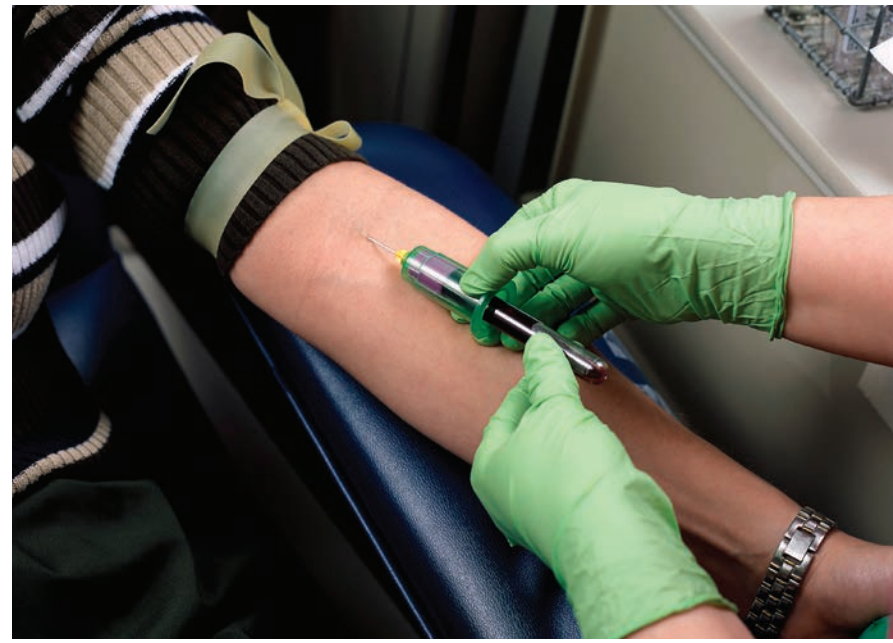
Implementing a phlebotomy training program for rural hospital systems

A proposed framework for clinical support services

By Atheena Therese Estrada, MBA, CPT(NHA), Jewel Goodman Shepherd, MPA, CHES, PhD, Marcie Sariol, MBA, PhD

It is well known within the medical field that phlebotomy is one of the most common invasive healthcare procedures performed. Laboratory results from blood collection play a significant role in assisting medical providers as they make critical decisions about patient care, including formulating proper diagnoses and treatments for patients. Although a common procedure performed daily in hospitals, the knowledge, skills and abilities needed to perform phlebotomy successfully may be underestimated by some healthcare leaders. Despite standards and guidelines set in place by organizations such as OSHA (Occupational Safety and Health Administration), CLIA (Clinical Laboratory Improvement Amendments), CAP (College of American Pathologists) and CLSI (Clinical and Laboratory Standards Institute), qualifications to apply for a position as a phlebotomist vary greatly among states.



As an example, to perform phlebotomy in the state of California, most healthcare organizations (HCOs) require applicants to: 1) be a high school graduate or have passed the GED test; 2) complete a phlebotomy program accredited by the California Department of Public Health; 3) pass a national certification examination from a certifying organization approved by the California Department of Public Health; and 4) apply and obtain a California Certified Phlebotomy Technician I certificate. Alternatively, to perform phlebotomy in the state of South Dakota, most HCOs require applicants to: 1) be a high school graduate or have passed the GED test, and 2) prior healthcare experience is preferred but not typically required.

Since applicant standards vary across the country, it may be extremely beneficial for a hospital system located in, or whose service area is in, a rural or medically underserved area to establish an in-house phlebotomy training program. This work presents a framework for implementation of a formalized phlebotomy training program for such hospital systems.

Proposed framework

The following framework will feature steps that are critical in establishing a phlebotomy clinical support service training program within a rural-based hospital system.

Part I: Identifying hospital system challenges

A collaborative effort with a hospital Health Services Administrator (HSA) to determine underlying challenges to implementing a training program is a priority. An HSA should have intimate knowledge of how each of the healthcare provider positions support healthcare delivery; including the formulation of goals, decision-making, planning, organizing, monitoring and controlling overall health system operations. Additionally, an HSA will be helpful in analyzing and integrating decision-making toward designing the organizational structures to meet regulatory standards and achieve optimal organizational performance specific to strategic planning, operations management and employee development.

Part II: Proposing objectives

The following are proposed objectives of implementing a formal phlebotomy training program for a rural hospital system. It's important to note that these objectives may be addressed in an order that best suits the HCO.

- Objective 1: Standardize phlebotomy training throughout the organization in order to consistently provide excellent patient care.
- Objective 2: Establish a local testing site in order to increase accessibility for laboratory caregivers wishing to complete a national certification examination.
- Objective 3: Identify process improvement opportunities related to phlebotomy processes and identify performance measurements.

• Objective 4: Reduce pre-analytical caregiver turnover rate.

The following are strategies for achieving the stated objectives:

• Develop a formal phlebotomy training program that is approved by the National Accrediting Agency for Clinical Laboratory Sciences (NAACLS) to ensure industry and academic standards are met.

• Partner with a national certification organization in order to offer testing for Phlebotomy Technician Certification (CPT) through the hospital system or a nearby testing site.

• Ensure the hospital system has the resources and support needed for implementing and sustaining a training program such as education coordinators, certified trainers, continuing education opportunities including recertification, technical support, training rooms, training equipment, etc.

• Develop quality measures for success such as tracking the number of successful draws phlebotomists have after completing formal training, tracking the number of on-the-job-related injuries due to needle stick injuries, collecting client/patient feedback pertaining to phlebotomy experience, collecting caregiver information regarding the level of confidence and preparedness they feel they have due to the training, etc.

Part III: Measuring and monitoring success factors

In order to ensure the success of a phlebotomy program, the hospital system should collect data prior to its implementation. Factors such as phlebotomists' first attempt success rate, quantity not sufficient (QNS) rate, phlebotomists' success ratio (number of successful draws versus number of draws attempted), number of years of phlebotomy experience (if any), program completion rate and pass rate of a national certification examination on the first attempt should also be considered.

In-house training program example

In response to increasing turnover rates and lack of qualified, properly trained phlebotomists, the Medical Center of Central Georgia in Macon developed an in-house training program that provides the necessary instruction to become a skilled phlebotomist.[1] A unique model was developed to satisfy the needs of the HCO by hiring phlebotomy students as full-time *temporary* employees, while completing a 16-week didactic-clinical externship onsite.[1] Phlebotomy trainees received a total of 115 hours of classroom instruction and completed 475 hours of clinical experience; covering material that allows trainees to sit for a national certification exam.[1]

Of the 73 phlebotomy students hired through this training program, approximately 80 percent completed the program and 90 percent passed the certification exam.[1] The phlebotomy training program had a significant impact on phlebotomist turnover rate, dropping from 57 percent to 20 percent.[1] The institution of this program also gave rise to a full-time phlebotomy instructor/directory position, with responsibilities such as providing specific continuing education for the phlebotomy department, individual retraining opportunities and in-service opportunities for nursing personnel.[1] The attention to phlebotomy education assisted the Medical Center with improving specimen quality and safe collection practices throughout the HCO.[1]

In conclusion

Formal education and training serve as intangible resources for hospitals because they provide economic value to individual workers. Human capital, also inclusive of individual experience, has been shown to not only increase productivity but also an organization's (i.e. hospital system) profitability. The ability of an organization to foster and increase the development of a work force's human capital is therefore a concerted effort that is expected to result in tangible outcomes. It stands to reason that training should be viewed as a human capital investment that can increase patient care, efficiency and ultimately, financial performance. Moreover, research indicates that formal onboarding and training is much more effective in helping new hires adjust to the organization, learn their role and increase retention rates. The Society for Human Resource Management (SHRM) defines formal onboarding as a written set of coordinated policies and procedures that assist an employee in adjusting to his or her new job in terms of both tasks and socialization.[2] Thus, it stands to reason that establishing a formal phlebotomy program may help maximize success and the overall attainment of the hospital system's laboratory personnel, particularly in rural areas.

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Neurodegeneration biomarkers

A paradigm shift in the definition of AD

By Iswariya Venkataraman, PhD

Neurodegeneration is the underlying mechanism behind many diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and motor neuron diseases (MND), as well as cases of traumatic brain injury (TBI) and stroke. It is often difficult to identify neurodegeneration. Reliable biomarkers are critically needed to diagnose specific neurodegenerative diseases at an early stage, and help differentiate patients with overlapping clinical conditions. The classic biomarkers for AD are beta-amyloid (Aβ) and tau, and their analyses in cerebrospinal fluid (CSF) are part of the 2011 National Institute on Aging and Alzheimer's Association (NIA-AA) diagnostic guideline.[1,2]

With scientific advances in the field, the NIA-AA updated the 2011 guidelines and published a research framework in 2018. This framework lays emphasis on the diagnosis of AD using biomarkers, shifting disease definition from a syndromal to a biological construct in the living.[3] Biomarkers in AD are grouped according to the amyloid, tau and neurodegeneration (ATN) classification system, which categorizes different biomarkers according to the underlying neuropathological change they measure, such as Aβ deposition, tau aggregation and neurodegeneration, respectively.[3,4] The research framework is currently intended for observational and interventional research, but not for routine diagnostics since the research framework is not a diagnostic criterion or guideline.[3]

Neurodegenerative process

Neurodegeneration refers to any pathological condition that results in the progressive loss of structure or function of neurons. This process is regarded as a continuum that starts with misfolding of proteins due to various causes such as genetic mutations, RNA translational errors and abnormal post-translational protein modifications,[5] that ultimately results in formation of neuro-toxic aggregates such as plaques and tangles (**Figure 1**). In AD, plaques consist of aggregates of the pathogenic protein Aβ 1-42 (Aβ42), which deposit extracellularly next to the nerve cell ends. In contrast, neurofibrillary tangles are made of tau proteins and are located inside nerve cells. All of these protein aggregates appear to acquire toxic properties damaging to neurons. As a consequence, there is a loss of synaptic integrity or degeneration of the synapses, leading to cognitive decline and other neurological symptoms.[6]

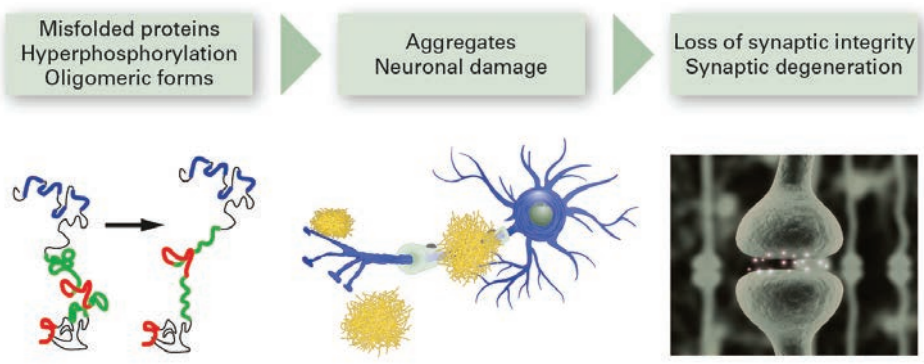

Figure 1. Representation of the neurodegeneration process

Alzheimer's disease

Alzheimer's disease is the most common cause of dementia in the elderly, and is observed in almost 70 percent of all dementia cases. AD is considered the sixth leading cause of death in the United States. Approximately 5.8 million Americans suffer from AD.[7] People with AD experience various symptoms that change over time, depending on the extent of damage to neurons in different parts of the brain. Therefore, the disease is divided into three consecutive phases: the preclinical stage, the mild cognitive impairment (MCI) stage and the dementia stage.[2] As the disease progresses, there is significant damage to the neurons causing cognitive decline and negatively impairing the individual's memory, thinking and behavior. On average, the life expectancy after onset of symptoms is seven to 10 years in patients aged 60-70 years.[8] Definitive diagnosis of AD is challenging and requires evidence of the neuropathological alterations in the brain. A diagnosis of probable AD is based on the clinical signs of memory loss and behavioral changes and the exclusion of possible reversible causes. Imaging techniques such as magnetic resonance imaging (MRI) or positron emission tomography (PET) for detection of specific protein aggregates are used to support differential diagnostics.[9,10] Clinical studies and recent biomarker data indicate that the pathology of AD begins to accumulate at least 10-20 years before the appearance of cognitive symptoms. Currently, the "gold standard" for the definitive detection of amyloid-pathology in a patient's brain is amyloid staining in post-mortem autopsy brain tissue.[11] Hence, there is an urgent need for pre-mortem biomarkers that demonstrate strong indications of amyloid pathology. Analysis of biomarkers in CSF aids diagnosis, especially in the early stages, and helps to discriminate AD from non-AD dementia patients.[11] CSF biomarkers that support the diagnosis of AD include Aβ42, Aβ40, total tau (Ttau) and phosphorylated tau (P-tau).[12]

Beta amyloid

One of the core neuropathological characteristics of AD is the accumulation of Aβ containing neuritic plaques in the brain parenchyma. Postmortem studies have indicated an inverse

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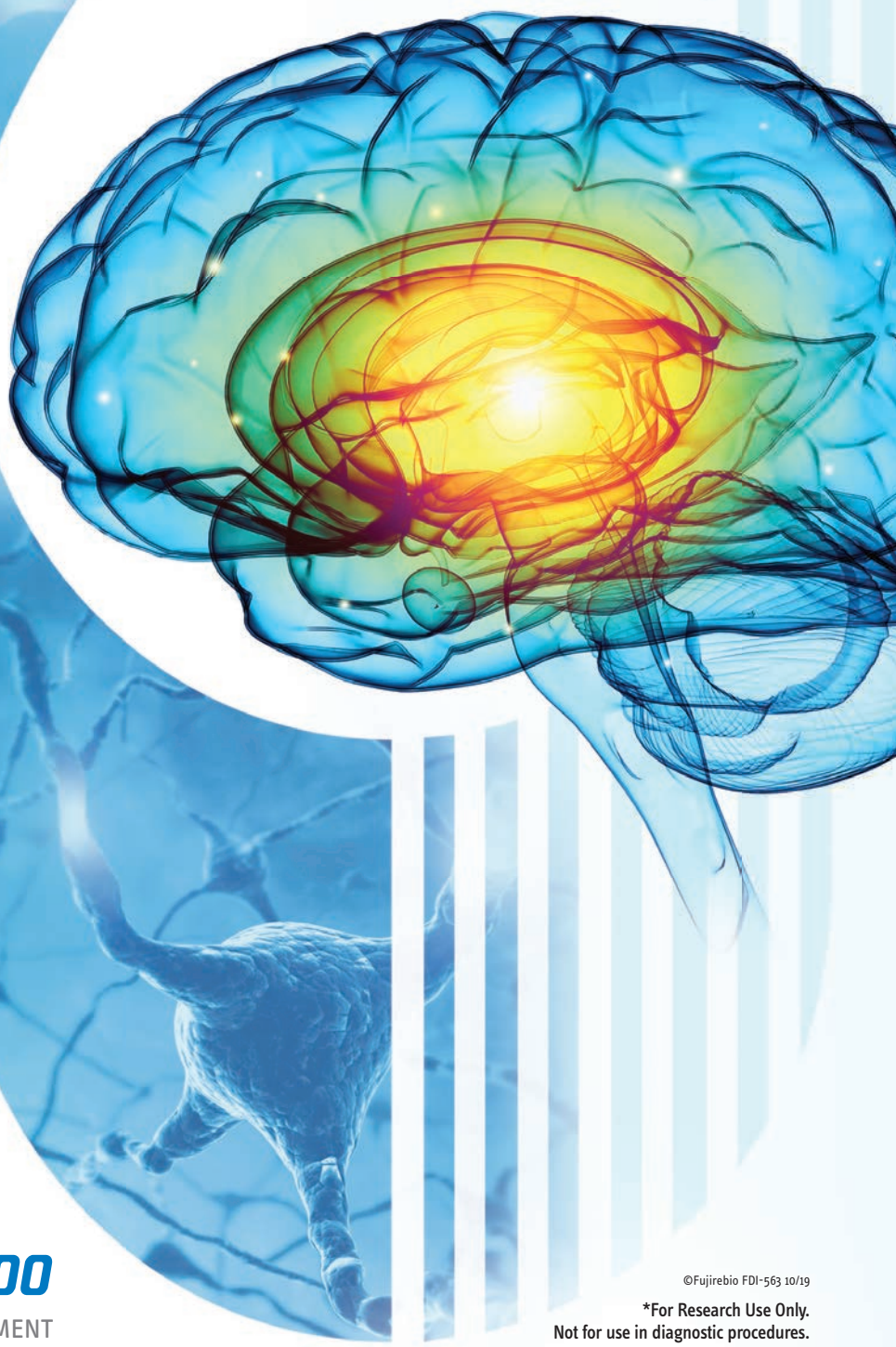

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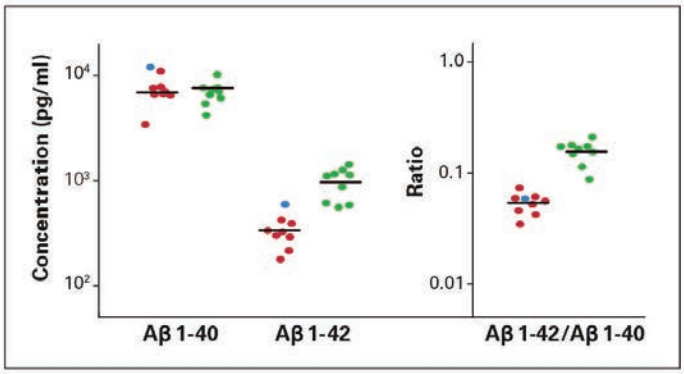

Figure 2. Case example of beta amyloid ratio calculation (Internal study)

association between CSF Aβ and amyloid plaque burden, caused by increased deposition of Aβ42 in the brain.[13] Therefore, individuals with AD show significantly decreased levels of Aβ42, which is detectable at least five to 10 years before the onset of cognitive decline. Unlike Aβ42, the values for Aβ40 in CSF remain stable in persons with amyloid-pathology, reflecting its utility as a marker of the individual's amyloid level. Many studies have reported better clinical performance with CSF Aβ42/Aβ40 ratio compared with CSF Aβ42 alone for detecting Aβ cortical deposition burden in prodromal AD.[14-16] CSF Aβ42/Aβ40 ratio can differentiate AD from other dementia-related disorders **(Figure 2),** and can improve progression prediction in subjects with mild cognitive impairment (MCI). Determination of ratio increases the efficiency of early diagnosis compared to analysis of the individual biomarkers alone, and can contribute to delimitation from other dementia syndromes (e.g. vascular dementia, frontotemporal dementia). Furthermore, studies have reported high concordance between Aβ42/Aβ40 ratio and PET measurements in determining abnormal cortical Aβ deposition.[17] The overall prediction success rate increases from 83 percent to 93 percent when using the ratio compared with Aβ42 alone. Therefore, CSF Aβ42/Aβ40 ratio is superior to Aβ42 alone and can be used as an alternative to amyloid PET (imaging with an amyloid-specific contrast dye) to estimate Aβ neural plaque density in adults with cognitive impairment who are being evaluated for AD.[18]

Tau

The major histopathological features of AD include not only deposition of amyloid plaques, but also accumulation of neurofibrillary tangles. In AD, tau becomes hyperphosphorylated causing neuronal dysfunction. Consequently, a combination of release from neuronal injury and active secretion of tau proteins results in increased CSF levels (2-3 fold) of tau in mild-moderate AD patients compared to agematched controls. T-tau is a general marker of unspecific neuronal damage which occurs in AD, but also in other conditions such as stroke, traumatic brain injury, etc. In contrast, hyperphosphorylated tau such as P-tau(181), is an AD-specific marker indicating tauopathy.[19] At EUROIMMUN, measurement of P-tau demonstrated a positive predictive value for AD at 88 percent and a high negative predictive value at 91 percent **(Figure 3).**

A paradigm shift in the definition of AD

The NIA-AA diagnostic recommendation for AD published in 2011 divided AD into three clinical entities: (1) preclinical, (2) MCI and (3) dementia stages.[2] The 2018 updated research framework is established based on the definition of AD as a biological construct. The ATN classification system for biomarkers was introduced in 2016, and was designed using both CSF and imaging biomarkers in each group. It is possible to characterize the research participants using either biomarker alone or biomarker with imaging when available. "A" refers to aggregated Aβ or associated pathologic state, which is measured by amyloid PET or CSF Aβ42or Aβ42/40 ratio, respectively. "T" refers to aggregated pathologic tau or associated pathologic state, which is measured by tau PET or CSF P-tau, respectively or tau PET (imaging with a tau-specific contrast dye). "N" refers to general biomarkers of neurodegeneration or neuronal injury including CSF T-tau, MRI and fluorodeoxyglucose PET.

Classic AD assays measure aggregates in CSF, which reflect the neuropathological changes in the brain. Synaptic protein biomarkers (see below), in contrast, provide a measurement of synaptic integrity and are assumed to be a more direct predictor of cognitive impairment. The ATN classification system is flexible since it is possible to add additional biomarkers that focus on neurodegeneration or synaptic integrity to the research framework as they become available.[3,4,20]

n = 110 clinically characterised samples		Clinical decision	
		AD positive	AD negative
EUROIMMUN P-Tau (pT181) ELISA	Positive	57	8
	Negative	4	41
	Sensitivity	93%	
	Specificity	84%	
	Positive predictive value	88%	
	Negative predictive value	91%	

Figure 3. Analytical performance of the P-tau(181) ELISA

Synaptic proteins

There is a direct correlation between synaptic dysfunction and memory disturbances including cognitive symptoms even at very early stages of AD. Synaptic proteins reflecting this pathophysiological process in the CSF could be useful biomarkers to monitor synaptic degeneration and, in turn, to monitor cognitive decline in AD. In the ATN classification system, all biomarkers that indicate neurodegeneration or neuronal injury in general are grouped into the "N" category. Neurogranin, Beta-secretase 1 (BACE1) neurofilaments, alpha-synuclein and other synaptic proteins are some of the promising biomarkers that belong to this category.[3] Recent evidence shows that patients with MCI, as well as AD-related dementia, have significantly increased neurogranin levels compared with healthy controls. Additionally, CSF neurogranin levels are elevated in the prodromal stages of AD depicting synaptic injury as a measure of cognitive decline in AD.[21] BACE1 is a pre-synaptic protein, whose levels are increased in AD patient brains.

When compared to the individual analytes, the ratio of neurogranin/BACE1 portrays significant correlation to cognitive loss with rapid decline in mini mental status examination scores (MMSE). A high ratio predicted a more rapid decline in MMSE scores. Therefore, the CSF neurogranin/BACE1 ratio could be potential progression markers of cognitive decline in AD.[22] Assays for these biomarkers are currently used only in a research capacity. Studies are underway to determine the diagnostic and prognostic value of these parameters in a clinical setting.

Perspectives

The global burden of neurodegenerative diseases is increasing progressively, particularly in countries with aging populations. As of 2019, approximately 5.8 million Americans of all ages are living with Alzheimer's dementia. By 2050, the number of people aged 65 and older with Alzheimer's dementia in the U.S. is projected to increase to 13.8 million, straining health care systems.[7] Due to the devastating nature of these diseases, early diagnosis is critical to enable therapeutic intervention and organization of adequate care. Therefore, there is an urgent need for reliable and robust biomarkers that support early diagnosis, differential diagnosis and prognosis in AD. The NIA-AA research framework stresses the diagnosis of A D using biomarkers, but is not based on the underlying clinical symptoms and signs. Currently, the biomarkers implemented in AD are invasive, as well as expensive. Therefore, the Alzheimer's biomarker consortium groups are keen on developing less-invasive and less-expensive blood-based biomarkers that, along with genetic and clinical information, would support screening target populations at an early stage.

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Iswariya Venkataraman, PhD, serves as Market Development Manager, Scientific Affairs, **EUROIMMUN U.S.** She's currently a member of the Alzheimer's Disease Neuroimaging Initiative (ADNI) Private Partner Scientific Board (PPSB) which aims to advance the standardization and validation of biomarker tests in AD and other neurodegenerative conditions.

What's with hot start, anyway? A tale of mispriming and how to avoid it.

By John Brunstein, PhD

Ever looked at a PCR thermocycle program and noticed it has a prolonged initial high temperature step before the repeated cycling occurs? In this month's episode, we'll delve into what that's for, and the several ways in which the same effect can be achieved.

Primer, meet template

If the whole mechanics of the PCR process aren't fresh in your mind, you might want to dip back in the archives to the April 2013 installment of this series, "PCR: the basics of the polymerase chain reaction." Key points here are that the process requires synthetic primers, or at least their 3′ ends, to anneal down onto a template to recruit DNA polymerase in and commence template-directed elongation of the primer. That primer annealing is a balancing act between random thermal motion of the primers and template (attempting to tear them apart) and bonding energy (primarily H bonds) between primer and template (attempting to hold them together). As temperatures go lower, less bonding energy is needed to tip the balance towards primer annealing; this means that shorter sequences, or in this case, less than all of the primer sequence has to match its complement on the template to anneal.

Imagine then that within a reaction tube, we have all the components needed for PCR—template, buffer, dNTPs, primers, and polymerase—and that we're going to be manipulating this at room temperature, which is where most lab techs like to work. Most PCR primers have annealing temperatures well above room temperature, often in the 50-65° C range. Put them at 21° C and guess what; they only need to match about half their length to effectively hybridize onto a template. If that half length is towards the 5′ end, or even in the middle—in fact, anywhere but including the 3′ end and its critical hydroxyl—this doesn't create a substrate for polymerase to do anything. So aside from the fact that this interaction effectively sequesters some primer in non-productive locations, it's not very harmful. If, however, the 3′ end of the primer is annealed down, a functional substrate for polymerase is formed, and it may be grabbed and nascent strand elongation begins. Your previously carefully designed, highly target specific primer is now starting to amplify someplace it shouldn't.

At this point, I suspect there's murmurs of discontent in the gallery. "But PCR uses thermostable polymerases, usually from thermophilic organisms, and they don't work at ambient room temperatures!" Well, that's not quite true. Yes, they generally work much better (faster, more processivity) at their evolved optimal temperatures, but they still can catalyze DNA synthesis from a proper template, slowly, at lower temperatures. How much slower probably depends on the buffer, the enzyme and the actual ambient temperature, but it's rather a moot point. Even if the non-specifically annealed primer is only extended a handful of base pairs, its now created a modified, elongated primer in which the initially mispaired region plus the extended section now are a perfect match for someplace undesirable in the template. That's right, now even at elevated temperatures designed for specific annealing of the initial primer, this modified primer can anneal it's lengthened 3′ end and effectively recruit polymerase. In fact, it can't act to prime amplification from its intended target anymore, as its 3′ end doesn't match the correct target.

Oops

Oh, it gets worse. If our undesired, single strand of misprimed product is able to find any floating DNA fragment capable of annealing to it, that in turn can prime synthesis back across what was the original primer. This creates a perfectly good binding site for any functional original primer, which now competes with the actual target sequence. While this might not seem a highly likely occurrence, recall we're dealing with literally billions of base pairs of template sequence in the average sample, and statistics is no longer our friend here. Bottom line, in reality, if you let your polymerases sit around in an active state with primers and template at temperatures well below the primer annealing temperature, you tend to lose assay sensitivity (primers get sequestered and rendered unusable), and you lose specificity (modified primers start priming amplification of off target garbage). Obviously, this is a bad thing, and should be avoided if possible.

Solving the problem: hot start

The techniques used to circumvent this problem are generally lumped together under the title of "hot start" methods, meaning the PCR reaction isn't made wholly functional until the reaction is well above intended primer annealing temperatures for the first time. No functional reaction mix means no modification of misprimed primers and we get a fresh, clean start as the reaction comes to the first programmed annealing temperature. This can significantly improve sensitivity and specificity, so let's look at some of the ways to do this. For completeness, we'll cover some methods no longer in vogue, as well as the most commonplace methods in the lab today.

First approach: set up the reactions on ice, and don't put the reaction tubes into the thermocycler until it's well above annealing temperature. Wait, what? Doesn't putting the reaction on ice make the primer mispriming issue worse, making it need even less nucleotides at its 3′ end to match something to anneal down? Yes, that's right, but the trade-off is that we're getting further away from the optimal activity temperature of the polymerase, and it may be that it's so sluggish at these temperature ranges that it just really hasn't got much or perhaps any chance to catalyze primer extension.

Lower temperatures also require less kinetic mixing in the sample, meaning productive collisions between a polymerase and (unwanted) substrates occur less frequently. Overall, while this doesn't unequivocally solve the problem,

at least it greatly reduces it. By not placing the reaction tubes into the thermocycler until it's hot, the small volumes of each reaction increase in temperature rapidly, meaning there's not much window of opportunity for both primers to be misannealed and for polymerase activity to occur. This method is probably the crudest approach to hot start; it requires hands-on activity and it's not perfect. But it's cheap and simple and at least it helps (empirically, sometimes quite a lot).

Second approach: don't make the reaction contents complete until the mixture gets hot. Variations on this approach hinge on encapsulating critical components (either the primers themselves, or sometimes, the divalent ions such as Mg^{2+} needed for DNA polymerase catalysis) in something like a wax bead sitting in the reaction tube. This wax has a melting point somewhwere above usual primer Tm ranges but below 95°C, such that the reaction doesn't become complete until it's above temperatures where mispriming can occur.

In the dawn of time from a lab PCR standpoint, before heated PCR machine lids or "hot bonnets" were common, this had an additional advantage in that after melting, the wax would float to the top of the reaction and serve as an evaporation barrier. You no longer had to drip mineral oil on top of each reaction vessel to serve this role as part of the reaction setup. While a clever approach (and one still in use in some settings) downsides of this are either that you have to custom-formulate primers into the wax beads for every assay (with appropriate control of concentrations and bead size per reaction), or for the divalent cation beads, lose ability to optimize cation concentration as the beads are premade. (A solution to this, of course, could be to vary the reaction volume such that the premade bead cation content provides the desired final optimum, so it's not an insurmountable challenge.) Overall though, these wax-embedded reagent methods aren't overly commonly encountered.

Third approach: involves doing something to the DNA polymerase itself such that it's actually unable to function prior to being heated well above primer annealing temperatures—a heat activation step. Since polymerases don't naturally come with an on/off switch, clever folks in the biotechnology industry have had to come up with a way to do this. Patents being what they are and human ingenuity being what it is, at least two different strategies to do this have been developed and both are referred to as "hot start polymerases." One of these works by developing thermolabile monoclonal antibodies which have a strong affinity for the DNA polymerase active site, and effectively block the site from accepting substrate while bound. (Nothing says this has to be an antibody; another engineered polypeptide could work as well, but it's probably technically easiest to do this via antibodies.)

During polymerase formulation, after it's purified, it is mixed with an excess of this blocking antibody and binding is allowed to proceed to completion, yielding intact but inhibited polymerase. Subsequent handling of the reagent is such that the antibodies remain bound right through until final reactions are made. When this sample is heated, however, the importance of the antibody being thermolabile becomes apparent; at some temperature above expected primer-specific annealing, the blocking antibodies denature or unfold. This means they no longer have affinity for the polymerase active site, detach and float off into solution. This denaturation is generally thermodynamically irreversible, meaning they won't come back to interfere with polymerase activity, regardless of subsequent thermal profiles. Hot start polymerase formulations based on this approach generally have fairly short hot start cycle requirements, on the order of less than five minutes.

The second route to this is via a reversible chemical modification of the polymerase, such as esterification of a critical active site hydroxyl (-OH) side chain. This modification completely abrogates enzyme function, but the blocking agent and buffer composition are chosen to make the blocking reaction chemically reversible at elevated temperature. Similar to the idea with antibody blocking, during reagent formulation the purified enzyme is treated with excess blocking agent to leave little or no active enzyme. After final reaction constitution, in a hot environment (e.g. 95°C), the blocking agent is hydrolyzed off to regenerate the critical side chain moiety, and enzyme activity is regained. Compared to conditions during formulation, concentration of released blocking agent is low. That, combined with at least a brief high-temperature denaturation phase in every cycle, acts to ensure enzymes don't become re-blocked. These chemical-based hot start polymerases usually suggest a somewhat longer initial hot start phase to each reaction (~10 minutes).

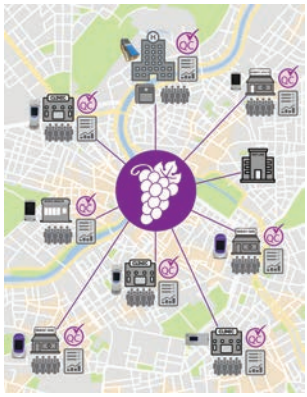
Do you always want to hot start, a hot start polymerase?

What if you use a hot start enzyme but dispense with the normal extended hot incubation on your reaction? In this case, the enzyme activation occurs more slowly over each of the cycle denaturation steps, with release of a little more active enzyme each time. This timed enzyme-release approach can actually be desirable sometimes—if only small amounts of template are expected in early reaction cycles, little functional enzyme is needed to sustain effective PCR, and it limits the amount of unoccupied functional polymerase, "wandering around the tube getting in trouble." This approach usually must be offset by increasing the total cycle number however, which can lead to its own problems. So selecting between this and a longer enzyme activation pre-step is another aspect best left addressed during assay optimization and validation steps, and before general use.

This third approach, in one of its two guises, is by far the most common hot start method seen in the average laboratory. It allows for ease of ambient sample and reaction handling while avoiding spurious side reactions. Thus, the next time you're looking at a reaction thermal profile you'll now know why so much time (sometimes as much as one-third of the total reaction time) is spent in a hot preactivation step, and what this is doing to maximize your assay utility.

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

Integrated software solution



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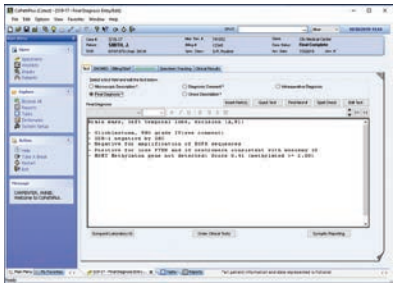
Customizable, task-based LIS



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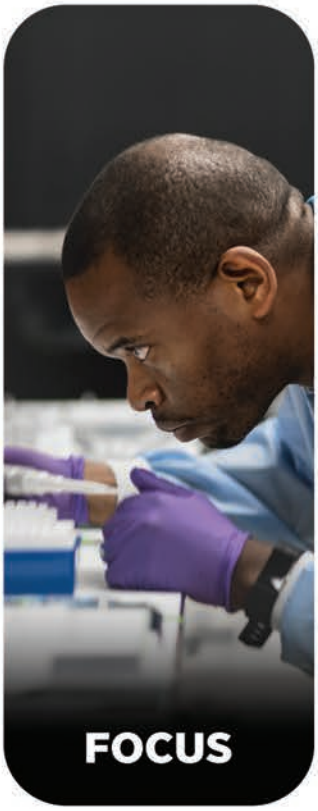

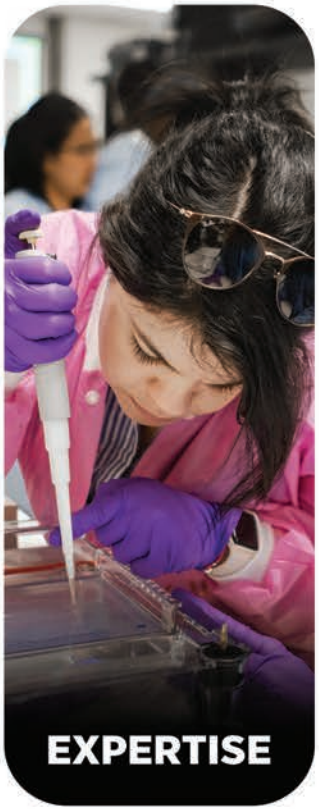

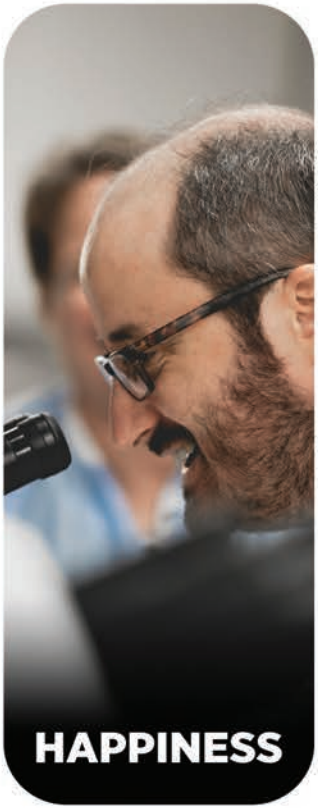

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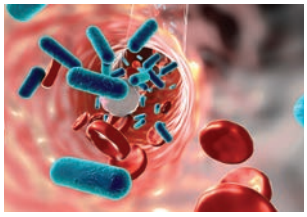

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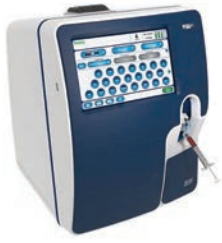

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Enzo Life Sciences

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A conversation with Dan Scungio, MT(ASCP) SLS, CQA(ASQ) aka "Dan the Lab Safety Man" (DLSM)

How did DLSM evolve? While serving as Sentara Healthcare's Lab Safety Officer (LSO) I worked with Terry Jo Gile, a seasoned clinical laboratory safety consultant known at the time as "The Safety Lady." She mentored me for five years. Terry Jo has since retired, however, I carry on her legacy of improving safety savvy in clinical, academic and research laboratories across the world as DLSM.

Tell us about your relationship with Sentara Healthcare. I've worked for Sentara, a multi-hospital system in Virginia and North Carolina, for 23 years. I began there as a lab generalist. I eventually managed two different labs before becoming the system LSO over 11 years ago. Today, I oversee the Lab Safety program for 12 hospitals and over 20 labs. I also chair the system Physical Environment committee, a group designed to oversee regulatory matters pertaining to Emergency Management, Hazardous Materials, Life Safety and several others.

What is the most asked question about lab safety? Most are centered around chemical management and hazardous waste handling. These are important areas of concern in the lab, and the regulations affecting them are vast and sometimes complicated. I find that many labs are following dangerous chemical and waste practices; placing staff and the environment in danger. One of my goals is to provide training to make sure labs understand the best safety practices, as well as the laws that regulate safety for chemical handling and hazardous waste management.

What are the most common threats to lab safety? Based on my experience, I would say the overall inattention to the proper use of Personal Protective Equipment (PPE). Personal safety in labs is often taken for granted. In many labs, staff do not wear lab coats, gloves or face protection when it is required. You can follow lab-specific groups on social media and see several posts each week, which include pictures of unsafe lab practices. In fact, the pictures themselves come from inappropriate cell phone use in the lab; another important infection prevention issue. Don't get me wrong, there are some laboratories where PPE use is enforced and followed, but there are so many more where it is not, and there are a great number of employee injuries or exposures just waiting to happen.

What kind of information can be found on your site? DTLSM, Inc., presents an annual Safety Academy (a virtual training series), provides on-site audits, safety procedure and process reviews, live education sessions and other customized services upon request. It also offers a free monthly lab safety newsletter, Safety Savvy. There are also monthly blog posts, as well as resources that can be used by lab safety professionals such as a chemical incompatibility chart, a Target Organ Poster and links to other lab safety resources. There is also a products page for those who need specific training materials such as CDs or books. For more details, visit http://danthelabsafetyman.com/products

Discuss new areas of lab safety. The face of lab safety has changed dramatically over the past 11 years. OSHA adopted the Globally Harmonized System for the Classification and Labeling of chemicals, changing how every lab manages their chemicals. The EPA put forth the Generator Improvement Rule in 2018 which affects hazardous waste management. CAP has also recently made changes to its safety standards which affect lab safety practices. The CDC will soon publish an updated version of its guide, *Biosafety in Microbiological and Biomedical Laboratories*. This document provides what are considered "gold-standard" practices for biohazard safety. The WHO and the International Organization for Standardization are both developing updated lab safety standards that will affect risk management and lab safety practices around the world. Lab safety is a constantly changing landscape, especially as technology and regulations evolve.

Is automation affecting lab safety? Some front-end automation has made the lab a safer place for employees. Automated specimen processing lines that centrifuge, remove caps and aliquot specimens without human intervention reduce exposure risks. The same is true for other automation such as slide makers and strainers which operate without staff. On the other hand, modern technology has created some lab safety challenges. Vendor representatives often use cell phones and laptops when diagnosing instrument problems. The use of these items inside and outside the lab presents many infection-control risks. Auditors are increasingly using electronic tablets to do their work. How are these devices properly disinfected? It's a difficult process to manage, and more issues will arise as technology continues to change.

How is recycling/sustainability affecting lab safety? Recycling in the lab affects safety in a number of areas. Overall waste management is tricky because there are several waste streams (regular trash, regulated medical waste, chemical waste, universal waste, etc.). Adding recycling to the mix can be done, but it takes good staff education and explicit procedures. For example, recycling paper is good, but consideration needs to be given to protected health information and patient privacy. As a result, many paper items in the lab need to be shredded rather than recycled. Recycled chemicals can bring savings to the lab and the environment, but handling the chemicals brings exposure opportunities to lab staff, and maintenance of recycling equipment becomes vital.

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