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MEDICAL LABORATORY OBSERVER

JANUARY 2020 ■ Vol 52 ■ No 1

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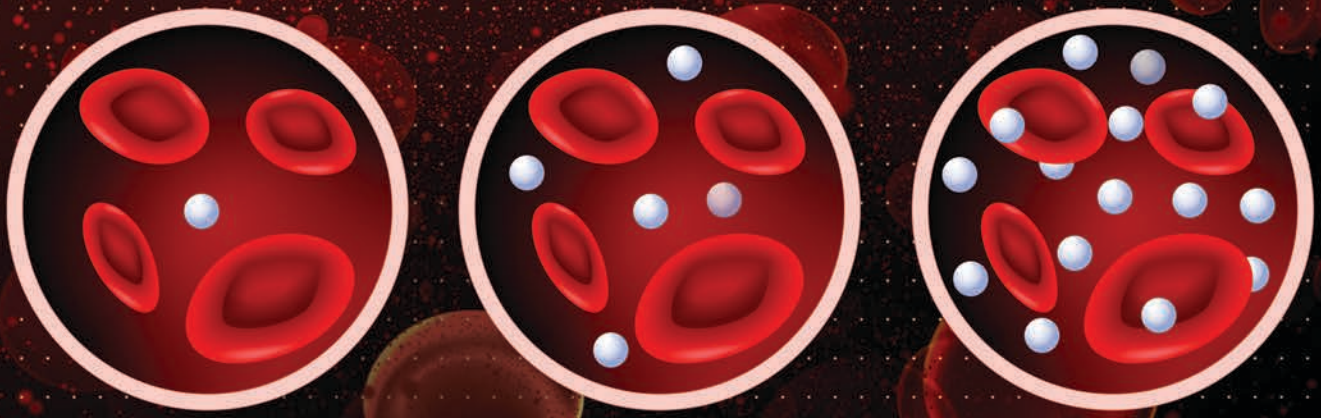
CE

Diabetes Mellitus

Clinical & Diagnostic Considerations



AI in laboratory diagnostics

State of the industry: IT solutions in the lab

Lab QC - from error detection to risk management

LAB INNOVATOR

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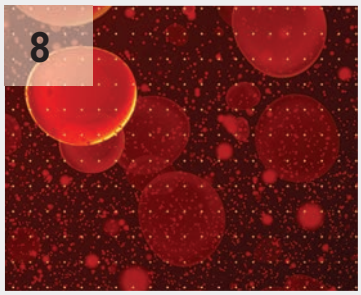

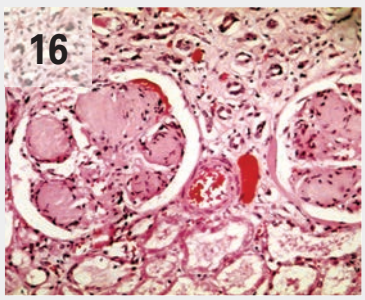

STATE OF THE INDUSTRY QUARTERLY REPORT

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2020 State of the Industry

By Brenda Silva
Editor

As 2020 begins, one of the top priorities predicted for the clinical diagnostics industry is an expanded role for information technology (IT) to assist with increasing demands for accurate patient test results. Ever since technology successfully moved into the clinical diagnostic environment, lab directors and managers have come to recognize its value, especially when faced with an overworked staff who was responsible for performing time-consuming test procedures. Today, the current decline of physical laboratorians has created a need to "hire" and integrate new technology and automation solutions to keep pace with test demands, while also maintaining test standardization and eliminating the historic risks of hands-on errors that can occur during testing protocols.

In an effort to learn more about how clinical labs are utilizing available IT solutions, *Medical Laboratory Observer (MLO)* recently conducted a survey of almost 300 respondents who provided insight and comments about their current IT usage and plans for future use. To see what the collected data showed, read our special *State of the Industry* insert entitled "IT solutions in the clinical lab," beginning on page 26. This feature is the first of four quarterly special reports *MLO* has planned that focus on important topics in clinical diagnostics. The remaining three reports scheduled for this year include best practices in lab management in the April issue, disease management in the July issue and molecular diagnostics in the November issue.

We've also highlighted artificial intelligence (AI) in the lab in this issue, as a complement to our focus on IT solutions, with research that describes how innovative approaches and algorithms are contributing to efficiency and efficacy in the lab. Current AI solutions and machine-learning options are helping to streamline workflow, in much the same way that existing LIS/LIMS and new IT solutions do, with all-things technological designed to work in digital concert to relieve both the physical and financial burdens faced by many labs today. Along with the challenges that accompany new technology and its adoption in the lab comes the issue of reimbursement that is a constant source of concern for administrations when considering the value of implementing digital solutions and training staff.

Another major source of concern for clinicians, as well as the subject of many lab tests, is the rising number of patients who are diagnosed with diabetes every day. According to the International Diabetes Foundation (IDF), there were 425 million diabetics worldwide in 2018, with that number expected to rise to 642 million by the year 2040. To further stress the importance of early treatment, this issue includes two articles on diabetes, with one that details clinical and diagnostic consideration for diabetes mellitus (DM), and another suggesting that biomarkers play an important role in early diagnosis of diabetes. Both offer insightful research that may provide answers to future disease management.

When looking at the best practices in lab management, administrations must consider not only the advantages of the new technology/equipment, but also what kind of reimbursement and/or return on investment (ROI) it may offer down the line. In addition, questions about scalability, data reliability and the potential for increased efficiency must also be answered satisfactorily before the first dollar is designated for the expense. In these situations, the new technology must prove itself worthy from day one to validate the costs they demand.

Likewise, as increasing patient tests demand more technology, diagnostic labs claim a more important percentage in the continuum of care. As the integral piece that connects many other parts in a new paradigm of healthcare, clinical labs must look to improve tomorrow by embracing the benefits of today's diagnostic advances. By doing this, new lifesaving technologies can truly save lives in the future.

I welcome your comments, questions and opinions -please send them to me at Bsilva@mlo-online.com

MEDICAL LABORATORY OBSERVER

MEDICAL LABORATORY OBSERVER Vol.52, No.1

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MLO - MEDICAL LABORATORY OBSERVER
(ISSN: 0580-7247). Published monthly, with an additional issue in August, by Endeavor Business Media, LLC., 2477 Stickney Point Rd, Suite 221B, Sarasota, FL 34231 (941) 388-7050. Subscription rates: $127.60/year in the U.S.; $154.88 Canada/Mexico; Intl. subscriptions are $221.43/year. All issues of *MLO* are available on microfilm from University Microfilms International, Box 78, 300 N. Zeeb Rd., Ann Arbor, MI 48106. Current single copies (if available) $15.00 each (U.S); and $20.00 each (Intl.). Back issues (if available) $17.60 each (U.S.); $22.00 each (Intl.). Payment must be made in U.S. funds on a U.S. bank/branch within the continental U.S. and accompany request. Subscription inquiries: subscriptions@endeavorb2b.com. *MLO* is indexed in the *Cumulative Index for Nursing and Allied Health Literature* and *Lexis-Nexis*. *MLO* Cover/CE, Clinical Issues, and Lab Management features are peer reviewed. Title® registered U.S. Patent Office. Office of publication: Periodicals Postage Paid at Nashville, TN 37209 and at additional mailing offices. **Postmaster:** Send address changes to Omeda (MLO Medical Laboratoy Observer), PO Box 3257, Northbrook, IL 60065-3257.
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Fast Facts

Healthcare Spending

4.6 percent

Is the amount that total national healthcare spending in 2018 grew, according to a study conducted by the Office of the Actuary at the Centers for Medicare & Medicaid Services (CMS).

4.5 percent

Is the average growth in overall healthcare spending 2016-2018, slower than the 5.5 percent average growth for 2014-2015.

$11,172

Is the amount per person spent as part of the national healthcare expenditures.

$3.6 trillion

Is the total US total healthcare expenditure. According to the report, private health insurance, Medicare, and Medicaid experienced faster growth in 2018.

5.8 percent

Is the amount private health insurance spending (34 percent of total healthcare spending) increased to $1.2 trillion in 2018, which was faster than the 4.9 percent growth in 2017.

21 percent

Is the amount of Medicare spending, which grew 6.4 percent to $750.2 billion in 2018, which was faster than the 4.2 percent growth in 2017.

16 percent

Is the amount of Medicaid spending which increased 3.0 percent to $597.4 billion in 2018. This was faster than the rate of growth in 2017 of 2.6 percent.

• **Source:** The 2018 National Health Expenditures data. CMS website at: *https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-andReports/NationalHealthExpendData/NationalHealthAccountsHistorical.html*

1 in 5 adolescents now living with prediabetes

Nearly 1 in 5 adolescents aged 12-18 years, and 1 in 4 young adults aged 19-34 years, are living with prediabetes, according to a new Centers for Disease Control and Prevention (CDC) study published in *JAMA Pediatrics*.

Prediabetes is a health condition in which blood sugar levels are higher than normal, but not yet high enough to be diagnosed as type 2 diabetes. The condition also increases the risk of developing type 2 diabetes, chronic kidney disease, heart disease, and stroke.

Monitoring the percentage of adolescents and young adults with prediabetes can help determine the future risk of type 2 diabetes. To do this, CDC researchers used data from the National Health and Nutrition Examination Survey covering the years 2005-2016.

"The prevalence of prediabetes in adolescents and young adults reinforces the critical need for effective public health strategies that promote healthy eating habits, physical activity, and stress management," said CDC Director Robert R. Redfield, M.D. "These lifestyle behaviors can begin early in a child's life and should continue through adolescence and adulthood to reduce onset of type 2 diabetes."

Key study findings:

Nearly 1 in 5 (18%) adolescents (those aged 12-18) and 1 in 4 (24%) young adults (aged 19-34 years) were living with prediabetes.

- The percentage of adolescents and young adults living with prediabetes was higher in males and participants with obesity.
- Hispanic young adults had higher rates of prediabetes compared to white young adults.
- Adolescents and young adults with prediabetes had significantly higher cholesterol levels, systolic blood pressure, abdominal fat and lower insulin sensitivity than those with normal glucose tolerance, which increased their risk of type 2 diabetes and other cardiovascular diseases.

Research shows that adults with prediabetes who take part in a structured lifestyle-change program, including weight management and exercise, can cut their risk of developing type 2 diabetes by 58% (71% for people over 60 years old). Participation in the CDC-led National Diabetes Prevention Program lifestyle change program can help prevent or delay type 2 diabetes in those at high risk. The program, available to those aged 18 and older, is taught by trained lifestyle coaches, and encourages healthy, whole-life changes to help participants address barriers to improved nutrition, increased physical activity and coping mechanisms for stress reduction.

Parents can also help encourage healthy eating and increased physical activity. They can aim for their children to get 60 minutes of physical activity a day.

HCV updates recommendations for identification and management of chronic Hep C

HCVguidelines.org — a website developed by the American Association for the Study of Liver Diseases and the Infectious Diseases Society of America to provide up-to-date guidance on the management of hepatitis C — was recently revised to reflect important developments in the identification and management of chronic hepatitis C (HCV).

Notably, the guidance includes an important new recommendation that all adults be screened for HCV. In addition to universal screening for hepatitis C, the guidance emphasizes universal treatment.

The update includes:

- A simplified treatment algorithm for patients without cirrhosis or with compensated cirrhosis, who have never been treated for HCV, for use by primary care providers.
- New treatment recommendations for children ages 3-11.
- A recommendation that patients with acute HCV be treated without a waiting period.
- Updates to all treatment sections, including removal of less efficacious, complex, alternative regimens, and regimens no longer available in the US.
- The update also includes new information about management of hepatitis C in patients receiving transplantation of organs from HCV-infected donors, an emerging area of the field.

"HCV has been called 'the silent killer' because of its ability to damage the liver while causing few or no symptoms. Identifying patients who don't know they are infected is key to stopping the spread of the disease. Our Panel has always recommended screening high-risk populations, but several studies now demonstrate that routine, one-time HCV testing among all adults in the U.S. would likely identify a substantial number of HCV cases that are currently being missed, and that doing so would be cost-effective. This is why we now recommend universal screening of adults," said HCV Guidance Co-Chairs, Drs. Marc G. Ghany, Kristen M. Marks, Timothy R. Morgan, and David L. Wyles.

"The good news is that once new HCV cases are identified, there are safe and effective treatments that can cure more than 95% of people. We believe that the

improved testing and treatment strategies described in the Guidance will bring us closer to achieving the World Health Organization's goal of eliminating HCV infection as a public health threat by 2030," they added.

AAAHC toolkit and benchmarking study offer tips for latex and penicillin allergies

The Accreditation Association for Ambulatory Health Care (AAAHC) has updated its Allergy Documentation Toolkit with an overview of challenges and improvement strategies, as well as more specific information on latex and penicillin/beta-lactam allergies, to help ambulatory healthcare organizations avoid patient complications.

More than 50 million Americans suffer from allergies each year, costing the healthcare system an estimated $18 billion. Penicillin allergies are the most common drug allergy in the U.S., with a reported prevalence of 10%, while latex allergies affect 1–7% of the U.S. population. For surgical procedures, specifically, hypersensitivity reactions may affect 1 in every 358 patients. The updated toolkit covers a wide range of allergic reactions – from severe and life-threatening, to sensitivities, intolerances, idiosyncratic reactions, and side effects.

"While documentation cannot always prevent adverse reactions, how healthcare providers approach documentation can help to reduce risk," said Naomi Kuznets, PhD, vice president and senior director of the AAAHC Institute for Quality Improvement. "Ambulatory organizations can use this resource to develop an action plan to improve allergy education and create a standard, consistent process to follow when documenting allergies."

While the Centers for Medicare and Medicaid Services (CMS) require documentation of allergies to medications in the pre-surgical assessment, allergy information on patient charts is often incomplete or inconsistent.

As shown in the 2019 AAAHC Quality Roadmap, accreditation survey data from 2018 surveys revealed two of the most common issues in allergy documentation are allergies not being updated during each visit and an overreliance on "No Known Drug Allergies" (NKDA).

"It is best practice for providers to note any severe reactions a patient has to any type of treatment and not just to drugs," said Kuznets. "Thorough documentation enables healthcare providers to take immediate action when a reaction occurs in the future."

To improve allergy documentation practices, AAAHC encourages ambulatory organizations to develop an action plan centered on education, consistency, and standardization. The updated toolkit provides organizations with current research and an action plan to educate staff, achieve consistent documentation, and standardize processes, prompts, and care transitions. Complementing the revised toolkit is an allergy documentation benchmarking study set to begin in January 2020.

Virtual reality could help flu vaccination rates

Using a virtual reality (VR) simulation to show how flu spreads and its impact on others could be a way to encourage more people to get a flu vaccination, according to a study by researchers at the University of Georgia and the Oak Ridge Associated Universities in Oak Ridge, Tennessee. This is the first published study to look at immersive virtual reality as a communication tool for improving flu vaccination rates among "flu vaccine avoidant" 18- to 49-year-old adults.

"When it comes to health issues, including flu, virtual reality holds promise because it can help people see the possible effects of their decisions, such as not getting a flu vaccine," said Glen Nowak, the principal investigator and director of the Center for Health and Risk Communication headquartered at Grady College. "In this study, we used immersive virtual reality to show people three outcomes—how if infected, they can pass flu along to others; what can happen when young children or older people get flu; and how being vaccinated helps protect the person who is vaccinated as well as others. Immersive VR increases our ability to give people a sense of what can happen if they do or don't take a recommended action."

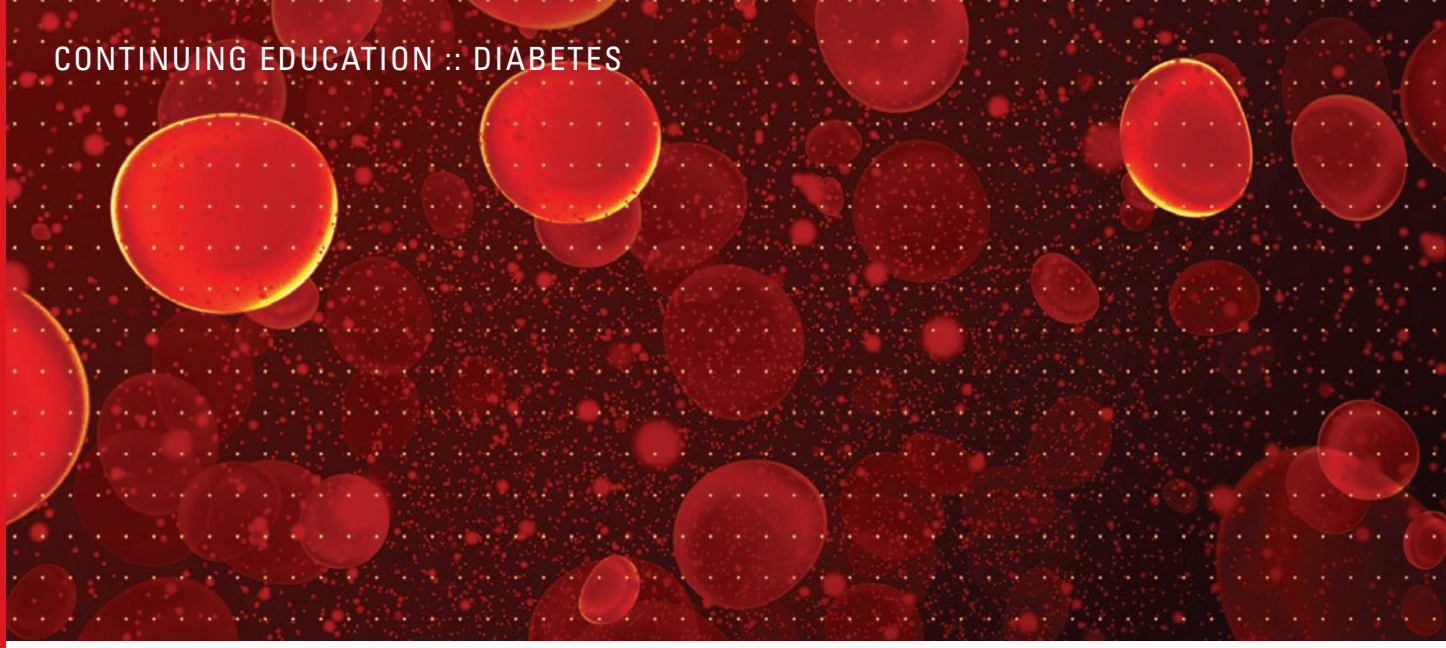
The research, "Using Immersive Virtual Reality to Improve the Beliefs and Intentions of Influenza Vaccine Avoidant 18- to 49-year-olds," was published by the journal Vaccine on Dec. 2, which falls during National Influenza Vaccination Week, Dec. 1 – 7. The research was conducted by faculty at Grady College of Journalism and Mass Communication, including faculty in Grady's Center for Health and Risk Communication. The research was conducted with support from a grant and researchers from ORAU.

According to the Centers for Disease Control and Prevention (CDC) during the 2017-18 flu season, only 26.9% of 18- to 49-year-olds in the U.S. received a recommended annual influenza vaccination even though it is recommended for all 18- to 49-year-olds. The low current acceptance of flu vaccination makes it important to identify more persuasive ways to educate these adults about flu vaccination. The findings from this study suggest one-way virtual reality can be more effective as it can create a sense of presence or feeling like one is a part of what is happening.

1. The 171 participants in this study self-identified as those who had not received a flu shot last year and did not plan to receive one during the 2017-18 influenza season. In the study, participants were randomly assigned to one of four groups: a five-minute virtual reality experience;
2. a five-minute video that was identical to the VR experience but without the 3-dimensional and interactive elements;
3. an e-pamphlet that used text and pictures from the video presented on a tablet computer; and
4. a control condition that only viewed the CDC's influenza Vaccination Information Statement (VIS), which is often provided before a flu vaccine is given and describes benefits and risks. Participants in the VR, video and e-pamphlet conditions also viewed the CDC VIS before answering a series of questions regarding flu vaccination, including whether they would get a flu vaccine.

In the VR condition, participants were provided headsets, which enabled them to vividly experience the information and events being shown as if they were in the story, and video game controllers, which enabled them to actively participate at points in the story. Compared to video or the e-pamphlet, the VR condition created a stronger perception of presence – that is, a feeling of "being there" in the story, which, in turn, increased participants' concern about transmitting flu to others. This increased concern was associated with greater confidence that one's flu vaccination would protect others, more positive beliefs about flu vaccine and increased intention to get a flu vaccination. Neither the e-pamphlet nor the video was able to elicit a sense of presence nor were they able to improve the impact of the VIS on the confidence, belief and intention measures.

"This study affirms there is much to be excited about when it comes to using virtual reality for heath communication," Karen Carera, senior evaluation specialist at ORAU, said. "However, the findings suggest that for virtual reality to change beliefs and behaviors, the presentations used need to do more than deliver a story. They need to get users to feel like they are actually in the story."



Clinical and diagnostic considerations for diabetes mellitus

By Thomas Lohmann, MD

The term diabetes, derived from the Greek word diabainein, meaning "to pass through," refers to any condition which is associated with the production of large amounts of urine. When the polyuria is associated with hyperglycemia, the term Diabetes Mellitus (DM) is used (Mellitus, from the Latin meaning "sweetened with honey"). The hyperglycemia results from reductions in insulin production, secretion or action on many cell types and with long-term hyperglycemia, there can be damage to nerves, blood vessels, retinas and kidneys.

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LEARNING OBJECTIVES

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

1. Recall the signs, symptoms and risk factors of the development of diabetes mellitus.
2. Describe the pathophysiology of the classifications of diabetes mellitus.
3. Discuss lab values in the diagnosis of diabetes mellitus.
4. Discuss the HgA1C test, its limitations and factors in interpreting results.

It is estimated that the direct cost of diagnosis and treatment of Diabetes Mellitus in the United States is over $350 billion annually. The cost of undiagnosed diabetes and the long-term sequelae is many times that number. Therefore, efficient approaches to the diagnosis of this heterogeneous group of diseases are crucial to identify and treat patients early in their disease cycle to reduce the high cost of managing the late-stage complications and to improve patient outcomes. There are multiple types of Diabetes Mellitus, with classification being based on the mechanisms causing hyperglycemia. As defined by the American Diabetes Association Standards of Care, the following categories are recognized:

Type 1 diabetes: due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency. Autoimmune markers include islet cell autoantibodies and autoantibodies to GAD (GAD65), insulin, the tyrosine phosphatases IA-2 and IA-2β, and ZnT8. Type 1 diabetes is defined by the presence of one or more of these autoimmune markers. The disease has strong HLA associations, with linkage to the *DQA* and *DQB* genes.

Type 2 diabetes: due to a progressive loss of β-cell insulin secretion frequently on the background of insulin resistance.

Gestational diabetes mellitus (GDM): diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation.

Specific types of diabetes due to other causes: e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY], diseases of the exocrine pancreas (such as cystic fibrosis

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and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation).[1]

How is DM diagnosed?

Patients in all categories of DM manifest symptoms of increased plasma glucose, resulting in polydipsia, polyuria, polyphagia with weight loss, increased numbers of yeast infections and impairment of growth. Uncontrolled hyperglycemia often leads to ketoacidosis or lactic acidosis from nonketotic hyperosmolar syndrome.

The underlying common element is a lack of insulin response at the end-organ cell receptors. This may result from a decreased production of insulin due to autoimmune destruction of the beta cells of the pancreas. Other patients with DM have a resistance to the action of insulin, sometimes associated with metabolic syndrome and obesity. The basis of the abnormalities in carbohydrate, fat, and protein metabolism results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.[2]

If a patient presents in a hyperglycemic crisis, or with clear signs and symptoms of chronic hyperglycemia, the diagnosis of DM can be confirmed by a single random plasma glucose which exceeds 199 mg/dL. Without this severe clinical presentation, the diagnosis depends on two abnormal glucose values.[7]

Criteria for the diagnosis of diabetes (at least one of the following criteria are met):

1. After at least 8 hours of no caloric intake, the plasma glucose is ≥126 mg/dl
2. After the administration of a 75-gram glucose oral challenge, the plasma glucose after two hours is ≥200 mg/dl
3. A1C ≥6.5 percent employing a method that is NGSP certified and standardized to the DCCT assay
4. In the clinical setting of marked hyperglycemia, a random plasma glucose ≥200 mg/dL alone is sufficient.

Screening with Plasma Glucose		
Diagnosis	Fasting plasma glucose	2 HR post load glucose
NORMAL	<100 mg/dL (5.6mmol/L)	<140 mg/dL (7.8mmol/L)
IMPAIRED	100-125 mg/dL (5.6-5.9 mmol/L)	140-199 mg/dL (7.8-11.1 mmol/L)
DIABETIC	≥ 126 mg/dL (7.0 mmol/L)	≥ 200 mg/dL (11.1 mmol/L)

In asymptomatic patients, who should be tested for DM or prediabetes?

1. Testing should be considered in overweight or obese (BMI ≥25 kg/m2 or ≥23 kg/m2 in Asian Americans) adults who have one or more of the following risk factors:
   - First-degree relative with diabetes
   - High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
   - History of cardiovascular disease
   - Hypertension (≥140/90 mmHg or on therapy for hypertension)
   - HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
   - Women with polycystic ovary syndrome
   - Physical inactivity
   - Other clinical conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans)
2. Patients with prediabetes (A1C ≥5.7 percent) should be tested yearly.
3. Women who were diagnosed with GDM should have lifelong testing at least every 3 years.
4. For all other patients, testing should begin at age 45 years.
5. If results are normal, testing should be repeated at a minimum of 3-year intervals, with consideration of more frequent testing depending on initial results and risk status.[1]

What is prediabetes?

There are a group of patients that do not fall into the "Normal" or the "Diabetic" categories when tested for fasting glucose or a 2-hour post load glucose challenge. These patients are recognized as having a relatively high risk of developing DM and cardiovascular disease in the future. They frequently are obese, with increased lipids, particularly triglycerides, and hypertension. Patients with prediabetes often have near-normal glycated hemoglobin levels and can only be classified using the standardized OGTT.

What is the role of HbA1c in the diagnosis and management of DM?

Two laboratory assays play a role in the diagnosis and monitoring of diabetic patients: plasma glucose and HbA1c. With glucose, results from many different assay methods can be combined to create a longitudinal patient record, with widely accepted reference ranges and standardized assays. In contrast, patient results for HbA1c should not be combined from different methodologies, due to variations in interference from variant hemoglobins. Red blood cell (RBC) survival times should also be considered, with shortened survival resulting in artificial lowering of the HbA1c.

The HbA1c result is used to provide an estimation of the patient's glycemic control over the last two to three months, assuming the RBC's have an average circulating lifespan of 120 days. During that time period, glucose in the blood permanently binds to the hemoglobin in the RBC by the Amadori rearrangement forming HbA1c from the wild type (or typical) HbA. The higher the level of circulating glucose the higher the percentage of HbA1c will be formed, in turn, an average estimated glucose level (eAG) can be calculated from the percentage of HbA1c.

The American Diabetes Association (ADA) has published Standards of Care for HbA1c:

1. To avoid misdiagnosis or missed diagnosis, the A1C test should be performed using a method that is certified by

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the NGSP and standardized to the Diabetes Control and Complications Trial (DCCT) assay.

2. Marked discordance between measured A1C and plasma glucose levels should raise the possibility of A1C assay interference due to hemoglobin variants (i.e., hemoglobinopathies) and consideration of using an assay without interference or plasma blood glucose criteria to diagnose diabetes.

3. In conditions associated with an altered relationship between A1C and glycemia, such as sickle cell disease, pregnancy (second and third trimesters and the postpartum period), glucose-6-phosphate dehydrogenase deficiency, HIV, hemodialysis, recent blood loss or transfusion, or erythropoietin therapy, only plasma blood glucose criteria should be used to diagnose diabetes.[1]

If one uses HbA1c with a diagnostic cutoff of 6.5 percent, the diagnostic sensitivity for DM is 30 percent, meaning, if only HbA1c is ordered, there is a 70 percent chance of patients being missed in the diagnosis of DM.[3]

Interpreting HbA1c results

When interpreting HbA1c results, one must consider biologic variation of this marker. There is normal genetic variation in the rate of hemoglobin glycation. Conditions that prolong or shorten RBC survival will also disrupt the direct relationship of average glucose to the level of HbA1c. Patient age must also be considered, as levels increase with aging. Lastly, patient ethnicity plays a role, as levels are higher in African Americans at the same degree of glycemic control.

The choice of methodology of an assay for HbA1c should take into consideration the following interferences:

1. **Analytical interference:** Most newer methods for HbA1c have minimal analytical interference from the presence of the major hemoglobin variants (HbS, HbC, HbE, HbD) in the specimen. The reader is referred to the NGSP website for a more detailed table by manufacturer and methodology.
2. **Clinical interference:** There are clinical conditions that will limit the ability to use the HbA1c value as an estimate of the degree of glycemic control. "This issue is of particular concern when using assays for HbA1c (e.g. immunoassay) that will produce an HbA1c result for homozygous Hb variants, without providing information that a Hb variant is present in the sample."[4]

The current interpretation of HbA1c values, which corresponds to the calculated (eAG), assumes that the RBC life span is the same for all patients. However, even modest variation in red cell survival, which would not be apparent in routine hematological studies, could have a significant impact on the HbA1c level.1 Therefore, the detection of some of the more common causes of decreased (or increased) RBC survival would be important in determining whether the HbA1c level was an accurate reflection of a patient's level of glycemic control. In general, a shorter RBC life span would yield lower levels of HbA1c at a given average whole blood glucose concentration as compared to that of a normal patient.

RBC Lifespan and impact on Hb A1c result	
Factors increasing RBC lifespan	Impact on HbA1c results
Hereditary elliptocytosis	Prolonged survival results in higher measured HbA1c for a given average glucose level
Factors decreasing RBC lifespan	Impact on HbA1c results
Renal failure, dialysis, hemolytic anemia, spherocytosis, cirrhosis, hemoglobinopathy, beta thalassemia, RBC enzyme deficiencies, pregnancy, transfusion, sepsis	Shortened survival results in a lower measured HbA1c for a given average glucose level

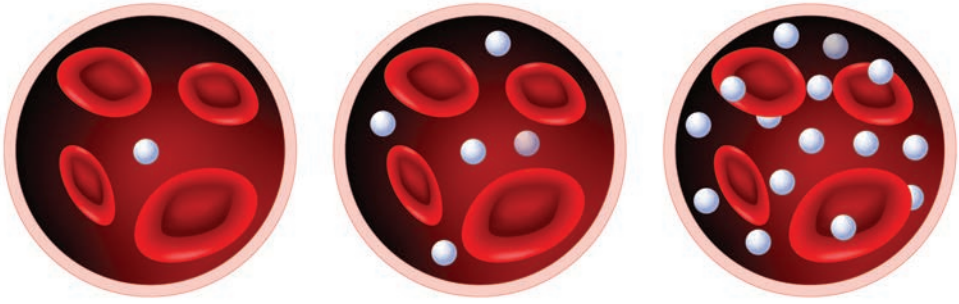
Extrinsic causes of decreased RBC survival include pernicious anemia, acquired hemolytic anemia, pregnancy, nephritis, hepatic disease, burns, sepsis, and anemia associated with malignancy. Intrinsic causes include hemoglobinopathy, paroxysmal nocturnal hemoglobinuria, congenital hemolytic jaundice and elliptocytosis. Renal and hepatic disease may be detected by scrutiny of the results of routine serum chemistry profiles. Hemolytic anemia is rare and may be suspected with a normocytic, normochromic pattern of anemia. Rarely will a patient with diabetes have testing which is specifically focused on determining if red cell survival is diminished due to congenital causes, with the most common condition being the presence of a hemoglobinopathy.[6]

Most methods are free from analytical interference from common hemoglobinopathies; however, the clinical interference may not be known if the patients' result does not indicate the presence of a hemoglobinopathy or other disease state that can alter the RBC lifespan.[5]

HbA1c methodology	Hemoglobinopathy detection?
Boronate affinity chromatography	NO
Capillary separation (CZE)	YES
Cation exchange HPLC	YES
Enzymatic	NO
Immunoassay	NO

Estimating glycemic control from HbA1c alone is applying a population average to an individual, which can be misleading. Although the mean of the average glucose concentration (AGC) is correlated with the HbA1c, there is a significant degree of inter-individual variation in AGC at the medical decision point of HbA1c (6.5 percent) which includes some AGC values well within the non-diabetic range. Likewise, some patients having HbA1c levels below 6.0 percent have AGC values which are associated with poor glycemic control. Therefore, should a single HbA1c less than 5.7 percent be relied on to rule out pre-diabetes or diabetes mellitus? If the patient has an unsuspected condition that

results in shortened RBC survival, this will falsely lower the HbA1c, to an extent that the patient will appear to be euglycemic. It is advisable that the method chosen for screening give information related to the presence of hemoglobin abnormalities, and that with homozygous or double heterozygous conditions, an alternate test be chosen for screening and monitoring of therapy. HbA1c can no longer be the only quality monitoring element for all diabetics. The key to effective utilization of HbA1c is knowing when this marker is most likely to be an inaccurate indirect indicator of glycemic control due to reductions in red cell circulation times. The choice of an analytical method for HbA1c is important, as methods that do not identify the presence of abnormal hemoglobin molecules may give erroneous results that, when reported, can wrongly indicate better glycemic control that truly exists for that patient.



Summary

Diabetes mellitus has several main subtypes, all having periods of hyperglycemia, and all, if left untreated, will result in damage to the kidneys, optic nerves, peripheral nerves and blood vessels. A third of patients with Type 1 DM have an initial presentation with ketoacidosis or lactic acidosis, while Type 2 and Gestational DM are more commonly found to have hyperglycemia on routine screening. The ADA has published criteria for the diagnosis of DM. Using these criteria, some patients do not fall into the "Diabetic" category, but who have Impaired Fasting Glucose or Impaired Glucose Tolerance. These prediabetic patients are usually obese, and have hyperlipidemia, low HDL and hypertension. Many of these patients will see reductions in fasting and post load glucose values with a proper weight loss regimen and regular exercise schedule. The category of Gestational Diabetes Mellitus has a specific screening algorithm, and these patients require monitoring of plasma glucose levels at three-year intervals during their lifetime.

Guidelines for screening and monitoring of Type 1 and Type 2 diabetics now includes the measurement of HbA1c levels. While there are many techniques utilized by labs for this analyte, there is an increasing awareness of the role played by RBC survival times in the creation of the glycated hemoglobin molecules. While some of the causes of altered RBC survival are readily apparent in the patient's clinical presentation, the presence of hemoglobinopathies or thalassemia may go unrecognized. In patients with homozygous or doubly-heterozygotic hemoglobinopathies, the red cell survival may be reduced to a degree that precludes the use of HbA1c as a marker of hyperglycemia. The ADA has issued guidelines that, "In conditions associated with an altered relationship between A1C and glycemia, such as sickle cell disease, pregnancy (second and third trimesters and the postpartum period), glucose-6-phosphate dehydrogenase deficiency, HIV, hemodialysis, recent blood loss or transfusion or erythropoietin therapy, only plasma blood glucose criteria should be used to diagnose diabetes."[1] It is therefore critical that patient testing for HbA1c should be performed initially using a method which can detect the presence of abnormal hemoglobin molecules or thalassemia's, and that the presence of these abnormalities is communicated to the ordering physicians to determine the effect, if any, on the measured HbA1c. The degree of glycemic control should then be measured using glycated albumin or continuous glucose monitoring meters.

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Clinical and Diagnostic Considerations for Diabetes Mellitus

JANUARY 2020 *[This form may be photocopied. It is no longer valid for CEUs after July 31 2021.)*

TEST QUESTIONS

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

1. **The diabetes mellitus term is historically used when**
   - ○ oliguria is associated with hyperglycemia
   - ○ polyuria is associated with hyperglycemia
   - ○ polyuria is associated with hypoglycemia
   - ○ oliguria is associated with hypoglycemia

2. **Long-term hyperglycemia can cause damage to**
   - ○ a. nerves
   - ○ b. retinas and kidneys
   - ○ c. blood vessels
   - ○ d. all of the above

3. **The estimated annual cost of diagnosis and treatment of Diabetes Mellitus is**
   - ○ a. 150 million
   - ○ b. 250 billion
   - ○ c. 300 million
   - ○ d. 350 billion

4. **There are 5 recognized categories of Diabetes Mellitus.**
   - ○ a. True
   - ○ b. False

5. **Which category of diabetes is caused by autoimmune β-cell destruction that leads to absolute insulin deficiency?**
   - ○ a. Type 1 diabetes
   - ○ b. Type 2 diabetes
   - ○ c. gestational diabetes mellitus
   - ○ d. diabetes of other causes

6. **Which category of diabetes is caused by the progressive loss of β-cell insulin secretion?**
   - ○ a. Type 1 diabetes
   - ○ b. Type 2 diabetes
   - ○ c. gestational diabetes mellitus
   - ○ d. diabetes of other causes

7. **Cystic fibrosis and/or pancreatitis can lead to**
   - ○ a. Type 1 diabetes
   - ○ b. Type 2 diabetes
   - ○ c. gestational diabetes mellitus
   - ○ d. diabetes of other causes

8. **Symptoms of diabetes mellitus include all but**
   - ○ a. weight gain
   - ○ b. polyuria
   - ○ c. polyphagia
   - ○ d. yeast infections

9. **Diabetes mellitus is diagnostic with a fasting plasma glucose of**
   - ○ a. > or = to 99 mg/dl
   - ○ b. > or = to 114 mg/dl
   - ○ c. > or = to 120 mg/dl
   - ○ d. > or = to 126 mg/dl

10. **Risk factors in asymptomatic patients include all but**
    - ○ a. ethnicity
    - ○ b. hypotension
    - ○ c. physical inactivity
    - ○ d. first-degree relative with diabetes

11. **Women who where diagnosed with gestational diabetes should**
    - ○ a. have life-long testing every 3 years.
    - ○ b. have life-long testing every 1 year.
    - ○ c. have life-long testing beginning at 45 years-old
    - ○ d. none of the above

12. **Individuals with prediabetes have**
    - ○ a. a normal oral glucose tolerance test (OGTT)
    - ○ b. an elevated A1C test
    - ○ c. a normal A1C test
    - ○ d. normal random glucose levels

13. **Different assay methodologies of HbA1C tests are widely accepted in creating a longitudinal patient record.**
    - ○ a. True
    - ○ b. False

14. **An estimated average glucose is calculated from**
    - ○ a. OGTT test results
    - ○ b. fasting plasma glucose results
    - ○ c. random plasma glucose results
    - ○ d. HgA1C results

15. **Only plasma blood glucose should be to diagnose diabetes in patients with**
    - ○ a. HIV
    - ○ b. sickle cell disease
    - ○ c. erythropoietin therapy
    - ○ d. all of the above

16. **The American Diabetes Association has published standards of care in order to avoid missing a diagnosis of diabetes**
    - ○ a. True
    - ○ b. False

17. **If only a HbA1C test is ordered to assess for diabetes, there is a _____% chance of patients being missed in the diagnosis of DM.**
    - ○ a. 25
    - ○ b. 75
    - ○ c. 80
    - ○ d. 95

18. **There are NGSP certified testing methods for HgA1C that have minimal interference in the presence of hemoglobin variants.**
    - ○ a. True
    - ○ b. False

19. **All but the following are conditions that will yield a lower measured HbA1C test result.**
    - ○ a. hereditary elliptocytosis
    - ○ b. beta thalassemia
    - ○ c. cirrhosis
    - ○ d. spherocytosis

20. **Which HbA1C testing method detects the presence of a hemoglobinopathy?**
    - ○ a. capillary separation
    - ○ b. enzymatic
    - ○ c. boronate affinity chromatography
    - ○ d. immunoassay

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   P ① ② ③ ④ ⑤ **E**
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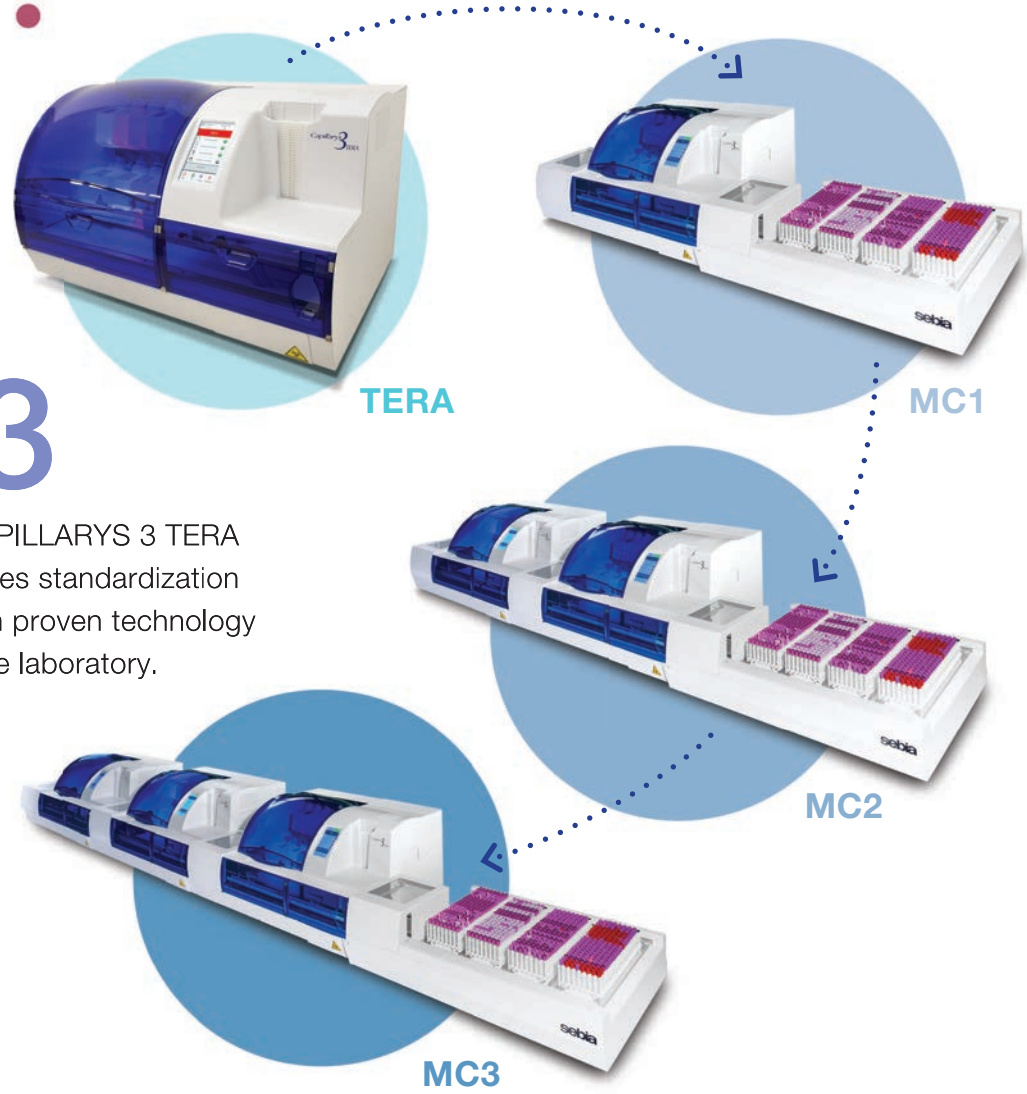

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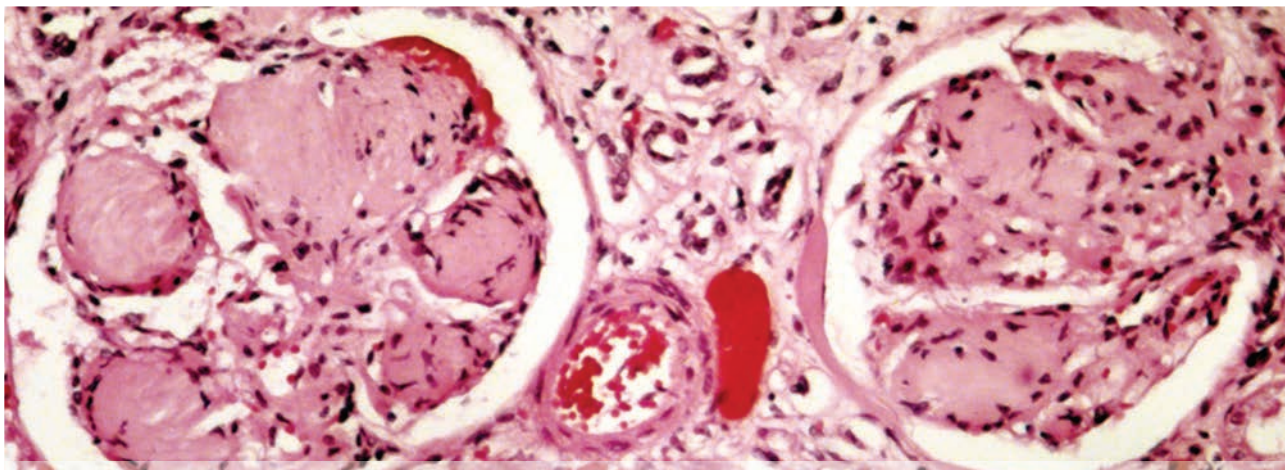
Biomarkers key in diagnosis and management of T2DM

By Jessica Pawlak



This photomicrograph from the CDC depicts some of the histopathologic details seen in a kidney tissue sample, in a case of nodular glomerulosclerosis, otherwise known as Kimmelstiel-Wilson syndrome, which is a form of diabetic nephropathy, associated with long-standing diabetes mellitus.

As the population of diabetic and pre-diabetic patients continues to increase, the need for diabetic biomarkers for earlier diagnosis, more effective treatment monitoring and earlier indications of pending disease complications is becoming more critical. The need to standardize methods to ensure accurate results across clinical platforms will remain critical to patient diagnosis, classification and care.

Insulin resistance and diagnostic biomarkers

Looking to the future of diabetes management, consideration for the multitude of factors affecting insulin production, insulin resistance and β-cells will lead to a better understanding of how individualized treatment and monitoring may work. Research into the pathophysiology of diabetes has led to the suggestion of novel biomarkers for diagnosing and monitoring diabetes.[3,4,5,6,7,8] The field of diabetic research has led to 10 Nobel prizes since 1923, and while diagnosis still relies heavily on glucose testing and HbA1c, the biological pathways involved in diabetes are complex and there may be a need for combination treatments for the best outcomes.[1,2] Typically, by the time a patient is diagnosed with diabetes, they have lost some of their β-cell function and are exhibiting clinical indicators, providing more evidence that the need for earlier biomarkers is key to treating at risk patients.

Complications caused by diabetes are most concerning, as its effects on the heart, liver, kidneys, brain and eyes lead to irreversible damage and, in some cases, death. The disease's pathophysiology has also lead to the discovery of a multitude of drugs for treating diabetes in conjunction with insulin or as a standalone treatment with life style changes. Metformin, an insulin sensitizing agent, is one such drug.

The exact mechanism through which insulin resistance occurs has not been identified; however, it has been identified that both overproduction of glucose and lack of glucose uptake play an integral role.[1,2,7] While this review focuses primarily on novel clinical chemistry biomarkers for diagnosing and monitoring T2DM, it is notable that in the last decade, there have been multiple T2DM associated genes identified. In his review, Defronzo discusses transcription factor 7 like 2 (TCF7L2), which plays a role in β-cell production and insulin secretion. The t-allele of a single nucleotide polymorphism of the TCF7L2 gene is associated with impaired insulin secretion and both CT and TT genotypes can predict T2DM.[2]

All types of diabetes are complex and involve multiple tissues and organs, with subsequent downstream effects on organ systems and metabolic pathways.[3] Around 2009, there was a switch from the typical Triumvirate theory to an Ominous Octet theory. The Ominous theory suggests that it is not only the muscles, liver and β-cells (triumvirate) that contribute to diabetic pathophysiology, but also adipocytes, pancreatic β-cells, and cells of the gastrointestinal tract, the kidney and the brain. Insulin sensitivity and uptake of glucose play critical roles in diabetes and yet it is still the failure of the β-cell that causes diabetes development to advance. The gold standard for measuring functioning β-cells is defined by: (change in insulin/change in glucose)/insulin resistance or $[(\Delta I/\Delta G) \div IR]$.[1,2,5] This switch to a multisystem theory, opened the doors to looking at biomarkers related to inflammatory, metabolic, gastrointestinal uptake/absorption and vascular and endothelial pathways, as well as, tissue biomarkers in skin and the retina.

So, what is a biomarker? The National Cancer Institute defines it as, "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease."[3] Biomarkers may be used to track response to treatments to determine if subsequent changes to the treatment plan are needed.

Table 1: Diabetic Biomarkers

Current		
Biomarker	**Advantage**	**Disadvantages**
HbA1c[3,4,5,6]	Non-invasive test, no fasting Available on automated platforms NGSP method standardization Indicates glycemic control over 2-3 month period Predicts diabetic diagnosis within 5 years	Interference in presence of hemoglobin variants Red blood cell turnover affects levels Thresholds don't consider ethnicity, BMI and age
Novel		
Biomarker	**Advantage**	**Disadvantages**
Fructosamine (FA) [4,6,7,8]	Non-invasive test, no fasting Available on automated platforms Indicates average glucose over 1-4 week period Not effected by red cell turnover Potential marker for monitoring treatment effectiveness Rapid, inexpensive, available on automated platforms	No standardization across assays Susceptible to ambient temperatures Extremely variable within single patient Not accurate if patient has high albumin turnover Levels may overlap with nondiabetic patients – not optimal for diagnosis High vitamin C interfere with results IgA levels effect results
Glycated Albumin (GA)[4,6,8]	Non-invasive test, no fasting Newer, automated assays are being developed More accurate in patients with renal failure and hemolytic anemia More accurate than FA in nephrotic, liver and thyroid diseases Current method is better standardized than FA	Measured directly by multiple chromatography methods, spectroscopy or electrophoresis which need special equipment and training Not accurate if patient has high albumin turnover Obesity causes falsely low values
C-peptide[4,5,7]	Non-invasive test, no fasting Available on automated platforms Predicted diabetic onset 10 years prior to onset Distinguishes between native and injected synthetic insulins	No standardization Recommended to test in same laboratory and with same method for patient monitoring
C-reactive protein (CRP)[5,6]	Non-invasive test, no fasting Available on automated platforms Predicted T2DM within 5 years of test* hsCRP indicates high CVD risk Associated with insulin resistance Associated with elevated levels of IL-6	Not as accurate as IL-6 Validated as indicator of CVD risk , role in IR needs more investigation
Leptin[4,5]	Non-invasive test, no fasting Available on automated platforms Connects more than 5 potential diabetic biomarkers Predicted diabetic onset 5-10 years prior to onset Associate with Insulin resistance	No standardization Role in diagnosis and/or monitoring still not fully characterized
Insulin[4,5,7]	Non-invasive test Available on automated platforms Predicted T2DM within 5 years of test* More common in type 1 diabetes management Can be used to indicate β-cell function	No standardization Requires fasting Does not distinguish between native and injected insulins
soluble leptin receptor (sOB-R)[4]	Present in diabetic patients 10 years prior to onset	More studies needed to confirm biomarker potential Measured by enzyme-linked immunosorbent assay (ELISA) methods
1,5 Anhydroglucitol (1,5 AG)[6]	Available on automated platforms Detects high glucose levels in the past 1 to 2 weeks	Race, sex and diet can affect results
β-hydroxybutyrate (β-HB)[7]	Rapid, inexpensive, available on automated platforms and handheld devices Indicator of DKA Measures blood ketones	Not optimal for diagnosis of T2DM, as presence occurs in severely unmanaged diabetes

*Not found to be a better predictor than OGTT and 2-h PG

For a biomarker to be confirmed, it must be shown to have utility in a least two independent populations.[3]

Biomarkers for disease management

While risk factors are often used in conjunction with biomarkers (and clinical presentation) in order to diagnose, monitor and manage disease states, it is important to note that they do differ. For example, patient characteristics such as age, weight and smoking history can inform the risk profile for diabetes; whereas HbA1c, a widely accepted biomarker associated with long-term diabetic outcomes, is a measurable biomolecule found within the blood and is indicative of the disease state and treatment management.

Together with the onset of new technologies in proteomics and genomics, combined with high-sensitivity imaging, and high-throughput clinical chemistry/immunoassay tests, the ability to find and validate diabetic biomarkers is increasing. Long-term outcome studies for these biomarkers will also be necessary to determine if the biomarker is a disease predictor or potential treatment target for long-lasting outcome changes. Fortunately, new technologies have provided a pathway for studies to be conducted retroactively on stored patient samples prior to diabetic diagnosis in order to analyze if these samples could have predicted the eventual onset of diabetes in the patients during set time periods from the initial blood draw. Given its manifestation as a complex, multisystem disease, it is important to note, that care must be taken to consider age, weight, BMI, fasting glucose and other potential variables that could alter the biomarker or influence its measurement.

While not all inclusive, Table 1 outlines some novel diabetic biomarkers taken from the overall review, including their advantages and disadvantages. A more detailed summary follows here.

Pros and cons of diabetic biomarkers

C-peptide is typically undetectable or low in type 1 diabetes with an initial decline followed by stabilization, while the marker is normal or high in type 2 diabetes.[3,4] Because it is derived in a 1:1 ratio when proinsulin is cleaved to make insulin, C-peptide an intriguing biomarker for insulin production in that high C-peptide levels can indicate high levels of insulin production.[7,8]

Fructosamine (FA) and glycated albumin (GA) are fairly new markers for early diagnosis of diabetic risk, but remain to be validated.[8] FA is created by the glycosylation of serum proteins (~70 percent of which is serum albumin), while GA directly measures the glycosylated albumin; high levels of either indicate high glucose levels over the previous 2-3 weeks. FA levels of > 2.5 mmol/L and GA ≥15.5 percent indicate diabetes, while GA levels of ≥ 13.35 percent indicate prediabetes.[6] Trends in FA could be used to determine if treatments are effective or if alterations to treatment plans should be made but it might be unsuitable for diabetes diagnosis.[8] Dorcely et al. suggest that combining GA with HbA1c is more sensitive in predicting prediabetes than HbA1c values alone. In addition, 1,5 Anhydroglucitol (1,5 AG) was also identified as a potential biomarker for diabetic treatment monitoring. 1,5 AG absorption is prevented when glucose levels are high, resulting in high urine and low plasma levels of this biomarker in diabetic patients, indicating high glucose levels in the past two weeks.[6]

Leptin has been identified, in a retrospective study, as a potential "hub" for multiple pathways including C-reactive protein (CRP) binding, glucose homeostasis, adipogenesis and insulin growth factor-binding protein 2 (IGFBP-2) interactions among others, leading to diabetic diagnosis and complications 5-10 years later.[4] The role of Leptin as a biomarker needs more controlled studies but provides interesting insight into the interconnected networks leading to T2DM and increased cardiovascular risk, and therefore should be studied as a potential early marker.

Initially, insulin would seem to be an obvious biomarker for diabetes, however, historical insulin results have not led to the accurate prediction of T2DM onset. T2DM is caused primarily by insulin resistance, so the amount of insulin is therefore less relevant than the actual estimate of resistance in a patient, done using the homeostatic model assessment (HOMA).[5,7]

Dorcely et al. outlined potential novel biomarkers related to insulin resistance such as α-hydroxybutyrate (α-HB), CRP, interlukin-6 (IL-6) and Acylcarnitine.[6] α-HB is significantly associated with insulin resistance independent of BMI, sex and age.[6] When there is insufficient glucose available for use (either due to endogenously low glucose levels or insulin resistance), the body metabolizes fat into ketones.[7] β-hydroxybutyrate (β-HB), another diabetic biomarker, is a measure of blood ketones and is likely not a candidate for early detection. Blood ketones consist of, acetoacetate, beta-hydroxybutyrate and acetone and each tests measures one or more of these ketones and is not interchangeable. IL-6 may be a better predictor of T2DM than CRP, as CRP may have a downstream role rather than being casual.[6]

Scirica's study on biomarkers, analyzed multiple diverse studies to determine the potential for biomarkers and their clinical implications. In two studies, he observed that high concentrations of NT-proBNP correlated with lower T2DM incidences, noting that in a separate transgenic mouse study, mice overexpressing BNP, were resistant to the effects of a high fat diet.[5]

Biomarker effficacy advances standardization

Once diagnosed, the need to test patients periodically for biomarkers has tremendous value, including to elucidate if there are changes in the pattern of expression that correlate to disease progression rates and the onset of diabetic complications. Changes in biomarker presentation at time of diagnosis and with disease progression could highlight when changes in treatments are needed.[4,5]

Further biomarker characterization should be undertaken to provide tools to predict eventual complications such as chronic kidney disease (CKD) and diabetic ketoacidosis (DKA) (both potentially irreversible and life threatening). Risk classification in the future could, and should, include the addition of cardiovascular disease (CVD) risk markers (N-terminal pro b-type natriuretic peptide, high sensitive troponin, high sensitive C-reactive protein), kidney function biomarkers (micro-albumin, creatinine, cystatin C), cholesterol markers (high density lipoproteins, low density lipoproteins, triglycerides, apolipoproteins, lipoprotein(a), ceramide) and liver function biomarkers (alanine aminotransferase, gamma-glutamyl transferase, plasminogen activator inhibitor 1, tissue

plasminogen activator, Fetuin A) to further differentiate the risk of diabetic diagnosis and treatment from downstream complications.[4,5,6]

If validated, certain biomarkers (i.e. FA, GA and 1,5 AG), could provide the physician with enough information to change treatment plans and track efficacy during a 2-3 week period (vs. every 3 months for HbA1c) and then modify treatments if response is not as expected. This would improve the monitoring of T2DM patients to ensure the treatments are effective and that downstream diabetic complications are not progressing well before they manifest clinically. Restricting monitoring to HbA1c, glucose and/or FA or GA levels as indicators of disease progression and regulation limits the knowledge of potentially life threatening implications of T2DM over time. As the diagnosis and monitoring of disease progression increases with the broader adoption of these new biomarkers, the next step will be to advance towards method standardization, much like what was accomplished for HbA1c.

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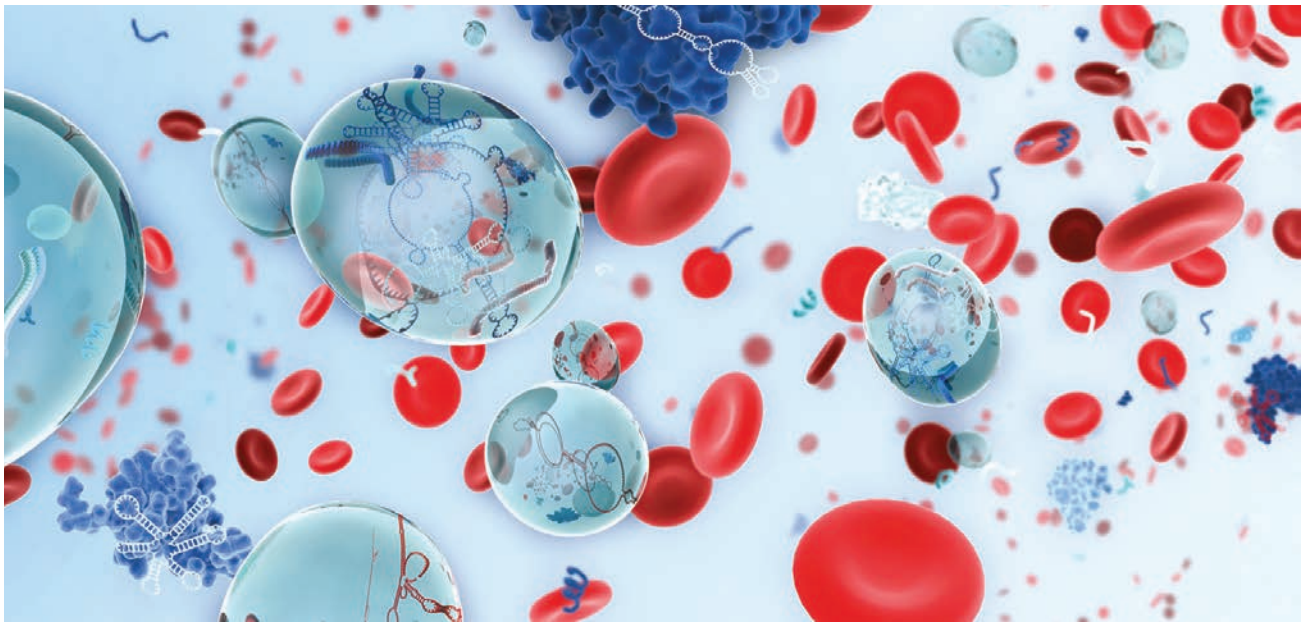

Liquid biopsy in cancer management

By Dr. Anke Homann

Historically, tissue has been the "gold standard" sample type for mutation profiling in oncology. More recently, analysis of blood and other body fluids has increased to complement tissue analysis and to support patient management in cancer. In patients for whom no tissue sample is available (e.g., due to the invasive and high-risk nature of the tumor biopsy procedures), a liquid biopsy is the only possibility to obtain mutation information for an optimal treatment decision. Collection of body fluid is less invasive and blood (plasma, serum), urine, cerebrospinal fluid, saliva, etc., can provide the relevant information about tumor heterogeneity. This information is almost impossible to obtain using tissue, since this would involve numerous tissue biopsies and necessitate knowledge of all existing lesions.

Despite these factors, liquid biopsy is not yet widely established as a routine diagnostic test within cancer patient management and there are challenges still to be overcome.

Analytes in liquid biopsy

Different circulating analytes, such as circulating cell-free DNA and RNA, circulating tumor cells (CTCs), exosomes and proteins have been identified in liquid biopsy and their diagnostic potential evaluated. The use of these analytes in cancer diagnostics has advantages, as well as limitations. An advantage of CTCs is their utility in cell morphology analysis, whereas ctDNA supports the use of highly sensitive detection methods.

Most advanced with respect to diagnostics is probably the analysis of circulating tumor DNA (ctDNA) in plasma, which is evidenced by several FDA-approved diagnostic tests. Standard methods for mutation profiling in liquid biopsy are currently PCR techniques, supporting a limited number of genes and short turnaround time, as well as next-generation sequencing (NGS) for a comprehensive panel of markers. Various mutation types (e.g., point mutations, small insertions and deletions, copy number variations or methylation markers) can be detected.

The use of ctDNA in cancer diagnostics has tremendous potential: early diagnosis, prognosis, treatment decisions, therapy and disease monitoring (including early identification of resistance mutations and recurrence testing). Liquid biopsies are used in various cancer types, especially lung, breast and prostate cancer. Even in brain cancer, recent data have shown that liquid biopsy can predict prognosis in patients. In addition to oncology, liquid biopsy can also be used in other applications, including reproductive health and other diseases.

At initial diagnosis, the mutation status is important for treatment decisions, since targeted therapies require mutation testing. Tumor heterogeneity is not (depending on the disease stage at diagnosis) the driver for liquid biopsy at initial diagnosis, rather the absence of tissue material. During therapy, monitoring of the disease can be supported by liquid biopsy testing, as the variant allele frequency (VAF) of driver mutations are expected to decrease and provide information related to therapy response. New resistance mutations may occur and can be detected in blood earlier than progression can be detected by other tools. Tumor heterogeneity at this disease stage is an important factor to consider for additional therapy decisions and liquid biopsy can reveal mutations of different lesions or metastases.

Challenges

The diagnostic workflow using liquid biopsy is not limited to just an assay: the workflow includes the complete preanalytical process from sample collection and handling through to data analysis. Each individual step within the workflow contributes to the robust result of a liquid biopsy procedure and therefore, the overall workflow needs to be standardized and optimized for the reproducible results that are a prerequisite for use in clinical routine testing.

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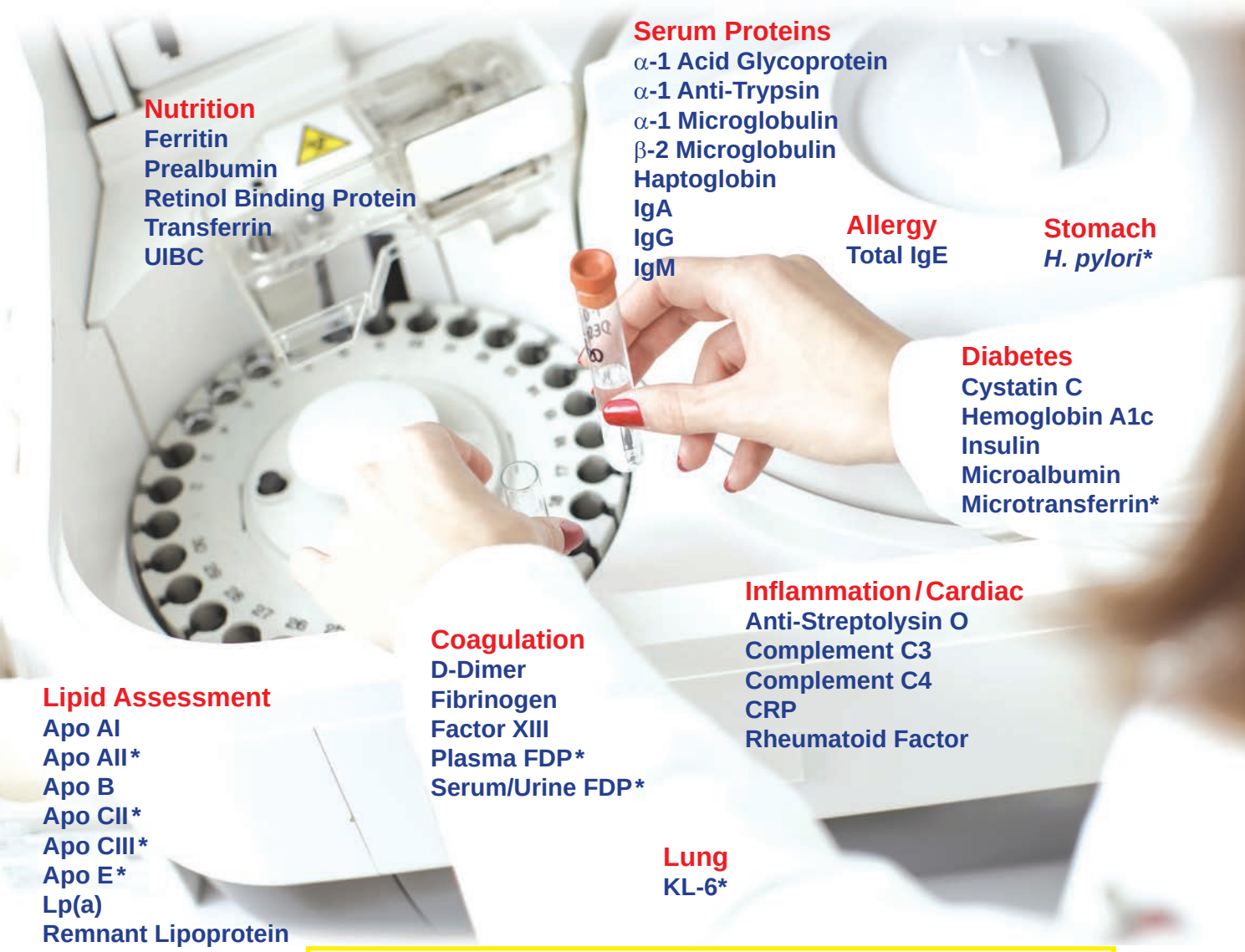

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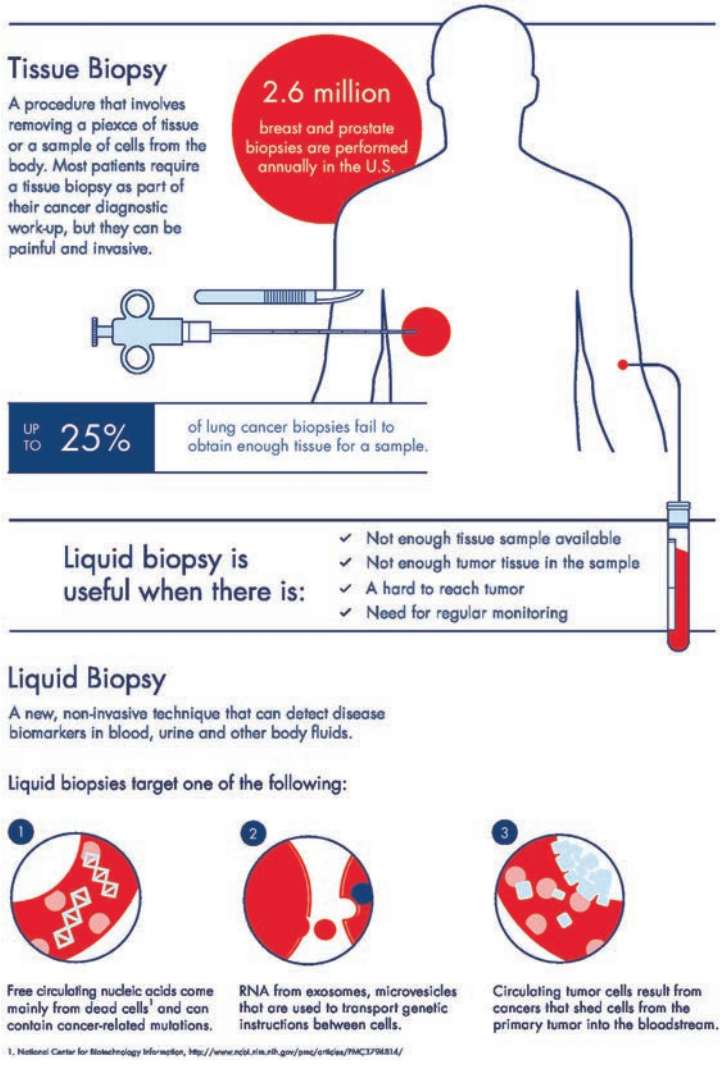
Limitations are known; for example, low concentrations of ctDNA in blood. Circulating DNA includes circulating tumor DNA, but also normal circulating DNA and sometimes genomic DNA from blood cells. The ctDNA is released by tumor cells of the primary tumor, circulating tumor cells (CTCs) or metastases. Therefore, ctDNA concentration fluctuates and data may be inconclusive; affected by tumor size or disease stage, for example. Clinical data have demonstrated that very low amounts of ctDNA (less than 10 copies per 5 ml plasma) or up to 100-fold concentrations can be detected in a patient specimen. Therefore, liquid biopsy assays require highly sensitive detection methods to identify individual tumor-derived molecules in a background of unmutated DNA. The preanalytical sample handling and processing is crucial to avoid degradation of clinically relevant ctDNA before even starting the detection step of the diagnostic workflow. Blood collection tubes that stabilize circulating tumor DNA in whole blood are essential to avoid ctDNA degradation or release of gDNA from blood cells. The half-life of circulating cell-free DNA is less than one hour. Therefore, the sample collection and tube handling processes also impact the outcome and therefore the diagnostic result. The nucleic acid extraction process is crucial to ensuring sufficient ctDNA and, of a quality high enough to allow the identification of low-frequency variants. In early diagnosis or minimal residual disease testing, low concentrations of mutated ctDNA are expected. Optimized data analysis tools are important to avoid false-positive or false-negative results that may lead to poor decisions in patient management. In addition, various parameters influence the rate of ctDNA shedding into the blood and more data, as well as standardization of sample collection is required. In routine diagnostic testing, a negative liquid biopsy is currently followed by a reflex tissue biopsy (where feasible), as the result may be influenced by the limitations discussed above.



Conducting clinical studies to support implementation of liquid biopsy into clinical routine is an ongoing activity. Reimbursement is limited and additional efforts, especially related to clinical validation, are required to ensure comprehensive mutation profile analyses using liquid biopsy. Inclusion in treatment guidelines requires adequate clinical efficiency of diagnostic tests. Reimbursement limitations are currently one of the biggest obstacles preventing utilization of liquid biopsy applications in cancer management.

Outlook

In addition to individual, approved liquid biopsy tests, comprehensive performance evaluation to support use of liquid biopsy in routine diagnostic is required. Additional tests for various analytes are required to cover the complete range of genomic alterations, including rearrangements. Clinical performance data are required to verify which allelic frequencies are clinically relevant, in order to ensure adequate analytical performance for individual use cases, cancer types and other diseases. As a high number of parameters from sample collection to data analysis influence the diagnostic result and therefore the patient management, studies are crucial to show concordance across available liquid biopsy tests and standardization of liquid biopsy workflows must be implemented. Consistency of results will support clinical guidance, which further impacts reimbursement decisions, as coverage is key to ensuring availability of these tests for all patients.

Standardization across the complete workflow has already begun, but more procedures and guidelines are required to support optimal patient management using these minimally or non-invasive procedures. To provide sufficient scientific evidence supporting the validity, clinical utility (as well as the effectiveness of mutation detection using ctDNA in cancer), a close collaboration of diagnostic test providers, pharma industry, as well as academic and clinical institutions, is required. This will make it possible to implement liquid biopsy applications in routine diagnostics and positively impact cancer patients. A broad use for multiple cancer types in diagnosis and disease monitoring based on guidelines for clinical practice will be a first milestone, and cancer screening applications will surely follow in the future.

Dr. Anke Homann, serves as Director of Medical and Scientific Affairs within the Oncology franchise at QIAGEN. She received her doctoral degree in cell biology from the Ruhr University of Bochum, Germany.

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FDA updates on Oncologic Diseases and NGS test use for HIV-1

FDA OHOP reorganizes, renamed office of oncologic diseases

The U.S. Food and Drug Administration's (FDA) office responsible for reviewing applications for new and existing cancer therapies has reorganized and been renamed as part of modernization plans approved in September 2019. The Center for Drug Evaluation and Research (CDER) Office of Hematology and Oncology Products (OHOP) has been reorganized and renamed the Office of Oncologic Diseases (OOD).

The OHOP contained three clinical divisions and one nonclinical division: Division of Oncology Products 1 (DOP1), Division of Oncology Products 2 (DOP2), Division of Hematology Products (DHP), and Division of Hematology Oncology Toxicology (DHOT). The new OOD structure consists of six divisions:

- DOP1 is re-named Division of Oncology 1 (DO1).
- SOP2 will be split into two divisions: Division of Oncology 2 (DO2) and Division of Oncology 3 (DO3).
- DHP will be split into two divisions to review products intended to treat hematologic malignancies: Division of Hematologic Malignancies 1 (DHM1) and Division of Hematologic Malignancies 2 (DHM2). DHP's review of products to treat non-malignant hematologic conditions will move to another office within CDER.
- DHOT remains the same.

DO1 will retain its responsibilities for products for breast, gynecologic and genitourinary cancers, as well as supportive care. DO2 will review products for thoracic and head and neck cancers, central nervous system cancers, pediatric solid tumors and rare cancers. DO3 will review products for gastrointestinal malignancies, melanoma and other advanced skin cancers and sarcomas.

DHM1 will be responsible for products for acute leukemia and myelodysplasia (includes myelodysplastic-myeloproliferative overlap syndromes), chronic myeloid leukemia and other myeloproliferative neoplasms with the term "leukemia," blastic plasmacytoid dendritic cell neoplasm (BPDCN), conditioning regimens for DHM1 indications, graft versus host disease, tumor lysis syndrome, cytokine release syndrome and CAR-T neurotoxicity.

DHM2 will review for products for lymphoma, chronic lymphocytic leukemia, multiple myeloma and other plasma cell malignancies.

Products for non-malignant hematologic diseases and conditions that DHP previously covered will be reviewed in the newly formed Division of Non-malignant Hematology (DNH) in the Office of Cardiology, Hematology, Endocrinology and Nephrology (OCHEN).

The Regulatory Project Management Staff are reorganized under the newly formed Office of Regulatory Operations (ORO) within the CDER Office of New Drugs (OND). Regulatory project management staff supporting the OOD will be in the newly formed Division of Regulatory Operations – Oncologic Diseases (DRO-OD), with individual branches supporting each of the five clinical review divisions in the OOD.

First NGS test for detecting HIV-1 drug resistance mutations

The U.S. Food and Drug Administration (FDA) authorized marketing of a test to detect human immunodeficiency virus (HIV) Type-1 drug resistance mutations using next generation sequencing (NGS) technology. The Sentosa SQ HIV Genotyping Assay is the first HIV drug resistance assay that uses NGS technology that the FDA has authorized for marketing in the U.S.

The current standard of care for patients with HIV-1 is antiretroviral therapy, also known as ART, the daily use of a combination of drugs to treat HIV by suppressing the virus. According to the NIH, it is a lifesaving treatment that can let patients with HIV lead long and healthy lives but it is not a cure.

Traditionally, monitoring a patient's viral load has been done to evaluate the effectiveness of treatments. Increasing viral loads indicate that the virus may have mutated and that a patient's current regimen is no longer effective at suppressing the virus. Once the virus has mutated and drug resistance develops, a person generally must change medications as different drugs will be needed to keep the virus from multiplying.

The Sentosa SQ HIV-1 Genotyping Assay detects HIV-1 drug resistance mutations in patients taking or about to start antiviral therapy. This assay detects mutations in genes of the HIV-1 virus from a sample of a patient's blood using NGS. Understanding the mutations in the virus can help healthcare providers select an effective combination of drugs in an ART regimen and indicate which drugs may no longer be effective against the mutated HIV-1 virus. The FDA reviewed data from performance studies, which demonstrated a greater than 95 percent sensitivity and specificity in detecting 342 HIV drug-resistant mutations and determined the Sentosa SQ HIV-1 Genotyping Assay provides a reasonable assurance of safety and effectiveness for its intended use.

The Sentosa SQ HIV Genotyping Assay is for use only in patients with HIV-1 who are about to start or already taking antiviral therapy and is not intended for diagnosing infection with HIV. Results of this test are intended to be used in conjunction with clinical observations, patient history and other laboratory evidence to make patient management decisions.

The FDA reviewed data for the Sentosa SQ HIV Genotyping Assay through the de novo premarket review pathway, a regulatory pathway for devices of a new type. Along with this authorization, the FDA is establishing criteria, called special controls, the requirements that test developers must meet for demonstrating accuracy, reliability and effectiveness of tests intended to identify virus mutations. These special controls, when met along with general controls, provide a reasonable assurance of safety and effectiveness for tests of this type. This action also creates a new regulatory classification, which means that subsequent devices of the same type with the same intended use may go through the FDA's 510(k) pathway, whereby devices can obtain clearance by demonstrating substantial equivalence to a predicate device.

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Clinical Lab IT Solutions

As the clinical lab industry faces increased patient testing, IT solutions provide an effective option to meet the demand for reliable and accurate results.

To learn more about how labs are taking advantage of the workflow benefits IT has to offer, *Medical Laboratory Observer* invited subscribers to take part in a survey and share their comments and usage.

The following special report shares that survey data and evidences current lab IT use, as well as planned use for the future, and looks at factors affecting both such as equipment costs and employee training, reliability of results and ROI. ▶

IT solutions in the clinical lab

Survey results show current usage trends and future challenges for IT solutions

By Brenda Silva

As clinical labs attempt to keep pace with testing and data management needs that show no sign of decline, administrations will be faced with finding new sources of revenue that allow labs to integrate solutions that focus on efficiency and productivity, such as IT software and automated systems.

When considering the history of practical medicine, patient care was limited to the equipment available and the knowledge of attending physicians, who often used best guesses as best practices to begin treatment. Today, however, physicians understand the importance of clinical labs as the first step in patient care, and often rely on lab-validated test results to help create a treatment plan that is specific to the clinical diagnosis of each patient.

As the number of patients who present with disease symptoms has increased, so have the demands on clinical labs to provide accurate and reliable test results for these patients. Such demands insist on fast processing with even faster turnaround times (TAT), provided on a daily basis by overworked and under-funded staff. The pressure on clinical labs to produce such results in a cost-effective way has had lab directors and managers struggling to meet the increased demands with decreased staff and budgets. As a timely option to address lab demands, many information technology (IT) solutions offer an option for all clinical and financial goals to be met.

For busy clinical labs, IT solutions present an opportunity to achieve consistent, accurate test results that are industry-standardized and reproducible. As a byproduct of using IT in the lab, daily workflow requirements become more efficient and effective with staff more productive. Using IT in the lab has also shown to be cost-effective and budget-friendly for labs that have taken advantage of the benefits that IT solutions offer, such as data organization and streamlined workflow.

In an effort to learn more about how today's clinical labs are using IT solutions, *Medical Laboratory Observer (MLO)* invited subscribers to respond to a 16-question survey, giving their feedback. In the end, almost 300 people responded, and data the survey collected showed that IT usage in the clinical lab is far from just a temporary trend. Rather, it's a valid and time-saving choice – one whose acceptance and implementation is growing every day, suggesting it will be an asset to clinical labs long into the future.

Demographics detail respondent roles

To better understand the demographics of survey respondents, *MLO* asked everyone who completed the survey to list the title they currently hold. Of the 273 survey respondents, more than half (53 percent) are in lab management positions (administration, supervisor, manager or director). Another 31 percent of respondents are medical lab scientists/technicians (MLS/MLT), section managers/department heads and clinical lab scientists/technicians (CLS/CLT). The remaining 16 percent of survey respondent hold positions in compliance, QA/QC, education, POCC/POCT, pathology, LIS/

EMR/EHR, IT, nursing, blood banking, regulatory affairs and consulting.

Survey respondents were also asked what type of organization best describes their lab, with hospital lab accounting for 68 percent of submitted answers. Of the other 32 percent of respondents, 30 percent are integrated clinical labs (11 percent), physician's labs (8 percent), government/public health labs (5 percent), group practice labs and blood banks (3 percent each). The remaining 2 percent were part of the 32 percent, and was made up of facilities listed as "other," which included community colleges, diagnostics companies, vocational schools, consulting, health centers, clinics, independent clinical labs, medical device companies, private labs, reference labs, research labs, teaching labs and university-affiliated labs.

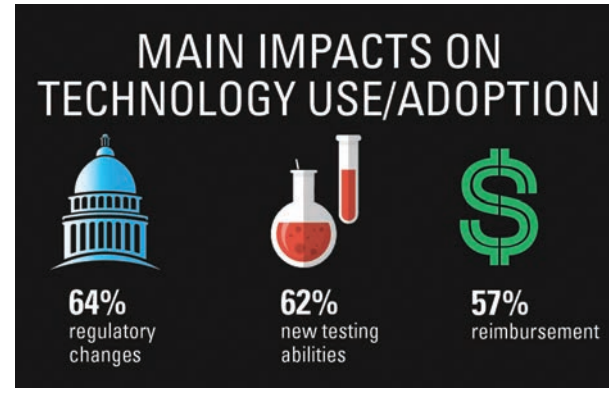
Within these labs and lab-related companies, the majority of survey respondents (44 percent) indicated their staff consists of up to 25 people, while another 20 percent of respondents noted their lab staff includes 26-50 people. Larger labs with 51-100 people on staff were represented by 17 percent of survey respondents, and the remaining 19 percent of labs reported more than 100 people on staff.

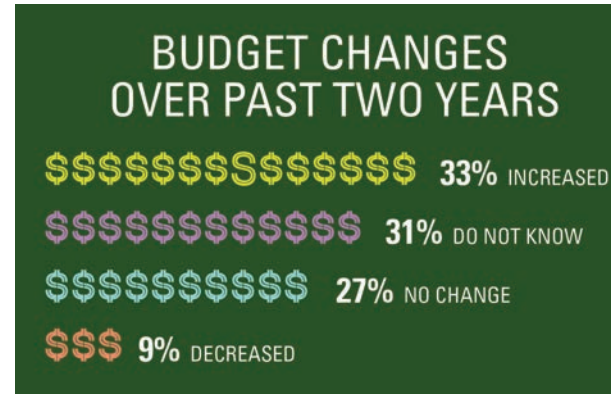
Factors affecting IT usage in clinical labs

According to clinical laboratory personnel shortage statistics reported by the American Society for Clinical Laboratory Science (ASCLS), clinical labs in the U.S. perform over 4 billion tests each year. Consider that many of these tests are being performed in clinical labs with declining resources – such as enough staff to meet testing demands – and the need for assistance becomes even more evident (https://www.ascls.org/advocacy-issues/workforce).

As such, the greatest appeal of IT is that it offers the needed assistance by way of software solutions that are designed to streamline lab workflows—increasing the accuracy of test results and overall productivity, while simultaneously reducing the risk of hands-on human errors. Yet, there are still labs that have not embraced the use of IT for a variety of reasons.

One of the biggest obstacles for installing and integrating an IT solutions system is usually the age and size of the existing lab/facility's infrastructure. For smaller, low-volume labs, the cost to replace existing infrastructure with new systems—assuming the new system would take up the same amount of space and not require more—has traditionally kept

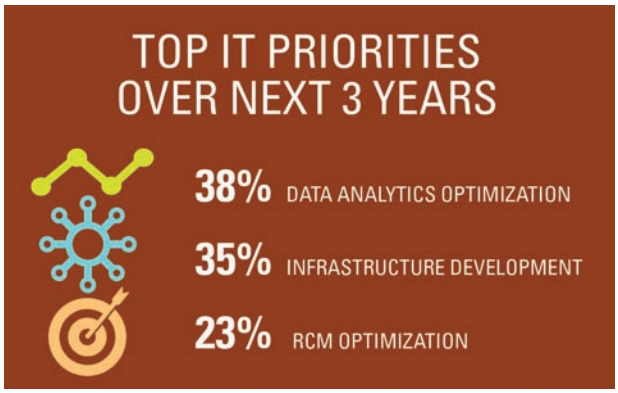


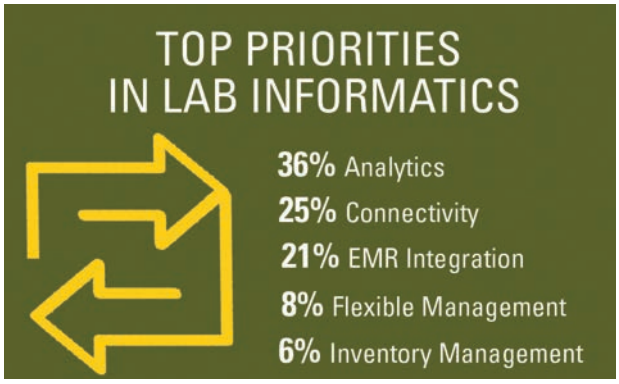


the idea out of financial reach. On the other hand, larger labs are prime candidates for using IT solutions simply because their demands are greater, their staff is bigger and the chances of seeing a successful return on investment (ROI) are also better, for the most part.

As part of the IT survey, respondents were asked to reveal current or changing factors that have affected their use or adoption of technology in their labs. The most common answer was new testing abilities, followed by an almost-equal number of respondents whose answer was state or federal regulatory changes. These choices were followed by reimbursement changes and patient demographic changes as the most popular answers. Among the write-in "other" option, respondents listed budget- and cost-related issues, as well as lab/hospital mergers, corporate issues, lack of staff and IT resources, space constraints and technology and training issues.

When asked how many staff members already use processes that incorporate IT such as laptops, desktops and automation equipment, 48 percent of respondents indicated up to 25 people. Among the rest of the respondents, 19 percent indicated 26-50 of their staff uses IT, along with 16 percent who have 51-100 people using IT and 17 percent of respondents who noted over 100 people use IT in their labs.





The importance of LIS/LIMS

In consideration of the huge amount of data generated by clinical labs, there is an overwhelming need for a place to house it all and organize it until it's needed. This is where labs can benefit from having LIS/LIMS systems in place – to ensure not only the privacy and security of patient data, but also the quick retrieval of patient data when diagnosis and early treatment are tantamount to life-saving efforts. These two types of systems serve to reduce repetitive manual lab tasks, allowing for greater hands-free data processing and results management.

Within the IT survey, respondents were asked how they currently use their LIS and 95 percent reported that they use it for electronic orders and results. Encouraged to select all answers that apply, respondents also chose clinical data connectivity as the second most popular answer, followed in importance by less manual intervention, customer service and scheduling. Additional answers from respondents included planning and inventory optimization, sales revenue, improved forecast accuracy, quality review and report generation.

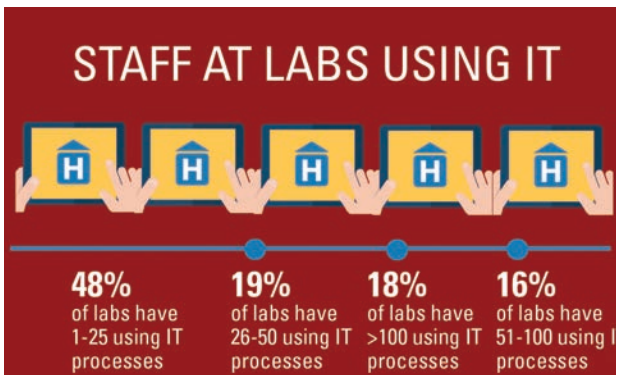
Respondents also listed their top priority when considering informatics including LIS (a lab

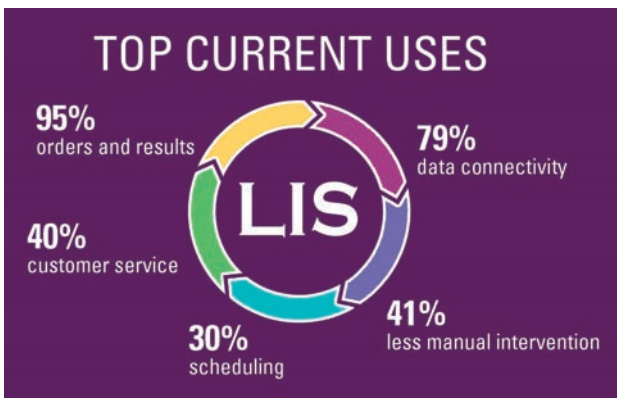
information system intended to store and track patient data) and LIMS (also for information storage, but specifically designed for sample management). Among the five answer choices offered, the most frequently chosen was analytic solutions for clinical/anatomical/molecular pathology (36 percent), followed by multi-lab networking/connectivity (25 percent), integration with EMRs (21 percent), flexible management capabilities (8 percent), real-time and/or automated inventory management (6 percent). The remaining percentage included cost, patient safety and training needs as additional priorities for LIS/LIMS.

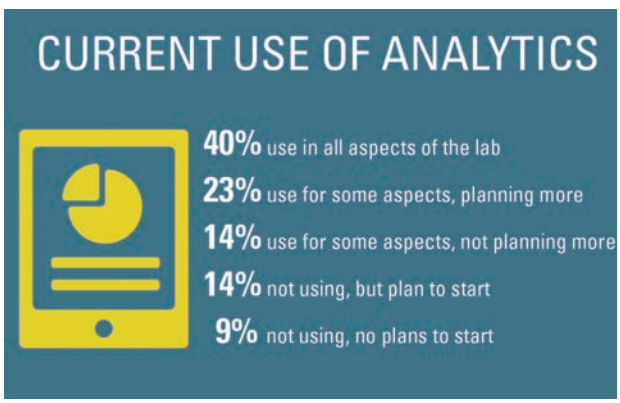
When asked what they felt was the most important reason was to invest in a LIS/LIMS, respondents were in agreement with their answers, which were increased patient safety, improved workflow, data integrity, security and scalability, as well as total connectivity. Other choices listed were maximized reimbursements and advanced reporting.

Budgets for IT and questions about analytics

Behind the doors of any clinical lab, certain conversations tend to be repeated over and over. These conversations usually begin and end with the same word: budget. For example, when a lab director asks for more staff they may be told there is no budget for it. Clinical labs are expected to produce reliable data under increased pressure and time constraints from clinicians who are eager to begin patient treatment once they have test results in hand. At times, it may seem like the







biggest obstacle in patient care.

But there is some good news reported by way of survey respondents, with 33 percent admitting that their budgets have increased compared to two years ago. Another 28 percent said their budgets stayed the same, and 29 percent did not know if their budget had changed from two years ago. Only 9 percent of respondents noted that their lab budgets had decreased from two years ago, with the remaining 1 percent revealing that they are not privy to budget-related information.

The good news continues regarding the usage of analytics to support lab operation and management. Survey statistics evidenced an overall industry openness to its use with 40 percent of respondents indicating analytics usage is already in place for some aspects of their jobs with plans for more. Another 37 percent also admitted using analytics with 14 percent of these respondents revealing they are not planning any additional usage. An added 14 percent said they have not started using analytics in their lab, but they would like to start. The last 9 percent of respondents reported they are not using data analytics for lab management, nor do they want to start in the near future.

One survey question that may cause concern was if respondents have tools to strategize product pricing, market share, maximize profit, etc. Only 31 percent said they have the tools to effectively manage their business, while 34 percent admitted they do not have the tools they need, but are interested in them. A disheartening 17 percent revealed they do have the tools they need, but they have not yielded the desired results, and the remaining 18 percent noted that the tools are not applicable to their business, and/or they do not see the value of these capabilities.

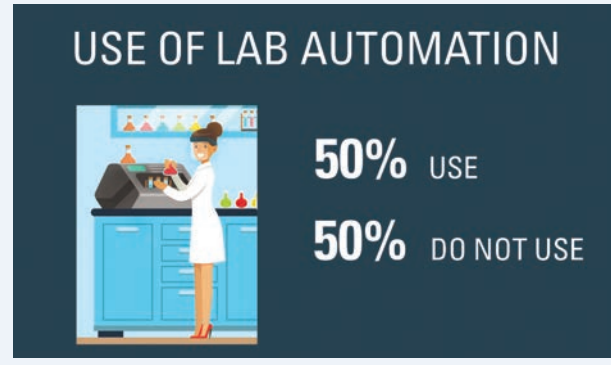
Future IT usage and challenges to overcome

In addition to the survey asking respondents about IT usage or lack thereof, as well as the status of their budgets, it also gave respondents a chance to divulge their lab wish lists, beginning with the areas they would like to see their labs expand in the future. The most popular answer – chosen by 42 percent of respondents – was chronic disease state monitoring. Answers that followed in order of popularity were scalable tools to expand molecular diagnostics (MDx), next-generation sequencing (NGS) testing, enterprise master patient index (EMPI) solutions, anatomic pathology testing and genetic analysis services.

Representing the answers of 14 percent of respondents, the "other" write-in option included wish list items such as better day-to-day lab management, better equipment, workflow and integration, emerging infectious disease testing, more staff, more space, more outreach tools, EMR integration, more interfacing between hospitals, increased efficiency, more wireless instrument interfaces, more IT support and new instrumentation to expand test menus.

When asked about their top strategic IT priority for their organization in the next three years, 38 percent of respondents said data analytics optimization to support lab

AUTOMATION: THE VALUE OF KEEPING THINGS MOVING IN THE LAB



Automated software systems and equipment are the unsung heroes of the clinical lab. True workhorses that stand ready to assist busy staff by offering simple, user-friendly operation – walkaway operation in many cases – that allow multiple procedures to take place simultaneously. These systems not only provide standardization of processing, but they also reduce the risk of human errors that occurs with manual testing procedures, such as pipetting. As such, the adoption of automation has proven itself worth the cost of equipment and training many times over for busy labs, who no longer have to rerun tests due to manual input and result inconsistencies.

However, when respondents of the IT survey were asked if their lab used a laboratory automated system, the answers were surprisingly split evenly down the middle with 50 percent saying yes and 50 percent saying no. For those that answered yes, they were asked to list the most valuable reasons to use an automated system. The answers chosen most by respondents, in order of their value were:

- Auto-verification, QC review and instrument flagging during the process;
- Flexibility and adapts to test volume levels;
- Scalable and can add modules (for example, centrifuges);
- Integrated IT connectivity;
- Test tubes and automated post-analytical tube management.
- Reduced consumable usage

For busy clinical labs, IT solutions present an opportunity to achieve consistent, accurate test results that are industry-standardized and reproducible.

management. This serves to remind us of the 91 percent of respondents who reported they are using or planning to use analytics in the future. Other answers in common among respondents were infrastructure and platform development (35 percent) and revenue cycle management (23 percent). The last 4 percent of respondents indicated that their top strategic IT priority over the next three years includes business intelligence, education, phlebotomy products and implementation of a new LIS.

Respondents were also asked to detail the challenges their organizations are currently facing or will face in the next three years in their planning and forecasting environment. As clear evidence of respondent budget concerns and lab demands, the most popular answers were funding, staffing and ROI/costs.

Conclusion – IT solutions are here to stay

Like many areas of medicine, the clinical lab is constantly changing, with products and technology intended to benefit and improve all aspects of patient care. As patients move through the cycle of care with physicians and specialists, the role of the clinical lab is arguably the most important part of a patient's treatment team. However, as clinicians continue to rely on the clinical lab for test results that help create treatment plans, the medical industry will need to provide the funding and staffing necessary to meet growing demands for diagnosis, disease and data management.

As clinical labs attempt to keep pace with testing and data management needs that show no sign of decline, administrations will be faced with finding new sources of revenue that allow labs to integrate solutions that focus on efficiency and productivity, such as IT software and automated systems. Trends in clinical labs prove it is only through forward-thinking options and lab industry professionals that these labs will be able to overcome the challenges that exist today, as well as meet any that appear in the future.

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Laboratory quality control – from error detection to risk management

By Jennifer MacCormack

Historically, laboratorians have had a habit of abbreviating "quality," reducing it to a one-dimensional activity when discussing things like daily QC results and QA reviews. Of course, that doesn't reflect the deep respect that laboratorians have for the word. In hospitals, reference laboratories and physician office laboratories, the concept of quality is ever-expanding to cover more and more aspects of laboratory operations in the form of effective Quality Management systems. While error rates in laboratory medicine are generally low, every error in the lab has the potential to end a patient's life with a missed diagnosis or an unnecessary course of treatment. In recent years, both CLIA and the deemed Accreditation Organizations have emphasized an increased focus on quality assessment and laboratory-wide risk management.

The CLIA regulations, and the accreditation criteria of all deemed Accreditation Organizations, require that all clinical laboratories implement a Quality Management system in order to continually assess quality in pre-analytic and post-analytic activities. Comprehensive Quality Management is essential because many laboratory errors fall outside of the analytic processes that can be monitored with what we traditionally see as QC, such as running external controls on a regular basis. Every lab is different and has different areas of risk, and so the laboratory Quality Assessment plan should ideally be an individualized, flexible, living document, continually adjusted as the laboratory learns about new or changing sources of potential lab errors.

Managing risk

Laboratories have always implemented measures for managing risk. We establish policies for specimen rejection based on our understanding of interfering substances, analyte stability and instrument limitations. We focus on detailed and thorough training of new employees and documentation of ongoing competency for all testing they perform. We document open dates and expiration dates of reagents; we use delta checks of patient results to detect error and we perform regular verifications of instrument calibrations. Many fail to realize that these actions are all based on an underlying risk assessment. It's just that in most cases laboratories have been taking someone else's word about assumed risk, whether it's the instrument manufacturer, an Accreditation Organization or published literature from professional clinical laboratory associations.

The Individualized Quality Control Plan (IQCP) was first implemented on an educational enforcement basis in 2014 to give laboratories more responsibility – and more ownership – over the assessment of the risks inherent in their own labs' processes. Better analyzing a laboratory's specific sources of error leads to the development of more effective and targeted strategies to mitigate them. In a sense, IQCP places quality control within the larger framework of quality assessment. Running QC samples at the frequency required by regulation or, in some cases at the frequency required by the manufacturer, at a minimum, will certainly reduce the risk of errors, but no test stands completely alone at the analytic phase. Quality must be considered throughout all phases of testing, including the laboratory's complete pre- and post-analytic processes.

Five years later, what has IQCP done for laboratory quality?

From COLA Inc.'s perspective as an Accreditation Organization, we can report that IQCP-related citations have continued to decrease as laboratories learn more about how to properly implement IQCP. This is likely due, at least in part, to the educational materials made available to laboratories who are putting IQCP into action. Guidelines for the proper elements to include in a risk assessment, for example, help laboratories to do a more thorough job in their data collection and development of a QC plan that fits in their laboratory environment, using their personnel.

But are we doing enough? Is IQCP actually making an impact on overall laboratory quality? It's difficult to say. Many other industries, especially those who are held to various stringent ISO standards, have been required for decades to collect and report data on errors, incidents and complaints. Medical errors are well-documented and widely reported, but there is a lack of accessible data on errors specific to the laboratory portion of healthcare. This is concerning as it is widely accepted that the majority of medical decisions are influenced, at least in part, by laboratory results. Without data to guide us, it is difficult to assess whether the implementations of new quality measures are making a difference in overall laboratory quality.

In 2019, risk assessment and IQCP are primarily being implemented for simple, moderately complex handheld or benchtop instruments whose systems and internal QC are robust enough for the manufacturer to recommend less QC than the regulatory requirements. While the risk management approach can be applied to any test system in the laboratory, in most cases it is only being applied to tests where it might lead to an immediate cost savings in QC.

However, labs that focus only on reducing QC when utilizing risk management have missed the boat. There may be circumstances where a laboratory should conclude, based on their analysis, to do more QC, not less, than the regulatory requirement.

Extra paperwork is a hard sell to laboratorians, especially when so many laboratories are already understaffed and overworked, and so much of the job is already dedicated to documentation. Clearly, when a majority of laboratories have concluded that IQCP is, "a way to do less QC," we've failed to properly make the point that a proactive risk management approach benefits every aspect of the laboratory. It is better, safer and more cost effective to implement risk mitigation instead of corrective action, but the process requires an investment in time and mental energy and is all too easy to leave on the back burner.

How do we turn the wheel and shift laboratory culture away from reactivity and towards proactivity? How can we make the case that improving laboratory quality through risk management is of ultimate benefit to even the smallest laboratories?

Redefining quality control

It's unfortunate that the term "quality control" has historically been reduced to controlling the analytic process via the testing

of materials of known value. While that is indeed an important step in quality control, truly controlling the quality of a test result requires a much broader view. An effective Quality Control plan must encompass every step surrounding the testing, from the requisition to the final report and beyond.

Quality Control is everything that a laboratory does to ensure accurate and reliable results, over the entire testing process. It includes all the required elements of a risk assessment under the IQCP framework: the specimen, the test system, the reagents, the testing environment, the testing personnel and reporting the results. QC is training and temperature monitoring and checking expiration dates on kits before use. QC is well-written procedures and regular instrument maintenance. And yes, QC can also be running external quality controls on the test system and ensuring they are in range before reporting patient results. Quality Assessment, on the other hand, is how a laboratory can check whether the current Quality Control plan is effective. It is a regular review of each of the measures that have been put into place to safeguard test quality, to see if those measures are appropriate.

Quality Control and Quality Assessment aren't separate processes; each is very much a part of the other. They need to be used together in order to truly effect change. When a robust Quality Management system, with buy-in from laboratory leadership, implements a risk assessment approach by collecting and sharing data, a laboratory can devise specific plans to improve the quality of their laboratory processes at every step of testing. It is then a matter of continually asking ourselves: is this working? Are these measures enough? By regularly collecting data around the QC measures that have been implemented, a lab can adjust as they go, instead of waiting for a laboratory error to force them into action.

Collaboration can lead to change

Data is a major driver of change. If we are dedicated to improving laboratory quality, we must work harder to collect and disseminate data about the current state of affairs in the laboratory world. Individual laboratories can collect data on their own errors, not only to know how many are happening, but also to pinpoint areas in their labs that are more prone to risk. Sharing this data with others can also lead to tremendous insight about laboratory testing overall; labs should be encouraged and empowered to share their failures and successes with others in their parent health systems and beyond, via articles, blog posts and presentations at laboratory conferences. This helps to support a culture of mutual growth and a holistic approach to error prevention, instead of one of shame, isolation and blame. No lab is an island, after all. We can, and should, learn from each other.

Jennifer MacCormack, is the Post Survey Team Leader with **COLA**, where she provides consultation to lab clients regarding regulatory compliance and quality lab practices, and guides member laboratories through the Accreditation process. She is a licensed Medical Laboratory Scientist with over a decade of experience in high-volume hospital blood banks and core laboratories. She holds a BS degree in Human Physiology from McGill University and a Medical Technology degree from Dawson College. She is also a freelance science writer whose articles are featured on websites dedicated to consumer safety, STEM outreach and science communication.

NRTI resistance testing – why and how?

By John Brunstein, PhD

A few months back, we looked into classes of antiretroviral (ART) drugs, particularly NRTIs and NNRTIs, which are key components of therapy for HIV. This month's episode will carry on from that by examining testing patient samples for ART resistance, using NRTI as the model. We'll consider why it's important to do this testing, what method(s) are used and when it's relevant to do. We'll also look at some technical aspects of the testing methods and their various pros and cons. Although our focus will be on NRTIs in the HIV setting where this is widely employed, the generic points covered (those not specific to a particular drug / target interaction) are applicable both to other classes of HIV ART drugs and, more broadly, to other viruses with associated ART regimens (for instance, HCV or CMV).

Why do ART resistance testing?

Let's deal with the easiest question first – why do NRTI resistance testing? The shortest answer is that long-term suppression of HIV replication is the current key to AIDS management; if it can be instituted early enough and is effective enough, an HIV infection can be supressed from either progressing to symptomatic AIDS, or from being readily transmissible; a win both for the patient and for public health. NRTIs are an important component of best practices (High Activity Anti Retroviral Therapy "HAART," applying a cocktail of NRTI, NNRTI and "Portmanteau inhibitors" targeting both viral protease and integrase).

Multiple NRTI class drugs are available, and while all target the same viral reverse transcriptase (RT) enzyme, they have unique variations in how they effectively bind and inhibit activity. This means that viral sequence changes in the region coding for this gene, leading to amino acid substitutions, can modify drug binding and effect. If the RT develops a mutation, or combination of mutations, which retain enzyme function while reducing binding of an inhibitory drug being applied, viral replication becomes unchecked and disease progression occurs.

The risk of this happening is not insignificant; like most RNA viruses, HIV replication is error prone, leading to frequent mutations and a constantly changing pool of viral variants subject to selective pressure. One biological fact works in our favor here: if within a cell, somewhere in the quasi-species swarm of viral sequence variants one is created which escapes inhibition by the current drug regimen, this resistant variant enzyme has no ability to selectively replicate just its own progenitor sequence. It will spend its time replicating the entire sequence pool, including all the non-adapted sequence variants.

This means that a drug resistance mutation doesn't immediately take over as the majority sequence, although it may begin to lead to an increase in net viral load. If a resistant variant virus inoculates a new host cell, however, genetic founder effect plays out and the drug-resistant form may rapidly expand. This delay in selection means that if we can detect an increase in viral load early enough – evidence that somewhere, there's functional RT enzyme forms escaping the drug – we can switch to a different NRTI. Because each NRTI has a slightly different physical interaction with the viral reverse transcriptase (RT) enzyme, mutations conferring RT resistance to one NRTI won't necessarily be effective against a different one. When this is the case, a therapy change can be employed and continue to drive virological suppression. Our goal is to be able to make these therapy changes when needed, and in an effective manner.

Why genotype and not phenotype – and how do we get this data?

If you're familiar with microbial antibiotic resistance testing, while molecular testing is a great rapid tool, it's not the final word; phenotypic testing is key to definitive assessment of resistance. The situation is different here, most simplistically due to pathogen genome size. For a bacterium, many gene products alone or in combinations may result in a phenotypic drug resistance. Tackling that from molecular tools would be a lot of targets to sequence; more importantly, we may not even know the relevant effect of many genetic variations and their combinations, so genetic data alone is not definitive in determining bacterial antibiotic resistance.

By contrast, the HIV genome is small and so extensively studied that we have at our disposal data on how almost any relevant mutation or combination of mutations in the only relevant target – the RT enzyme - effects binding and activity of the available NRTIs. This data is available in databases such as the Stanford HIV Genotypic Resistance Interpretation Algorithm.[1] This means that by sequencing the virus present in the patient, and checking against this data, it's possible to make informed decisions as to which NRTIs will be effective. (A question we won't delve into here is source of sample. Generally, peripheral plasma is used for simplicity; however, it may not fully represent the same viral sequence population(s) in particular cellular subpopulations such as memory T-cells. The clinical relevance of this unclear at present and out of our scope).

For completeness, it's worth mentioning there's a second line of argument against phenotypic testing in this context – cost and complexity. Conducting phenotypic antibiotic resistance testing is generally straightforward and inexpensive for many bacteria; it would be much more expensive and complex, not to mention all the biosafety headaches, to perform on HIV cultures. We're fortunate that molecular will suffice.

OK, so we want molecular data – how?

If we know we want molecular data to address this, the question becomes what method to use. If we were only looking for a very few specific mutations, simple allele specific PCRs could be used. The reality here, however, is that we must consider the possible impact of many possible mutations

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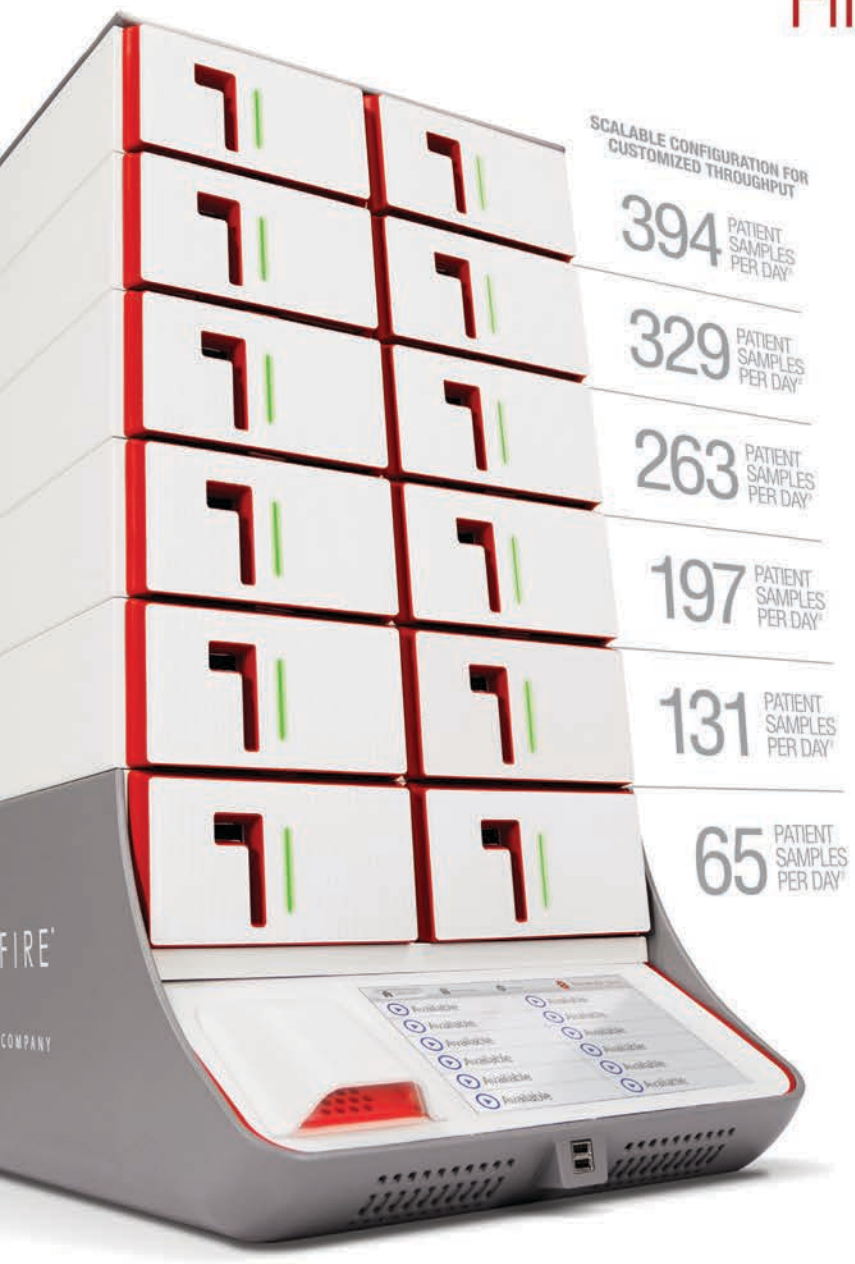

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scattered across the viral pol gene, so sequencing is the rational approach. Since we're starting with an RNA genome, and usually at low numbers, we'll want to do an RT-PCR process to both amplify viral material and convert it to more readily handled DNA. This can of course create headaches of its own, both by potentially biasing the pool (amplifying some viral sequences more than others) or by PCR errors occurring, which can then masquerade as true viral sequence variants. In general, we'll try to avoid these by using high-fidelity (low error rate) PCR enzymes where possible and be suspicious of any very low-abundance sequence variations (these are more likely PCR errors than high-abundance variations). All of this has only given us starting material for DNA sequencing; we still have to decide what method to apply.

Older technology in the form of Sanger sequencing has been (and at present probably still is) the most common approach. This is partly predicated around the relatively low cost of the platform, the low per-reaction cost and the relatively simple sample preparation (if you can do a regular PCR, you're equipped to do Sanger cycle sequencing and all that's left to do is load products on a benchtop capillary electrophoresis machine). The resulting data is human readable. A deficiency of Sanger sequencing, however, is that it works on an entire population of sequences as template for each reaction, with the results representing a "population average" of each base position. Generally, an individual base position variant has to reach something around 20 percent of the population to be detectable in this approach. The consequence is that a small subpopulation of drug-resistant forms could exist in a specimen, but not be seen by this technology. Multiple examples exist in the literature demonstrating Sanger sequencing not detecting HIV variants in 10 percent of population range. Since there's also published data indicating levels as low as 1 percent of drug-resistant viral forms is enough to have a demonstrable negative impact on therapy, that's a bit concerning.

Next Generation Sequencing (NGS) methods offer a means to avoid that particular problem. While there are multiple competing NGS platforms, the key in this application is that they all work around the concept of capturing massive numbers of sequence reads, each derived from a single template. We can thus consider this class of assay generically without reference to particular platforms. By putting a sample through an NGS library preparation and then analyzing it, a much more detailed picture of viral sequences present can be obtained. While we may not reliably capture extremely rare variants, reliable detection can be made down into the 5 percent of population range. (Exact lower bounds depend on a number of factors, including sample size, viral load, depth of sequencing, platform employed and particulars of bioinformatics workflow. While these methods can almost all, in theory, detect down to ~0.2 percent or lower, our previously stated concern that low-abundance results might be PCR artifacts is also at play. Five percent is a reasonable generic cutoff for our purposes.)

Further, the NGS approach allows the identification of multiple variations at single sites and may, in certain cases, be amenable to considering "phasing"; that is, being able to determine which of multiple variants at different sites in the target gene are associating together. This can, for instance, be helpful in assessing if individual viral sequences may have multiple resistances. Such linkage information is not available from Sanger sequencing, even if all the variations are visible in the sequence trace files. Finally, NGS data is inherently quantitative in a relative sense, meaning it's possible to see what the relative viral population proportions are of various sequence isoforms.

The downsides of NGS are that the platforms are generally fairly expensive, and the library preparation methods (while improving) remain relatively complex and labor-intensive. The cost per instrument run is also fairly high in most platforms but note that when multiplexed across enough samples (with attendant implications for batch sizes and possibly assay turnaround times), the cost per individual sample can actually be less than with Sanger sequencing. Bioinformatics workflows remain an issue as well, as the data is not as readily interpreted by non-expert users. Prepackaged workflows such as that proposed are a solution to this, if regulatory requirements in appropriate context are met.[2]

Which is better, Sanger or NGS?

The answer for most labs today, is "which one does your clinical lab already have access to in a suitably robust form?" Sanger is, however, a mature method or maybe even long in the tooth, whereas NGS methods continue to improve in accuracy, cost and ease of use. Its inherent capability to resolve lower-abundance viral forms will probably prove useful. If you're planning for the future, the answer is almost certainly NGS but the longer you can wait to take the plunge, the cheaper and better it's going to be.

Guidelines – when?

We've left the easiest part for last – when should NRTI resistance testing be done? In the U.S, the NIH's Aidsinfo program's most recent guidelines (October 2018) for ART resistance testing (including, but not limited to, NRTI) in HIV includes the following key points:[3]

- For patients just starting on ART, testing at outset is recommended (or if drug therapy is deferred for some reason, testing immediately prior to therapy commencement). This allows for an evidence-based selection of best efficacy agents; note, however, that initiation of empiric therapy shouldn't be delayed while waiting for test results.
- For patients already on ART, testing should be repeated when there is evidence from viral load testing of "virologic failure" (increases in viral load above 1,000 copies/ml plasma) or lack of response (significant drop in viral load) to current drug regimen.

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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.

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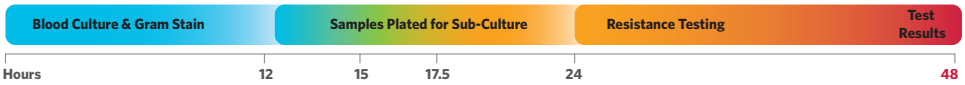

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AI advances efficiency in the lab

By Sue Sharp, PhD, (D)ABMM, (F)AAM, MS, MT(ASCP)

The clinical microbiology laboratory is exploding with new technology. Among the most exciting are the use of artificial intelligence and interpretive algorithms (AI/IA) to assist in the work up of bacterial cultures. Not having sufficiently trained personnel to staff our laboratories around the country, digital imaging along with AI/IA will help fill those gaps. AI/IA will assist the laboratory technologists and technicians with their workload by 'handling' negative and insignificant cultures, allowing the technical staff to spend their time and resources on those cultures that need their educated assessments. This article will review and discuss the AI/IA software currently available that make this possible, as well as what will be coming soon.

Chromogenic agars and AI

With the advent of liquid-based microbiology collection devices, automated culture processing instrumentation together with smart incubators and digital imaging, we can advance into the field of AI in the microbiology laboratory with culture interpretations. First, let's review current AI studies that look at the use of AI with chromogenic agars.

A study by Faron *et. al.* looked at methicillin-resistant Staphylococcus aureus (MRSA) screening cultures at four clinical sites which comprised over 57,000 specimens and using three different manufactures' agars. They showed that the sensitivity of the AI software reading of the culture images was 100 percent, as compared to manual image reading by the microbiologists[1]. This means that the AI software never called a culture negative that manual reading called positive. The AI software also detected an additional 153 positive cultures that manual reading missed.

A similar study looking at vancomycin-resistant Enterococcus (VRE) screening cultures reviewed over 104,000 specimens at three cites using two different manufacturer's agars[2]. Again, the AI software showed a sensitivity of 100 percent, as compared to manual image reading, and detected an additional 499 positive VRE cultures that were missed by manual reading.

In addition to screening cultures, AI software has also been used very successfully with chromogenic agars for the detection of group A streptococci (GAS) from throat specimens, as well as the detection of group B streptococci (GBS) from vaginal/rectal pregnancy screening cultures. The study by Van *et. al.* showed that AI software had a 100 percent sensitivity as compared to manual image reading using GAS chromogenic agar, and that the AI detected additional positive specimens that were missed by manual reading[3].

These investigators also compared the detection of GAS using chromogenic agar and AI to the detection of GAS by a molecular assay. Using a composite true positive definition (culture positive with GAS confirmed by MALDI identification and/or PCR x2 positive), the molecular assay had a sensitivity of 96.9 percent, chromogenic agar plus AI software had a sensitivity of 90.6 percent, while manual image reading has a sensitivity of 87.5 percent. Thus, using chromogenic agar with AI software is not only more accurate than manual image reading, it approaches the sensitivity of PCR testing.

Another study looking at GBS culture screening during pregnancy showed chromogenic agar used with AI software had a sensitivity of 95.5 percent as compared to manual image reading, which showed a sensitivity of 90.3 percent and molecular detection sensitivity of 96.8 percent[4]. The sensitivity of the chromogenic agar plus AI software was comparable to that of detection by molecular techniques.

Two studies using chromogenic agar with AI software have also been presented for the evaluation of urine cultures. The first showed 99 percent accuracy of the AI software in segregating urines into groups with no growth (29 percent of all urine cultures), those with insignificant growth (27 percent), those that contained significant growth of *Escherichia coli* (3 percent), and those that contained significant growth of another urinary pathogen (2 percent)[5]. These four categories entailed 62 percent of their urine specimens, 59 percent of these specimens could be reported in batch mode of 30 cultures with one computer click by the microbiologist, and 56 percent required no hands-on time by the staff.

The second study showed that AI software with another urine chromogenic agar had a 99.8 percent sensitivity as compared to manual image reading[6]. In addition, when AI software plus chromogenic agar was compared to conventional agar with manual image reading, a significant ($p<0.01$) reduction of

06:23 for positive urine specimen results and 04:48 for negative urine specimen results was observed.

AI and routine urine cultures

But AI is not just for use with chromogenic media. A recent study by Faron *et. al.* utilized AI software to segregate significant growth in urine specimens plated to standard media[7]. Briefly, nearly 13,000 urine specimens submitted for bacterial culture from three different sites were plated on sheep blood and MacConkey agars. All specimens were processed using a 1µL loop and images were captured after zero and 18 hours of incubation. The AI software quantitated each plate and reported the specimen as "non-negative" if either plate contained more than 10 colonies (>10^4 CFU/mL).

Results were then compared to manual interpretation as either positive or negative for pathogens, based on each laboratory's urine culture policy. All manual (M) positive (P), automation (A) negative (N) cultures were reviewed by a second technologist. Overall, the AI software was highly sensitive with an average sensitivity of 99.8 percent (range 99.7-99.9 percent). These data included 5,678 specimens that were positive by both methods (MP/AP), and only nine specimens that were MP/AN.

Specificity showed an overall rate of 72 percent, which included 5,598 MN/AN specimens and 2,180 MN/AP specimens. The 9 MP/AN discrepancy results were found to fall into two categories. The most common cause for discrepancy (eight of nine cultures) was due to the presence of microcolonies that were counted as positive by the technologist but were programed to be ignored by the software. Allowing the software to take microcolonies into account, all eight of these cultures would have been detected and placed into the AP category.

The one remaining MP/AN specimen was due to a difference in bacterial count near the reporting threshold for this laboratory (threshold of 50 colonies or greater). For this specimen, the laboratory report had a bacterial count of 55 and the AI software counted just under the threshold of 50 CFU (49 colonies). Interestingly, allowing the software to count the microcolonies and using a threshold of 10 CFU/mL for each laboratory resulted in 100 percent sensitivity of the software. The authors found significant utility in the ability to remove negative specimens from the microbiologists' review queue. In this study, 43.3 percent of all specimens were resulted as MN/AN, so for a laboratory that processes 350 urine specimens each day, the AI software would reduce the work load by 151 cultures/day, which equates to 55,000 urine cultures annually that would not need individual technologist review.

AI and AST

AI software is also under investigation for the ability to read and interpret disk diffusion results with as few as six hours of incubation. The study by Hombach *et. al.* showed that rapid disk diffusion antimicrobial susceptibility testing (AST) read at six hours, as compared to standard disk diffusion incubated for 18 hours, showed agreement of 97.2 percent, 97.4 percent and 95.3 percent for *Enterococcus faecalis*, *E. faecium* and *Acinetobacter baumannii*, respectively[8].

With Pseudomonas aeruginosa the average readability of inhibition zones was 68.9 percent at eight hours with an overall categorical agreement of 94.8 percent.

A second study by Hombach and colleagues showed that the vast majority of zone diameters for *Escherichia coli* and *Klebsiella pneumoniae* were readable after six hours of incubation, and reliable reading for Staphylococcus aureus was possible after eight hours of incubation[9]. These studies demonstrated that early disk diffusion reading is possible, and that the precision of disk diffusion AST results are not hampered by early reading.

Summary

There are also many efficiencies to be gained by using laboratory automation and AI software in microbiology. Laboratories will see a decrease in their cost per test, an increase in their productivity and be able to handle additional specimen workload without the need to increase staffing. AI is revolutionizing the microbiology laboratory by segregating chromogenic agar screening cultures into positive and negative groupings, counting colonies for our quantitative urine cultures, discriminating morphologies on routine bacteriology media and will soon read our disk diffusion AST results in a rapid fashion.

The promise of AI is that this software will move to the next step of automated release of negative cultures, whether that is on chromogenic medium or traditional culture medium. The utilization of AI in microbiology will allow a future where clinical microbiologists can spend their time on more complex cultures that require their expert attention and, at the same time, save valuable resources for the laboratory.

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Artificial intelligence and digitalization are revolutionizing laboratory diagnostics

By Stuart Kerty

So much is in silos across diagnostic laboratories: data, analyzers, devices—even people—divided by walls, by processes, by distance or simply by force of habit. The result can be partial visibility, limited insight and duplication of effort. Artificial intelligence (AI) and digitalization are proving to be the tools to help transcend these barriers. According to a 2018 survey of 200 laboratory executives, 69% expect widespread adoption of AI in the IVD lab within four years.[1]

While the workload is growing, diagnostics and treatment are also becoming more complex, relying on and producing massive amounts of data. Healthcare providers are now looking to AI to operationalize the data to support more-objective, data-driven treatment decisions that are tailored to the needs of each patient.

Digitalization is the foundation

Automation and diagnostic software have been commonplace in the lab for years. But many tasks are still performed manually with no digital records. Even if the data is electronic, often it is in a format that cannot easily be exchanged with other systems. In fact, 80% of data remains unstructured[2] and stored away in closed systems that are physically or technically disconnected from each other. To effectively use this information, it must be made interoperable, e.g., in a secure, open-sourced, vendor-neutral environment that can synthesize from disparate sources and systems. Digitalization is transforming these isolated and often-hidden sources of information into structured, sharable, actionable data.

Labs today use software to automate management of samples, operations and results to optimize workflow and staff utilization. For example, rules-based autoverification evaluates patient results against multiple parameters to validate and speed up reporting or reflex actions. Concurrently, intelligent systems actively analyze operations to predict bottlenecks and warn of potential issues, such as STAT sample delays or impending reagent expiration. Beyond the core lab, healthcare systems rely on digitalization to manage hundreds or thousands of point-of-care (POC) testing devices and their data. These rules-based applications follow predefined logic, performing tasks and calculations explicitly as they are programmed. AI is the next step in the evolution of laboratory software.

Today and tomorrow: what AI brings to the lab

Artificial intelligence-based software uses neural networks designed to emulate human thought processes. AI can recognize patterns beyond defined rules and analyze significantly higher volumes of information than humans could manage. Consequently, the diagnostics IT of tomorrow will build on and supercharge the capabilities of the technology we work with today. For example:

Streamlining laboratory operations

System failures are disruptive and costly. Today, AI can reduce unscheduled downtime by monitoring critical analyzer components in real time to detect system failures weeks before they occur. Imagine a future in which AI monitors predictive maintenance data, as well as inventory consumption rates, supply chain information and disease trends to automatically schedule just-in-time delivery of replacement parts, reagents and consumables. AI could also schedule repairs or installations to be completed in the most convenient and effective manner, whether by remote assistance or in-person service.

Process-management systems today can visualize lab operations from a centralized dashboard to provide real-time status and analyze trends across all tests, connected analyzers and locations within a health system. These systems can uncover inefficiencies and optimize clinical operations. With AI, visibility could be expanded across geographies, health systems and modalities to establish and benchmark best practices and expertise, and thereby, improve productivity and staff utilization.

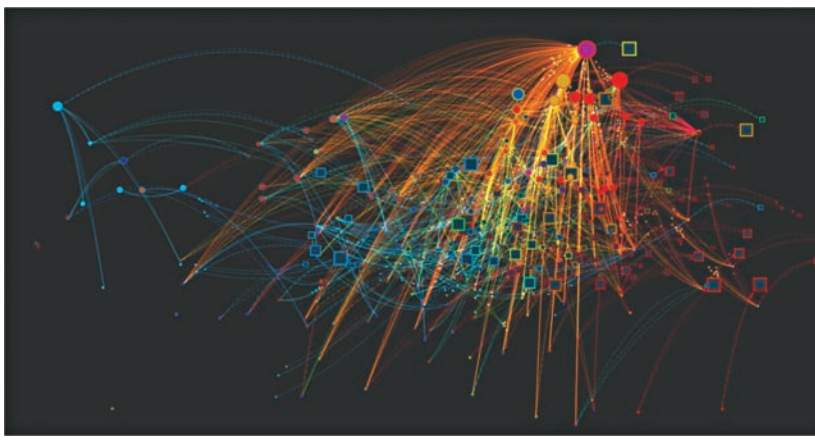

Figure 1. Screen capture of an artificial intelligence analysis and visualization mapping 300 disease trajectories over time. The purple circle in the upper-right corner represents sepsis. The size of the circles and squares depicts the relative number of patients represented, and categories of disease are differentiated by color.

To reduce manual tube handling and sorting, today's AI-enabled camera systems can instantly identify and characterize each tube using machine learning trained on an extensive image library of container types. Cameras detect sample container parameters, and the sample-handling system dynamically adjusts to meet the unique needs of each tube, such as routing and STAT prioritization, aspiration position or special handling requirements for tube-top sample cups or pediatric samples. Innovations in computer vision will transform many operational tasks that previously relied on human sight, but also hold incredible potential for performing clinical analysis.

Clinical analysis and decision support

Digital microscopy analysis leverages machine-learning algorithms trained using tens of thousands of specimen images. It can quickly, consistently and accurately identify and classify particulate and cellular objects in urine, serum or tissue samples, emulating and augmenting the expertise of humans, while easily scaling to support higher volume. With AI, images could be compared with specimens from across health systems, demographics and geographies to identify and diagnose more rare diseases.

Today, the majority of patient results are quickly and consistently autoverified and reported to physicians with minimal human intervention. In the future, AI has the potential to expand precision medicine by adapting reference ranges and reflex-testing guidelines to the unique needs of each patient. To accomplish this, patient and LIS data would be augmented with insights from demographic, population and diagnostic outcomes data.

Further expanding personalization, clinical decision-support systems are currently using AI to aggregate individual patient results from imaging, laboratory, pathology and genetic testing. In coming years, AI could integrate these sources with clinical studies and population health data to recommend treatment pathways and provide more-comprehensive insight to help physicians make best-practice decisions.

Can artificial intelligence influence changes in testing and treatment protocols?

Sepsis afflicts more than 1.5 million patients annually in the U.S., killing over 250,000, and is responsible for one out of every three hospital deaths.[3] This is well-known. But what if AI could reveal just how pervasive fatal sepsis outcomes are across disease states? How might this influence testing and treatment protocols?

Atul Butte, MD, PhD, chief data scientist at University of California Health, recently presented findings[4] that applied artificial intelligence to analyze data from 10.4 million patients from 350 California hospitals. Disease-progression mapping for 300 disease states revealed some surprising trajectories. Chronic liver disease and myocardial infarction are common morbidities, but unexpectedly, both led to death from sepsis; in other words, the ultimate cause of death for these patients with liver and heart conditions was an infectious disease, not a primary problem in the liver or heart. (**Figure 1**)

With mortality from sepsis increasing 8 percent for every hour treatment is delayed, as many as 80 percent of sepsis deaths could be prevented with rapid diagnosis and treatment. Findings like these have the potential to drive testing protocols. As an illustration, hospital admission guidelines might indicate procalcitonin (PCT) testing for the early identification of sepsis.

Advanced data-mining analytics such as those presented by Dr. Butte are increasingly being used to identify often-unexpected disease associations, and fill gaps in our current medical understanding. These insights can help physicians determine the appropriate protocols – such as diagnostic tests like high-sensitivity cardiac troponin or noninvasive blood tests based upon serum markers of liver fibrosis assays – to aid in identification of at-risk patients. Then, through intervention, physicians can alter the disease trajectory, potentially reducing the incidence of heart attacks, advanced liver disease or sepsis. While this technology remains nascent, such data-driven insight holds tremendous potential to inform diagnostic and clinical models. (**Figure 2**)

Intelligence-based medicine

Not too long ago, conversations about artificial intelligence seemed more like science fiction than a pragmatic approach to managing the business of the lab. While progress in the last few years has been remarkable, we are only at the very beginning of transforming care delivery with AI.

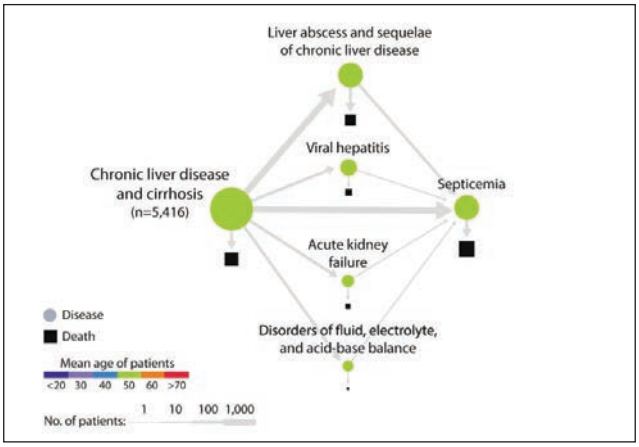

Figure 2. Graph tracing diagnosis trajectories of 5,416 patients with chronic liver disease and cirrhosis. Circles represent primary disease diagnoses and corresponding squares indicate fatal outcomes. Arrows represent patients within that path. The size of the circles and squares and the thickness of the arrows depict the relative number of patients represented.

Enhancing our digital capabilities with artificial intelligence can be daunting, but this evolution holds incredible potential to harness the power of information to help guide clinical decisions.

Anthony Chang, chief intelligence and innovation officer at Children's Hospital of Orange County, summed it up well: "For us to fulfill our vision of precision medicine and population health, we need to change the paradigm of evidence-based medicine to that of a data-science-driven, intelligence-based medicine."

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Stuart Kerty serves as the Global Solutions Marketing Manager for **Siemens Healthineers**.

Urine Time – Past, Present and Future

By Brian Fernández

The practice of laboratory medicine began 6,000 years ago with the analysis of urine as the primary diagnostic tool available to ancient physicians. Often referred to as the "divine fluid," urine was considered the golden liquid window through which they could glean information on mysterious inner workings of the human body. While often prone to fallacies and misinformation, many of the ancient inferences were indeed accurate. Early Hindu physicians correlated sweet tasting urine with the characteristic disease-state symptoms of diabetes mellitus. The recognition that black ants were attracted to this urine was probably the first diagnostic test in medical history. In the 4th century BCE, Hippocrates accurately described that urine was a filtrate of the humors (filtration of blood through the kidneys), that bubbles on the surface of freshly voided urine indicated long-term kidney disease (a physical symptom of proteinuria), and that an increase of urine sediment correlated with a worsening fever (leukocytes and bacteria from a urinary tract infection).[1]

No Simple Solution

In the most basic terms, urine is mostly water with some nitrogenous compounds, electrolytes and metabolic waste components. This generalization, however, dramatically underemphasizes the true complexity of urine and its importance as a noninvasive tool to monitor homeostasis and a myriad of different disease states. It is, quite literally, a veritable fountain of valuable diagnostic information.

Urine is an ultrafiltrate of blood plasma, representing the principal route of waste removal of products of metabolism from the body. Blood is constantly being filtered by the kidneys, receiving about a quarter of the total cardiac output. Over the course of a given day, the kidneys filter a staggering 180 liters of filtered plasma into a final urine volume of about 1.2 liters.[2] The composition of urine is arguably as complex as the blood from which it is derived, however, the concentrations of those compounds are often substantially different from one another.

For example, serum creatinine, a byproduct of muscle metabolism, is tightly regulated by the kidneys. Normal serum levels are about 0.9-1.3 mg/dL in adult males and 0.6 – 1.1 mg/dL in adult females. The creatinine concentration of a random urine sample, however, can range from 40 to 300 mg/dL.[3] The hyper-concentration of creatinine in the urine makes sense considering that it's the pathway of excretion of this nitrogenous metabolic byproduct. The physiology of creatinine makes it a very useful and convenient endogenous substance to assay when evaluating for kidney function. A decrease of urine creatinine levels coupled with an increase of serum levels provides a strong indication of declining kidney function.

Conversely, metabolically useful compounds such as glucose, amino acids and inorganic phosphate are initially part of the tubular fluid (pre-urine) ultrafiltrate, but are mostly reclaimed back into the blood by the tubular reabsorption process. **Table 1** below compares typical fasting serum versus random urine analyte concentrations from healthy individuals.[4]

Urine is a bewilderingly complex and constantly variable biofluid. The Urine Metabolome Database[5] (http://www.urinemetabolome.ca) currently lists 4276 small molecule metabolites that can be found in human urine using current assay technologies, and this list is constantly expanding. Urine contains metabolic byproducts of all the food, drink, vitamins, drugs, environmental agents and contaminants that enter our system. It also includes byproducts from the metabolism of the trillions of microorganisms that cohabitate our bodies.

Urine + Analysis

Urinalysis is a catchall term for the various diagnostic tests that may be performed on a urine sample. Broadly categorized, these tests include the physical, chemical and microscopic examination of urine.

Physical examination has a long and storied history wrought with erroneous inferences; however, it is still used today to provide important information. The most important aspects include the evaluation of color, foam, clarity, odor and concentration via physical specific gravity techniques.

Chemical examination mostly commonly employs the use of urinalysis dipsticks that contain various reagent pads to semi-quantitatively test for the presence of ascorbic acid, bilirubin, blood, creatinine, glucose, ketones, leukocytes, microalbumin, nitrite, pH, protein, specific gravity and urobilinogen. General chemistry analyzers can more precisely and

Table 1 – Typical Fasting Serum versus Random Urine Analyte Concentrations [4]

Analyte	Fasting Serum/	Random Urine	Serum:Urine Ratio
Chloride	100 mEq/L	200 mEq/L	0.5
Creatinine	1 mg/dL	150 mg/dL	0.0067
Glucose	90 mg/dL	10 mg/dL	9.0
Phosphorous	4 mg/dL	100 mg/dL	0.04
Potassium	4 mEq/L	80 mEq/L	0.05
Protein	7000 mg/dL	8 mg/dL	875
Urea	20 mg/dL	3000 mg/dL	0.0067
Uric Acid	5 mg/dL	80 mg/dL	0.0625

quantitatively evaluate urine for analytes such as amylase, calcium, chloride, creatinine, glucose, magnesium, osmolality, phosphorous, potassium, sodium, protein, blood urea nitrogen, urea and uric acid, to name a few. As per the current status of the urine metabolome project, these commonly tested analytes only scratch the surface of the totality of possible chemical examinations.

Microscopic examination of urine sediment traditionally involves the centrifugation, concentration and slide preparation of the sample to identify the presence of a variety of formed elements via manual microscopy. These include RBCs (erythrocytes), WBCs (leukocytes), Epithelial cells (renal, transitional, or squamous), Casts (hyaline or various pathological sub-types), Crystals (various), Mucous, Bacteria, Yeast, Trichomonas, Lipids, Sperm, etc. While manual microscopy techniques are still widely utilized, the emergence of automated urine sediment analyzers, using either flow cytometry or digital imaging methodologies, are rapidly being adopted by clinical laboratories. These systems do not require centrifugation, or other special processing, leading to an improved workflow. They serve to decrease the number of samples that require more labor-intensive and time-consuming confirmatory manual microscopy and allow for process standardization. Another major advantage with automated urine sediment analysis is that more patient samples can be screened, including those that are negative by reagent strip analysis. Using traditional laboratory algorithms, these samples would not have been examined further, possibly missing pathologic samples. A study from the University Hospital Zurich found that the combination of dipstick and automated urine sediment analysis using the Iris iQ200 and Sysmex UF-100 increased the sensitivity of screening to about 98 percent.[6]

Quantimetrix offers an excellent free mobile app called *Urinalysis Made Simple* that serves as a convenient urinalysis reference tool for laboratory professionals, teachers and students.

The Right Urine for the Job

The analysis of urine for disease characterization and monitoring is favored by physicians due to convenience and non-invasiveness. Due to the inherent hour-by-hour variation in the composition of urine , it's critical to collect the most appropriate specimen type, volume, and handling for the analyses to be performed. Not every urine sample is fit for the purpose of every type of test.

The three basic urine specimen types are random, first void (or first morning) and timed samples.

Random urine analysis is the most frequently performed for routine screening as it is the most convenient specimen to obtain. It can be collected anytime and does not require special patient preparation or instruction. These samples are the most affected by changes in fluid intake and exercise so they may not be the best refection of a patient's condition but are typically satisfactory for routine screening. Typically, random urine samples should be midstream (or clean catch) which is collected after the urine flow has started, helping to prevent contamination from bacteria and epithelial cells.

First void urine, as the name implies, requires that the patient voids before going to bed then collects a specimen first thing in the morning. While not the most convenient specimen to obtain, this urine has been retained in the bladder for about eight hours and is ideal for cytology analysis of RBCs, WBCs and epithelial cells. It's also ideal for analytes that require concentration or incubation for detection, such as casts, nitrites and protein.[2]

Timed urine samples are collected over a specified time period to help normalize for the variability of urine composition. These are typically collected over a continuous eight- to 24-hour period, and are particularly well suited for quantitative determinations for analytes such as creatinine, albumin, urea nitrogen, glucose, sodium, potassium, etc. Strict adherence to the timing and collection protocol is critical to obtaining useful results. A preservative may be necessary for timed urine to maintain sample integrity. Refrigeration is a common preservation method but may result in the precipitation of crystals. A variety of chemical preservatives may be used but they are not always suitable for all testing needs. There are also several commercially available urine transport tubes that contain preservative cocktails that are well suited for certain tests.[2]

Golden Future

Laboratory medicine's oldest practice continues to be relevant to this day. Urine is easily obtained and will continue to provide valuable information that is not available from any other source. Advances in urinalysis automation will certainly improve sensitivity, specificity and standardization. The recent push toward fully automated systems, such as the new Siemens Atellica 1500, will set the new standard for accuracy and efficiency in the clinical laboratory. The vast number of metabolites being compiled by the Human Urine Metabolome database project will undoubtedly reveal new biomarkers of disease and exciting new breakthroughs for this divine fluid.

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LABORATORY INNOVATOR

New president looks to champion growth of cellular therapies and biotherapies.

Beth Shaz, MD, President of AABB, is also executive vice president and chief medical and scientific officer at the New York Blood Center.

What made you decide to pursue transfusion medicine for your career?

I started off as a general surgery resident. Although I loved caring for patients and helping them get better, I realized I didn't love being in the operating room, so I switched to pathology. In transfusion medicine, I was still able to interact with patients and make a difference through diagnosing and managing their conditions in the lab.

Can you describe a clinical impact of your academic achievements?

At Grady Memorial Hospital in Atlanta, we designed, implemented and continuously improved a massive transfusion protocol, which resulted in more patients surviving trauma. We also studied the pathophysiology of early trauma induced coagulopathy with the goal of improving treatment. The impact of this research stays with me as I continue to participate in and support the development of new products to improve patient outcomes.

Your career has also included work in cellular therapies. How are blood centers positioned to advance into this space?

The cellular therapy space is an ideal area for blood centers to grow. Blood centers are experts in donor recruitment and eligibility, collection, processing, storage and distribution, which happen to be the core competencies of cellular therapies. Some blood centers are expanding their collections to mononuclear cell collections used for cellular therapies, while others are building and participating in cellular therapy manufacturing. At New York Blood Center, we launched Comprehensive Cell Solutions to help advance these new therapies. We provide the gamut of cell therapy services to our medical center partners, as well as biotech and pharmaceutical companies. New ventures also engage the employees who are excited to learn and want to be part of the growing industry.

You've written many publications about the recruitment and retention of African American blood donors. Why is blood donation from underrepresented minorities so important?

There are two major reasons why underrepresented minority donation is so important. First, red blood cell antigen matching between the donor and recipient is critical to prevent red blood cell alloantibody formation. These alloantibodies can result in severe adverse outcomes, including hemolytic transfusion reactions, in patients with sickle cell disease. Currently, there are not enough antigen-matched units for these patients. Antigen matching requires donors who have similar genetic backgrounds. Thus, minority donors are needed to support the needs of patients with sickle cell disease. Second, U.S. demographics are changing with increasing numbers of underrepresented minorities while the blood supply needs remain. Therefore, all individuals who can donate are needed to meet this continuing demand. The industry cannot rely on the current aging, donor pool.

Prior to becoming AABB president, you served on the Board of Directors and as a member of various committees. How did this prepare you for your new position?

I was fortunate to be on the Accreditation Program Unit early on my career, and from there became its chair, and co-founded the transfusion medicine fellowship sub-section of the current transfusion medicine section. These experiences created a wonderful network of colleagues. Throughout the years, I have had the opportunity to learn about collaborative projects and written multiple documents with colleagues who have different backgrounds and expertise. Through these opportunities, I developed leadership experience and learned how to manage a committee of dedicated volunteers. AABB has also provided rich academic experience by enabling me to be an associate editor of *Transfusion* and overseeing the "How Do I" section for about 10 years. Research support through the National Blood Foundation has further allowed me to study minority blood donation.

As AABB president, what are the main goals you hope to achieve?

My primary goal is to serve the community and make AABB an even more vibrant organization. I am looking forward to working on and supporting the implementation of AABB's new strategic plan, particularly by promoting the ability of the blood system to meet patient needs, championing the growth of cellular therapies and other biotherapies, and driving quality and safety in blood and biotherapies.

AABB's new mission is to be "a connected community dedicated to advancing transfusion medicine and biotherapies. From donor to patient. From lab to bedside." Why did AABB update its mission this year?

AABB's new mission, vision and strategic plan were guided by the thoughts, opinions and experiences of our members. The fields of transfusion medicine and biotherapies are rapidly evolving, and this renewed commitment ensures that AABB is well-positioned to help our community address challenges and embrace new opportunities. AABB's updated vision — "Improving lives by making transfusion medicine and biotherapies safe, available, and effective worldwide" — showcases our focus on advancing patient and donor safety both in the U.S. and internationally.

What are some of the biggest challenges you anticipate for AABB?

AABB needs to make decisions that are best for the community at large, and that can pose challenges in the short term. My hope is that by advancing AABB's mission and vision, everyone will feel that they have contributed and have been heard. I believe that through listening and discussing we can come to a stronger and more unified decision.

SEE WHAT'S COMING UP IN OUR 2020 EXCLUSIVE SURVEY REPORTS

We hope you enjoy the first in a series of State of the Industry reports found in this issue on page 25. We look forward to sharing the results of our next survey: "Best Practices in Lab Management" in the April issue. In July, we feature "Disease Management," and in November the "Molecular Diagnostics" results will take center stage to further engage and enlighten today's lab professional.

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