DETECTING PREDIABETES IN AFRICANS

Sensitivity

HbA1c 41%
Fructosamine 50%
Glycated Albumin 42%
HbA1c + Glycated Albumin 78%

The Big Deal about Small Volumes

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Serum osmolality and urinary sodium measurements are integral to the diagnosis and management of [hyponatremia]. p32
FDA Suggests Data Sharing for LDT Woes

With the Food and Drug Administration (FDA) focused on clinical validity in its proposed regulation of laboratory-developed tests (LDT), many in the lab community have observed that accruing the necessary data is a major challenge. At least in the case of molecular genetic testing, FDA officials are suggesting that more data sharing among laboratories would alleviate the problem.

Were these data silos broken up, new evidence might organically support the robust clinical validity for testing that FDA is seeking, according to Jeffrey Shuren, MD, JD, director of FDA’s Center for Devices and Radiological Health.

Speaking at the American Clinical Laboratory Association (ACLA) annual meeting in March, Shuren emphasized that much of the data that would support validation of new or existing tests already exists, but institutions are keeping it confidential. “If only we could free that data up and move to a place where we are sharing and aggregating that information, there would be lots of things that we could understand—and have significantly lower costs than we do today,” he said.

FDA also envisions more collaboration around developing standards for analytical and clinical validity, according to Shuren. He held up the model FDA is pursuing for regulating next-generation sequencing tests, and suggested that with the right model of standards for clinical validity and clinical claims—and open databases that FDA can review and certify—some tests might not require prior FDA review as high-risk tests. “Then you have the opportunity for [clinical] claims to evolve in line with the evolution of the science,” he said.

In the meantime, it appears that the final guidance on LDTs may come later in the year. Asked about previous comments in which he predicted the final guidance could come out in early 2016, Shuren responded: “Here are the realities: there is a current administration, and that administration is here until early calendar year 2017.”

COALITION PURSUES FEDERAL FUNDING FOR HARMONIZATION

AACC, joined by other laboratory associations, in vitro diagnostics companies, and clinical laboratories, is asking Congress to set aside funds in 2017 appropriations bills to harmonize laboratory testing. For those tests without a gold standard for reporting comparable results, harmonization allows for uniform results regardless of which assay or instrument is used.

The group wrote to senators and members of the House of Representatives, requesting $9.2 million for the Centers for Disease Control and Prevention (CDC) to carry out this work. “The undersigned organizations believe that every patient should have access to dependable and accurate clinical laboratory test results and that those test results should be harmonized,” the letter said. “The CDC has already done incredible work harmonizing the results for a limited number of tests, but we believe with additional funding CDC could expand its efforts—benefiting clinicians and patients alike, and contributing to overall efficiencies in public health and healthcare.”

Harmonized test results are critical if clinical guidelines are to actually improve care, the letter emphasizes. Harmonized tests also can prevent unnecessary, expensive follow-up testing caused by misinterpreted test results.

AACC succeeded in getting report language included in the Consolidated and Furthering Continuing Appropriations Act of 2015. This statement urges CDC to work with the private sector in accomplishing greater harmonization.
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AVOIDING THE TEMPTATION TO SWEEP ERRORS UNDER THE RUG: The Necessary Ingredients for Successful Lab Event Reporting

In our current era of transparency, healthcare organizations have been strengthening their event reporting as they strive to improve patient safety. There are many factors to consider when evaluating your institution’s success (or not) in reporting potential safety events. At Seattle Children’s Hospital we have found that the two most important ingredients are a blame-free culture and a robust review system.

CULTURE
The first ingredient in successful event reporting is to foster a safe environment for staff to disclose errors or problems with the system. This does not happen overnight, and it requires a lot of communication and assurances from hospital and laboratory leaders. Even the terms, “event” and “incident” can be off-putting, as they perhaps have scary or punitive overtones, or mask the intent of the process. At Seattle Children’s, we prefer the word “eFeedback,” and its intentions are clearly explained on our internal staff website (Figure 1). We discuss eFeedbacks daily with staff when issues arise either internal or external to the lab, and supervisors or directors often ask, “Did you eFeedback that?”

We encourage and expect staff at all levels to document concerns, errors, and good catches (or near misses) at the time of discovery. For example, if we report a test result in error and discover it only when a physician calls to question the result, the staff person who takes the call and investigates the problem uses the eFeedback tool—not necessarily the same person who entered the incorrect result. This is important for timely reporting and as a key element of a safe, blame-free environment. In addition, we encourage eFeedback inputters to provide suggestions on how we might prevent similar types of errors. Indeed, eFeedback has a specific section titled Ideas for Improvement, and we have used these suggestions to drive many improvements. Since Seattle Children’s began a refreshed patient safety campaign in 2012, hospital-wide we have seen a doubling of events reported monthly—from about 500 per month to approximately 1,000 per month, of which about 125 per month are lab-related.

ROBUST REVIEW SYSTEM
Both our patient safety department and a designated reviewer for each event type review and code each eFeedback entry. In the lab, our quality manager reviews each entry that involves a lab location or specimen—half of which lab staff self-report, and half come from outside the lab. In our review we task division-specific staff or supervisors to investigate the report, while medical directors assess any potential patient harm. We expect files to be closed within 2 weeks, including reports that document follow-up and details of the investigation. Finally, we thank the person who entered the report and provide that individual details of the follow-up when available and appropriate.

The goal of our patient safety department is to assess the degree of harm using Healthcare Performance Improvement standards, which Seattle Children’s employs to assess and report how safe we are as an institution. In cases involving patient harm, the patient safety department decides whether apparent or full root cause analyses are warranted. They then organize a team—including front-line staff—to work through the process and identify steps to prevent such an error from occurring again.

CHALLENGES AND SUCCESSES
We have had to tweak efeedback throughout the years. For example, the hospital customized specific event types for various departments, including the lab, to add appropriate fields that help with tracking while also considering the time required to complete an entry. Too many

---

What is eFeedback?

eFeedback is an online tool that helps Seattle Children’s Hospital staff and faculty members document issues that affect patient care and the safety of our patients, families, visitors, and staff.

Everyone who works at Children’s has a responsibility to use eFeedback to document what goes wrong—and what goes right. The system helps us learn from our mistakes, make our systems safer, and support our patients, families, staff, and providers.
required fields make it onerous for busy, front-line staff to complete in a timely manner. In eFeedback’s current state, it can still be challenging to document some lab-specific events. For example, there isn’t an easy way to handle reporting of a problem with an entire run affecting several patients, because our entry system is patient-centric. We have to use a workaround, entering the issue for one patient, then commenting in the free text field about the additional patients impacted.

Our department relies on eFeedback to help us escalate system-wide issues. For instance, our lab technologists noticed an increasing trend of specimen quality issues (hemolyzed, clotted, quantity not sufficient) collected from the emergency department (ED). We partnered with the ED leadership who requested that we document every ED patient specimen issue. This enabled the ED leaders to identify a correlation between specimen quality and newer nurses. This data demonstrated a need for more education to help our colleagues improve collection practices to benefit patient care. Similarly, we document any patient complaints we receive related to the cost of laboratory testing, to help hospital leaders with their cost transparency initiatives.

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- Ceftriaxone
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Kidney Recipients Fare Better With HLA-Incompatible Donations Than Waiting for HLA-Compatible Organs

A 22-center study found that kidney transplant recipients who received organs from human leukocyte antigen (HLA)-incompatible living donors after undergoing desensitization had significantly better 1-, 3-, and 8-year survival rates than controlled matches who either remained on a transplant waiting list, received a transplant, or remained on a transplant waiting list and did not receive a transplant (N Engl J Med 2016;374:940–9). The findings have “revolutionary” implications for kidney transplant protocols, according to the authors of an accompanying editorial.

Researchers at Johns Hopkins University in Baltimore led the effort, based on their own experience showing survival benefit for patients who received kidneys from HLA-incompatible living donors after undergoing desensitization. Since the Johns Hopkins kidney transplant program has a very high volume, the researchers wanted to determine whether other transplant centers could achieve similar results.

An enduring imbalance between the supply of and demand for HLA-matching kidney donations prompted Johns Hopkins’ initial research as well as the broader investigation. More than 32,000 people awaiting kidney transplants in the United States have anti-HLA antibodies, making it “very difficult” to find a match with a compatible donor, according to the authors.

The study involved 1,025 patients who received kidney transplants from HLA-incompatible live donors, and two control groups each comprised of 5,125 patients and matched with the intervention group on a variety of factors, including panel-reactive antibody level. The first control group included individuals who remained on a transplant waiting list and/or received a kidney transplant, while the other included patients who remained on a transplant waiting list and did not receive a new kidney.

The investigators used three methods to detect and define the degree of anti-HLA activity: cytotoxic cross-match, flow-cytometric cross-match, and Luminex antibody testing. They defined low donor-specific antibody level as a positive Luminex result and negative flow-cytometric cross-match, moderate as a positive flow-cytometric assay and negative cytotoxic cross-match, and high as a positive cytotoxic cross-match. The authors cited this heterogeneity in antibody testing as a limitation of their study.

Overall, kidney recipients from HLA-incompatible donors had a higher survival rate than either control group at 1 year, 3 years, and 8 years of follow-up. This survival benefit at 8 years extended across low, moderate, and high donor-specific antibody levels.

The authors of the accompanying editorial opined that the findings underscore “the numerous contradictory opinions raised by the transplant community.” HLA desensitization involves “extremely powerful immunosuppressive regimens,” putting them at risk for opportunistic infections and cancer. This regimen also costs about $30,000, while transplants cost around $100,000. In contrast, dialysis costs approximately $70,000 annually for life. “The differing opinions about who should undergo transplantation and the risks involved call for a certain degree of caution,” according to the editorial.
HBA1C PLUS GLYCATED ALBUMIN IDENTIFIES NEARLY 80% OF AFRICANS WITH PREDIABETES

A study of equatorial African immigrants living in the U.S. found that as individual tests hemoglobin A1c (HbA1c), fructosamine, and glycated albumin (GA) identified no more than 50% of individuals with prediabetes. However, combining results for HbA1c and GA increased the sensitivity for detecting prediabetes significantly, to 78% (Diabetes Care 2016; 39:271–7).

The authors conducted the study because of the burgeoning diabetes epidemic in sub-Saharan Africa, where prediabetes and diabetes prevalence is expected to rise within the next 20 years by more than 100%, the highest anticipated globally. At the same time, the transition from prediabetes to diabetes can be delayed with effective treatment, yet screening for prediabetes in Africa is underfunded and inadequately studied. In addition, HbA1c cannot be used in homozygous hemoglobinopathies common in Africans, such as sickle cell anemia.

This led the researchers to explore how well not only HbA1c but also fructosamine—a measure of all glycated proteins in plasma—and GA—a subfractin of fructosamine—detected prediabetes in Africans living in the U.S.

The researchers used oral glucose tolerance testing (OGTT) as the gold standard test for prediabetes, while also measuring fasting plasma glucose, insulin, HbA1c, fructosamine, GA, and hemoglobin electrophoresis. OGTT identified 34% of subjects as having prediabetes. As independent tests HbA1c, fructosamine, and GA identified prediabetes in 50%, 41%, and 42% of subjects, respectively. Combining either fructosamine or GA with HbA1c improved sensitivity, but only the combination of GA and HbA1c was statistically significant in contrast to HbA1c alone, with sensitivity 78% versus 50% and P value <0.001.

FAMILIAL HYPER-COLESTEROLEMIA MORE COMMON IN U.S. THAN GENERALLY RECOGNIZED

Familial hypercholesterolemia (FH) is much more common in the U.S. than previously thought, with approximately 1.5 million people affected (Circulation 2016;133:1067–72). These findings should be a clarion call for providing state-of-the-art care for patients with FH to prevent the coronary heart disease it causes, according to an accompanying editorial.

According to the investigators, published estimates of FH have varied widely, from 1 in 500 to 1 in 137, depending on the populations assessed and the criteria for defining FH. In their analysis, the authors included 36,949 participants in 1999 to 2012 National Health and Nutrition Examination Surveys (NHANES) who were at least 20 years old, along with a secondary analysis of 13,343 adolescents between ages 12 and 19.

The authors also used a modified version of the Dutch Lipid Clinic (DLC) criteria to define FH. The DLC assigns points based on low-density lipoprotein-cholesterol (LDL-C) levels. DLC also assigns 8 points for having a known gene defect for FH, but the authors were unable to consider this criteria as well as several others that were not collected as part of NHANES.

The authors found that the overall U.S. prevalence of probable or definite FH based on NHANES participants was 0.40%, which equates to about 1 in 250 individuals. They found a similar rate in adolescents, 0.42%, or about 1 in 237. The rates varied by race and ethnicity, ranging from 1 in 414 for Mexican Americans to 1 in 174 in other Hispanics.

COMPREHENSIVE MOLECULAR TESTING FOR CAP OUTPERFORMS CULTURE IN DETECTING CAUSATIVE PATHOGENS

Comprehensive molecular testing for community-acquired pneumonia (CAP) using a combination of multiplex, rapid, real-time PCR assays identified causative pathogens in 87% of cases, compared with 39% in culture-based methods (Clin Infect Dis 2016;62:817–23). “The significantly greater sensitivity in pathogen detection could make this technology the standard approach for microbiological diagnosis in hospitalized CAP patients,” according to an accompanying editorial (Clin Infect Dis 2016;62:824–5).

The Edinburgh, U.K.-based investigators conducted the study to explore whether a comprehensive molecular diagnostic approach examining 26 respiratory bacterial and viral pathogens along with bacterial quantification would provide better information for clinical decision-making around CAP. Quantitative molecular assays for respiratory bacteria have been used in patients with pneumonia, but they often lack key targets, use targets with sub-optimal specificity, or are only semi-quantitative, according to the authors.

Culture-based testing identified a bacterial pathogen in 39.3% of patients, all of which were positive by PCR for the human target gene GAPDH. Molecular testing methods detected bacteria in 81.1% of samples. Considering respiratory virus results as well, the molecular methods detected pathogens in 86.7% of cases.

The researchers found that molecular testing could enable physicians to scale back initial empirical antibiotic therapy for 77.2% of patients. They concluded that their findings show “the feasibility of providing the physician with significantly more information on which to base treatment decisions than is currently available.”
In 1999, Jocelyn M. Hicks, PhD, FRCP, a past AACC president hospitalized for Guillain-Barre syndrome, was shocked by the amount of blood drawn for her lab tests during her admission. This prompted her to survey 19 community, university, and children’s hospitals about blood-drawing practices. Hicks discovered that most respondents collected 10 times the volume of blood necessary for routine laboratory testing (1). Responses demonstrated 2.5–10 mL of blood was collected for testing that required just 1.5 mL or less.

Little has changed over the ensuing 17 years, despite the fact that modern chemistry analyzers require less than 50 µL of plasma for a routine comprehensive metabolic panel. Recent publications have equated laboratory blood draws as “contemporary bloodletting” and have shown a direct relationship between patients’ clinical fragility and the volume of blood removed for laboratory testing (2,3). Given these findings, it is not surprising that phlebotomy has been implicated as a primary cause of hospital acquired anemia (HAA). One study found that the rate of HAA was approximately

Challenges to Small Volume Testing

by Khushbu Patel, Sarah M. Brown, and Dennis J. Dietzen
45% in patients older than 18 years admitted to an internal medicine inpatient service (4). A prospective, multicenter observational study of 30 pediatric intensive care units (PICUs) reported a similar rate of 41% (5). HAA also has been associated with increased in-hospital morbidity and mortality.

Due to these concerns, there is a constant push to reduce blood sampling in pediatric patients, and lately, a new impetus to decrease blood draw volumes in adult care settings. Demand for laboratory testing on smaller blood volumes poses several challenges for laboratories, given the current limitations in automated sample processing and testing equipment. In this article, we address through the lens of our experience in pediatric testing several pre-analytical, analytical, and post-analytical issues that need to be considered when dealing with small sample volumes.

**How Low Can you Go?**
The amount of blood that can be drawn safely from an individual depends on his or her total blood volume estimated from total body weight (6). Consensus is lacking on how much blood can be obtained safely from a single draw. Most recommendations suggest no more than 3% of total blood volume be drawn per day in patients younger than 2 months of age and no more than 10% in patients older than 2 months (7). This means that approximately 150 mL of blood can be drawn per day from a healthy adult, while only 9 mL per day can be obtained safely from a healthy, 7-pound infant. Smaller amounts are recommended for ill and hospitalized children (8). Table 1 shows the permissible collection volumes as a function of total blood volume estimated from body weight in both healthy individuals and hospitalized patients. In PICUs, blood draws account for 73% of blood loss, and a single patient typically experiences 7.1 ± 5.3 mL of blood loss per day (5,9). This daily loss closely approaches the recommended total loss for an entire month in a hospitalized 7-pound infant.

Collecting the very minimal blood volume required for testing reduces the risk of HAA. The minimum blood volume needed for each test depends on the sum of individual test volume requirements, the volume necessary to measure interference indices, and the amount necessary for an analyzer to accurately pipet a sample (often referred to as the “dead volume”). The dead volume can be significant depending on assay and instrument platform. For example, an assay might require only 30 µL of sample but an instrument’s dead volume might be 10 times greater. Dead volume varies considerably among instruments and also depends on the sample container being used. Table 2 displays the dead volume requirements for various sample cups of two common instrument platforms.

Another volume requirement for chemistry analyzers relates to the measurement of hemolysis, icterus, and lipemic indices. These parameters are usually measured prior to assays on chemistry instruments, and can add approximately 10 µL to the total volume requirement. In addition to these volume requirements, certain instrument platforms have an aliquot storage unit, which stores additional sample in a refrigerated chamber for reflex testing. Such instruments require considerably more volume than recommended minimums and are not a feasible option for laboratories trying to accommodate low sample volume testing.

**Small Samples with Big Problems**
Most lab result errors occur in the pre-analytical phase of testing owing to specimen collection, specimen handling, and patient variables (10). These pre-analytical errors are behind the high sample rejection rates typically associated with small volume samples. Common small volume sample-related errors include incorrect blood-to-additive ratios, sample evaporation, hemolysis, and human error during manual specimen processing.

Under-filled collection tubes lead to decreased blood-to-additive ratios. Incorrect ratios can cause inaccurate test results, hemolysis, and altered cell morphology. For example, accurate measurement of prothrombin time depends on a specific blood-to-sodium citrate ratio. When citrate tubes are under-filled, the abnormally high concentration of citrate falsely prolongs clotting times. Higher concentrations of additives contribute to other pre-analytical problems, such as hemolysis and changes to cell morphology. Red blood cells exposed to high concentrations of glycolytic inhibitors like sodium fluoride are more likely to hemolyze (11). Likewise, cells exposed to hyperosmolar concentrations of ethylenediaminetetraacetic acid (EDTA) may shrink and exhibit an artificially decreased mean corpuscular volume.

Evaporation also is a major concern for small volume samples given that smaller volumes have a greater surface area-to-total volume ratio. Evaporation in small volume samples can occur during or after sample processing, creating large differences in analyte concentrations. Laboratorians must consider the effects of evaporation before adding on a test. For instance, a sample containing 5 mL of serum (a typical adult volume) that sits open to the air might, after several hours, show an increase in glucose concentration that is up to 10% higher than the initial measurement (7). In contrast, a sample containing only 0.1 mL under the same conditions might show a 50% increase over the initial analysis. To reduce the effects of evaporation, laboratorians should cap samples tightly during storage. Even frozen samples are prone to evaporation when stored for long periods of time.

High surface-to-volume ratios also significantly impact dissolved gases. Labs typically measure total CO₂ (TCCO₂) in plasma via enzymatic techniques following alkali treatment. Alkali treatment quantitatively converts circulating forms of CO₂ (HCO₃⁻, H₂CO₃, and dissolved CO₂) to HCO₃⁻, which serves as the limiting substrate for phosphoenolpyruvate carboxykinase.
At high surface-to-volume ratios, extended exposure of a small volume sample (pCO₂ ~40-50 mmHg) to air (pCO₂ ~0.3 mmHg) can rapidly deplete the pool of dissolved CO₂, reducing apparent TCO₂ concentrations. These changes are often incorrectly assumed to reflect metabolic acidosis in the patient, leading to unnecessary additional testing to explain the apparent acidosis. Figure 1 illustrates the magnitude of this phenomenon.

Small volume samples can be collected in reduced vacuum tubes or in microcollection tubes often referred to as “bullets” (Figure 2). These tubes, containing lower quantities of additives, are designed for specimen collection of 0.5-1 mL. However, using these miniature collection tubes poses several obstacles to laboratory workflow, especially for highly automated laboratories. To begin with, bullets do not fit most analyzers and automated robotic systems. Standard labels also are usually too large for bullets, requiring labs to purchase specialized labels and printers. Otherwise, labs have no choice but to manually enter patient information into their laboratory information system and analysers. Adding these manual processes to lab workflows can introduce errors, prolong turn-around times, and increase the number of employees needed.

One potential solution to this problem is to place bullets into larger tubes that can be barcoded and positioned into instruments. Some manufacturers make false-bottom tubes that fit on some instrument platforms, such as the purple top microtainer tube pictured in Figure 2. More often, labs have to transfer small volume samples into a compatible sample cup prior to analysis. Measures taken to accommodate small volumes, such as false-bottom tubes, sample cups, or tube-within-a-tube, all require validation prior to reporting patient results.

Small volume samples are often obtained via capillary blood collection from a finger or heel. Capillary
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blood collections historically have been used in pediatrics, but this practice is increasingly attractive in adult care. Indeed, the reduced pain (12) and anxiety associated with capillary sampling has been cited as the primary motivation for founding the start-up blood testing company, Theranos. However, dependence on capillary samples may lead to inaccuracy and exaggerated variability. Twenty five years ago research demonstrated that cholesterol measurement in capillary blood is positively biased compared to venous blood (13). A recent study comparing white blood cell (WBC) counts, three-part WBC differential, and platelet counts from successive drops of capillary blood reported the average drop-to-drop coefficient-of-variation was 5 times higher than obtained with well-mixed venous blood (14).

Capillary samples are also associated with high rates of hemolysis and clotting. Proper collection technique is essential to minimize hemolysis of a sample from a finger or heel stick. “Milking” or squeezing a finger or heel is an absolute non-starter because it can result in hemolysis. In addition, exposing slow flowing capillary blood to disrupted tissue can trigger clot formation. Coagulation testing, therefore, is contraindicated on capillary specimens.

**Volumetric Challenges**

Drawing minimal volumes often increases the frequency of a lab’s quantity not sufficient (QNS) specimens. In pediatric labs, a common reason for QNS is an elevated hematocrit, which can be as high as 70% in a newborn but normalizes to adult levels by 3 months of age (15). Consequently, newborn samples can yield much less serum or plasma after centrifugation when compared to an identical volume of adult blood which has a hematocrit around 45%. In such cases, more whole blood must be provided so that sufficient plasma is available for analysis. High hematocrit in newborn samples also complicates routine coagulation tests because sodium citrate only distributes into plasma, and not blood cells. In samples with a hematocrit greater than 55%, the resulting plasma citrate concentration is higher than normal, leading to falsely prolonged clotting times. Often this can be corrected by redrawing a sample using a reduced volume of sodium citrate.

Hemolysis also is a very common problem in pediatrics. While not fully understood, this phenomenon may be related to heightened osmotic and mechanical fragility of the neonatal erythrocyte population (16,17). Regardless of mechanism, capillary sampling and the use of small gauge needles undoubtedly exacerbate the problem. Multiple studies have found that drawing blood from a 20-gauge needle or larger helps reduce hemolysis (18,19). However, in children and elderly
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patients with small or difficult veins, smaller 23- or 25-gauge needles may be required. An analysis of hemolysis rates from various pediatric and neonatal units at St. Louis Children’s Hospital found that the nursery, emergency, and neonatal intensive care units—areas in which heel sticks and small gauge needle use account for the majority of sample collections—had the highest rates of hemolysis.

Even mild hemolysis may compromise many analyses. Potassium and lactate dehydrogenase released from erythrocytes are notable physiologic interferences that may impact clinical decision-making. In other analyses, the interference is spectral. For example, hemoglobin concentrations as low as 50 mg/dL (Roche Cobas 6000) affect the frequent measurement of direct bilirubin in nursery residents.

Mild-to-moderate hyperbilirubinemia may compromise other analyses as well. Bilirubin concentrations as low as 10 mg/dL routinely observed in neonates affect common enzymatic ammonia assays. Ironically, these ammonia measurements are indicated in patients with liver disease typified by hyperbilirubinemia. Table 3 lists common analytes associated with mild hemolysis and mild hyperbilirubinemia.

Conclusions
Small volume samples introduce several challenges for laboratory testing processes, including numerous pre-analytical, analytical, and post-analytical considerations. To meet these unique requirements, labs must educate staff continually about specimen collection, tube additives, and how minimum volume draws affect repeat or add-on testing. Along with these improved education and communication efforts, new instruments designed to accommodate small samples sizes will be critical in overcoming challenges associated with routine use of small-volume samples.

References

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Health disparities persist in the U.S. for a variety of reasons, including lack of access to care, cost, and caregiver biases. Even beyond social factors, there are situations in which a person’s race, ethnicity, disability, geography, or gender may influence his or her risk of disease and ability to recover. Health disparities also extract a financial cost, heaping a financial burden on families that can least afford it.

Clinical laboratories can and should play a role in reducing such health disparities, according to laboratory medicine experts. “If we know that there are disease processes for which disparity exists, we would be selling our physician colleagues short by not trying to determine the appropriate clinical testing parameters available in the clinical lab, and how they may differ among populations,” said Octavia Peck Palmer, PhD, an assistant professor of pathology at the University of Pittsburgh School of Medicine.

While researchers are well on their way to developing new diagnostics and therapies to tackle disparities, there are several steps laboratories can take right now to improve care.
BETTER REFERENCE RANGES
A reference range is defined for each clinical test as 95% of the normal population. Each laboratory must determine what the normal population is and make sure it reflects the patient population served, Peck Palmer said. Several tests warrant ethnic/race–specific reference ranges. “When laboratories establish or review references ranges, it is imperative that the laboratory has appropriate representation of individuals from different ethnic groups,” she said.

Case in point, researchers at the University of Hawaii John A. Burns School of Medicine analyzed data from Americans of European, African, Asian, and Hispanic ancestry to evaluate the distributions of common clinical lab results of health subjects using the 2011–2012 National Health and Nutrition Examination Survey (NHANES). Of 38 common biochemical and hematological tests, normal ranges for 33, when stratified by sex and race/ethnicity, showed statistically significant differences from NHANES laboratory manual reference ranges (Hawaii J Med Public Health 2015;74:302–10).

“There are many racial/ethnic differences,” said co-author Eunjung Lim, PhD, an assistant professor in biostatistics. “That’s why we are proposing that in the future we should develop racial/ethnic-specific reference intervals that would be more accurate and help improve quality of patient care.” Inaccurate reference ranges can lead to misdiagnosis or delayed diagnosis. They can also render minorities ineligible for specific pharmacological interventions, organ transplants, and clinical trials, experts said.

Such issues arose in Africa with black participants in National Institutes of Health-funded AIDS drug trials, according to Timothy Kien Amukele, MD, PhD, an assistant professor of pathology at the Johns Hopkins University School of Medicine in Baltimore. “They kept having to do all these investigations on people who had neutropenia,” Amukele said. “It was costing all this money. They would stop the drug, which meant the people weren’t getting the drugs they needed, and it would not resolve.”

Trouble was, the reference ranges didn’t reflect the fact that healthy people of African ancestry tend to have lower white blood cell (WBC) counts than Caucasians, he noted. This has appeared in the medical literature at least since the 1960s, when it was coined “benign neutropenia.”

Amukele, himself an African American, once had a low WBC count when he was a teen. Doctors thought maybe he had HIV. “My mom was completely freaked out,” Amukele recalled. “She was crying. It was a total mess. And then it turns out it was nothing. Nothing. It was normal. So these things have real impacts, and we haven’t fleshed them out completely.”

After his experience with the drug trials in Africa, Amukele searched the medical literature to identify common laboratory tests for which typical reference ranges would misclassify at least 10% of people of African ancestry. Twelve analytes made his list: immunoglobulin G, magnesium, neutrophils, total serum protein, total WBC, serum albumin, lactate dehydrogenase, blood urea nitrogen, alanine transferase, creatinine, CD8+, and total serum bilirubin.

“The response I got was, ‘Everybody knows that reference ranges vary based on biology, it’s just not that interesting,’” Amukele explained. “But the point I was making is, ‘No, the care providers don’t know about this difference’.”

UPDATED REQUISITION FORMS AND REPORTS
Another step laboratories can take to reduce health disparities is to gather more information on laboratory requisition forms. For example, if a patient is undergoing transgender hormone therapy, that patient’s laboratory results may appear abnormal.

“When laboratories establish or review reference ranges, it is imperative that the laboratory has appropriate representation of individuals from different ethnic groups.”

• Octavia Peck Palmer, PhD
A study published in the American Journal of Medicine identified reference ranges that should be adjusted for patients transitioning from male to female (2014;127:159–62). Those differences cannot even be considered if the laboratory is not aware of the patient’s transition status, Peck Palmer said. “We have begun this conversation at our institution and are working to ensure that all meaningful patient information can be recorded,” she said.

Another opportunity to reduce disparities is to append interpretive clinical information to patient test results. Deborah Cragun, PhD, CGC, an assistant professor at the University of South Florida and Moffitt Cancer Center in Tampa, has been studying why black breast cancer patients are less likely than white patients to get tested for BRCA mutations and to receive genetic counseling (Breast Cancer Res Treat 2015;151:169–76).

One reason, she found, was simply that nobody told them to. Perhaps the doctors assumed black women wouldn’t want testing or that they couldn’t pay, she said. Regardless, reformatted lab reports could help close that gap. “Especially now, when individuals are often getting copies of their test results, if there’s language on there—it doesn’t say ‘we recommend’ because labs shouldn’t recommend—but it can say that according to National Comprehensive Cancer Center Network [NCCN] guidelines, women with these certain risk factors outlined by NCCN should be referred for a genetic risk assessment,” Cragun said.

Laboratories can also advocate for Lynch syndrome tumor screening at their institutions, according to Cragun. About 3% of colorectal cancers are caused by Lynch syndrome, which is hereditary, and detection can prevent future cancers. Lynch syndrome tumor screening is being adopted at certain university and academic medical centers, but less so at community hospitals and places that have large minority patient populations. “My concern is that as this gets rolled out unevenly—it’s just the hospital system’s decision—that we’re going to increase disparities,” Cragun said. She noted recent research that looked at Lynch syndrome screening in Louisiana (Am J Gastroenterol 2015;110:948–55). The researchers found low rates of public hospital screening and of screening in young, high-risk patients. They suggested as possible contributors inadequate provider education and disparities in patients’ access to specialized services.

NEW DIAGNOSTICS AND PERSONALIZED MEDICINE
In the future, laboratories may play a role in fighting health disparities by measuring ancestry–informative markers to determine a patient’s racial admixture. Work by Melinda Aldrich, MD, PhD, an assistant professor at the Vanderbilt University Medical Center Genetics Institute in Nashville has shown, for example, that lung function reference equations—which already take race into account—are even more accurate when the clinician knows how much of the patient’s ancestry is African and how much is European.

There are also many clinical areas in which researchers are discovering biological differences between racial and ethnic populations that could lead to personalized therapies, along with related new laboratory tests. For example, Peck Palmer’s research has shown that severe sepsis occurs more frequently and leads to more deaths in blacks than in whites or Hispanics, even among patients with similar economic backgrounds and similar access to healthcare.

Her preliminary work in patients hospitalized with community-acquired pneumonia revealed that the zinc finger protein 816 gene is downregulated in black patients compared to white patients. Furthermore, black patients with specific polymorphisms in the ZNF816 gene had a higher risk for severe sepsis compared to white patients. Renä Robinson, PhD, an assistant professor of chemistry at the University of Pittsburgh, is embarking on a study to identify what, if any, biological markers are involved in racial disparities in Alzheimer’s disease, which is two to three times more common in African Americans than Caucasians. “We may be able to identify some potential target for therapeutic intervention or maybe diagnosis,” Robinson said. “That would be the hope.”

Still, it is hard to separate the biological causes of health disparities from the social factors involved, according to Jesse Roman, MD, chairman of medicine at the University of Louisville School of Medicine who also chairs the health equality subcommittee of the American Thoracic Society. “To get to the genes and all that kind of thing, that’s great, but I would advocate let’s deal with the socioeconomic and discrimination first,” Roman said.

While genetics and epigenetics certainly play a role in disparities, social conditions are often tightly intertwined, Roman said. For example, in his native Puerto Rico, the higher instance of asthma might be linked to stress—a social factor—but stress influences a gene related to responsiveness to bronchodilators.

In his opinion, the greatest impact laboratory medicine could have right now is in hiring a diverse workforce and pushing, as a profession, to lower the cost of laboratory tests. “Many of the health disparities today are still related to access to care,” he said.

Robinson, too, is concerned about access. “I think there is a role and room for genetics and molecular studies to make a difference, but I am also a realist,” she said. “You can come up with a diagnostic assay but if it isn’t affordable to people or heavily subsidized by insurance companies, then it isn’t helpful. And so that issue would need to be addressed as well.”

Yet Robinson is hopeful that bioscience research like hers can make a difference. “I’m definitely optimistic in that regard,” she said. “If there are findings that come out of the molecular studies that show clear ways to intervene and to help a population of people—that would be a big deal.”

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**THE CASE FOR POPULATION-BASED BRCA1 AND BRCA2 TESTING**
GENETICISTS WEIGH IN

Geneticists and genetic counselors say the goal of population-based BRCA1/2 screening is worthy, but implementing wide-scale testing would be more difficult here than in Israel, which has a high concentration of Ashkenazi Jews with well-defined pathogenic mutations. The U.S. is more diverse racially and ethnically, so its population will have many more types of BRCA1/2 mutations than the three included in King’s study. Meanwhile, many variants’ pathogenicity isn’t well-established, said Bruce Korf, MD, PhD, chair of genetics at the University of Alabama at Birmingham (UAB) and past president of the American College of Medical Genetics and Genomics.

“Knowing how to handle variants of unknown significance is a particular challenge,” Korf noted, adding that the proportion of individuals with a pathogenic variant who will actually develop clinical symptoms is unknown for many BRCA1/2 mutations and can vary by race and ethnicity. “With a diverse U.S. general population, you have to decide which pathogenic mutations to include,” he explained.

Among the fast-growing population of U.S. Hispanics descended from various Latin American countries, there is great diversity that could make choosing particular variants difficult. A recent review paper suggested that in Latin American countries there is significant variation both in rates of BRCA1/2 mutations and prevalence of specific ones. The authors proposed different testing strategies for particular countries.

Pal, whose own research found that young black women in Florida diagnosed with invasive breast cancer have higher BRCA1/2 mutation frequency than previously appreciated, emphasized that discussions related to universal screening should focus on the population as a whole rather than particular ethnic groups.

Mary-Claire King, PhD—a professor of genomic sciences and of medicine at the University of Washington in Seattle—based her stance on her findings in 8,222 Israeli Ashkenazi Jewish men tested for three pathogenic BRCA1/2 mutations known to commonly cause breast and ovarian cancers in Ashkenazi Jewish women. Subsequent testing of female relatives revealed that the mutations were equally common in both sexes. Fifty percent of families that harbored the mutations had no history of cancer that would have prompted testing (JAMA2014;312:1091–2).

Population screening would identify women who wouldn’t be offered testing based on their family history, King and her team wrote, adding that clinicians don’t always follow the USPSTF guidelines. Widespread testing “enables mutation carriers to be identified independent of physician referral or family involvement,” they maintained.

COST-EFFECTIVENESS?

Researchers from UCLA have argued that the scenario suggested by King would be too costly and inefficient in the U.S., principally because of a dramatically lower prevalence of pathogenic BRCA1/2 mutations here. About one in 40 Ashkenazi Jews has a pathogenic mutation, versus a more modest prevalence of one in 400 among the general population. Population screening would prevent just four breast cancers and two ovarian cancers for every 10,000 women screened, according to the researchers’ analysis (JAMA Oncol 2015;1:1217–8).

For 99.75% of women screened, a negative genetic test result confers no gain in life expectancy, does not eliminate the need for regular mammograms, and may provide false reassurance, they noted.

In the time since the UCLA researchers conducted their analysis, BRCA1/2 test prices have dropped owing to the U.S. Supreme Court having invalidated most gene patents. This has enabled multiple labs to offer the test, creating price competition. One lab, Color Genomics, even offers a 19-gene test, including BRCA1/2, for $249.

While that price is unusually low, costs are dropping at a rate that could make BRCA1/2 testing common in 5 years, said Steven Katz, MD, MPH, a professor of medicine, health management, and policy at the University of Michigan in Ann Arbor.

Cheaper tests aside, one of the UCLA authors pointed to several other costs associated with universal BRCA1/2 testing. These include genetic counseling, appointments with surgeons and gynecologists to discuss possible reconstructive surgery and fertility options, and the procedures themselves, said Elisa Long, PhD, an assistant professor at the UCLA Anderson School of Management. Long, who is a breast cancer survivor with a deleterious BRCA1/2 mutation, added that few studies have addressed how women act on test results.

Costs and benefits are important considerations under the Wilson and Jungner criteria usually used in population screening decisions, noted Tuya Pal, MD, a clinical geneticist at the Moffitt Cancer Center in Tampa, Florida and an associate professor of cancer biology at the University of South Florida. These criteria call for considering the cost of finding those who need treatment in relation to possible expenditures on medical care as a whole. “We would need a national discussion about whether [population-based BRCA1/2 screening] is worth pursuing.”

“Medicine is all about targeting those at high risk in a way that gets the biggest bang for the buck,” Katz added. With the overall low prevalence of pathogenic BRCA1/2 mutations in American women, population screening will not enhance life expectancy and quality of life for a large proportion of the population, he contended.

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SPEAKERS: William E. Winter, Michael Laposata

HOSTED BY:
William E. Winter, MD
Department of Pathology, Immunology, & Laboratory Medicine, University of Florida, Gainesville, FL
SCREENING AND COUNSELING: HAND-IN-HAND

Regardless of any screening paradigm that might be adopted, counseling would be key. Each woman must understand that a negative BRCA1/2 test result doesn’t mean she absolutely does not harbor a disease-causing genetic variant in one of those two genes or potentially in other cancer predisposition genes, said Meagan Farmer, MS, director of cancer genetic counseling at UAB.

“A negative BRCA test result doesn’t negate the need for mammograms. Women with family histories of breast cancer may qualify for advanced breast cancer surveillance, such as earlier breast imaging or breast magnetic resonance imaging in addition to mammograms, even after a negative BRCA test result,” she added.

Before any population screening, the country must have adequate infrastructure, genetics professionals emphasized. Right now, there is a shortage of both geneticists and genetic counselors in many parts of the U.S., according to Farmer. Meanwhile, Long asked who would handle the conversations about risks associated with BRCA1/2 and other potentially pathogenic variants.

“Do you have primary care doctors step in? They might not be familiar with BRCA or have enough time to counsel patients.”

Any plans to improve screening infrastructure also should consider the needs of women with less access to follow-up care and ability to pay for it, Pal added. Those women are disproportionately nonwhite. She also pointed out that while the federal Affordable Care Act generally covers preventive care, including BRCA1/2 testing, insurers have varying personal and family history criteria for coverage. While the federal Genetic Information Non-Discrimination Act makes illegal genetic discrimination in health insurance and employment, it does not protect against such discrimination in life and disability insurance.

Questions related to population BRCA1/2 testing may be a preview for the day when most patients get some form of genomic sequencing as part of preventive care, Korf said. “As we learn more about genetic risk factors for common diseases with actionable prevention methods, consideration of wide-scale population screening will become more and more important. Decisions to initiate screening need to be based on rational, evidence-based studies of cost-effectiveness, infrastructure readiness, and consideration of social issues.”

Deborah Levenson is a freelance writer based in College Park, Maryland.

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Diabetes Care 2016;39:271-277 DOI: 10.2337/dc15-1699
FDA Authorizes Use of CDC Zika Tests, Clamps Down on Unapproved Tests for Virus

As the range of the Zika virus continues to expand, the Food and Drug Administration (FDA) has issued emergency use authorizations (EUA) for two Zika tests, while also taking enforcement action against unapproved tests for the virus. The two tests designated for emergency use are the Centers for Disease Control and Prevention’s (CDC) Zika Immunoglobulin M (IgM) Antibody Capture ELISA (MAC-ELISA) and Trioplex Real-Time RT-PCR assay. The Zika MAC-ELISA detects Zika virus-specific IgM in human sera or cerebrospinal fluid (CSF), while the Trioplex assay detects and differentiates RNA from Zika, dengue, and chikungunya in human sera or CSF, and also detects Zika virus RNA in urine and amniotic fluid.

At the same time, FDA has sent a letter to Texas Children’s Hospital and Houston Methodist Hospital requesting more information on the Zika test they recently began offering to their patients. The two hospitals have developed the first rapid assay for the virus, known as the Zika direct test, which detects genetic material from Zika in blood, amniotic fluid, urine, or CSF and provides results in a few hours. In its letter, FDA states that, due to the current public health emergency, it believes that it is appropriate for the agency to review information related to this test’s design, validation, and performance characteristics. In an article in Regulatory Focus, FDA spokesperson Eric Pahon clarified that this means the agency is requesting that all tests for Zika go through the expedited EUA regulatory process. Some members of the healthcare community see this action as a reflection of FDA’s broader goal of bringing laboratory-developed tests under its oversight.

The agency’s letter also states that CDC and the Centers for Medicare and Medicaid Services asked FDA to evaluate the science behind the Zika direct test. FDA sent similar requests for information to First Diagnostics and MD Biosciences regarding Zika tests these companies have developed.

FDA Clears BioFire’s Multiplex PCR System for Use with Respiratory Panel

The Food and Drug Administration has granted bioMérieux’s molecular biology affiliate, BioFire Diagnostics, special 510(k) clearance to market the FilmArray Torch for use with the FilmArray Respiratory Panel (RP). The FilmArray Torch is a fully integrated, random, and continuous access multiplex PCR2 system that provides up to six times more sample throughput compared to the company’s existing system. It requires 2 minutes of hands-on time and has a total run time of about an hour. The FilmArray RP is a panel of 20 respiratory viruses and bacteria that is performed directly on nasopharyngeal swab-associated viral transport media. BioFire has also submitted special 510(k) applications for the FilmArray Torch’s use with other panels, including blood culture identification, gastrointestinal, and meningitis/encephalitis panels.
**BIO-RAD DROPLET DIGITAL PCR SYSTEM BECOMES FIRST TO EARN CE IVD MARK**

Bio-Rad Laboratories has received CE IVD marking for its QX200 Droplet Digital PCR (ddPCR) system, making this the first digital PCR system in the European Union that can be used for clinical applications. ddPCR had been available since 2011 for research use only. The technology partitions a DNA or RNA sample into 20,000 droplets and amplifies targeted sequences within each droplet, enabling laboratories to detect and quantify low concentrations of target DNA and RNA sequences. It has a range of genomic applications, including cancer mutation detection, gene copy number determination, viral load monitoring, and gene edit detection. In particular, the QX200 system is intended to aid in clinical decision-making for diseases ranging from cancer to transplant rejection and viral infection. According to Bio-Rad, it might also be an effective tool for liquid biopsy, and prospective trials are currently underway to evaluate the utility of combined ddPCR and next-generation sequencing in analyzing genomic alterations in circulating cell-free DNA.

**ROCHE GETS APPROVAL FOR HPV AND HCV TESTS**

Roche has received regulatory authorization for two tests: the China Food and Drug Administration has approved the CINtec Plus Cytology test and the U.S. Food and Drug Administration (FDA) has approved the expanded use of Roche’s cobas AmpliPrep/cobas TaqMan HCV test, v.2.0.

The CINtec Plus Cytology test was designed to detect two biomarkers associated with persistent HPV infections that may lead to cancer, distinguishing them from infections that are likely to resolve on their own.

The cobas AmpliPrep/cobas TaqMan HCV test, v.2.0 is a quantitative test for hepatitis C virus (HCV) RNA that is intended for use as an aid in the diagnosis of HCV infection for certain patient populations. With this expanded FDA approval, results from this test can now be used to confirm an active HCV infection, in addition to providing an accurate measurement of how much virus is in a patient’s blood. This is the first quantitative HCV RNA test that FDA has approved for this purpose.

**FDA CLEARS VERMILLION’S OVARIAN CANCER RISK TEST**

Vermillion has obtained 510(k) clearance from the Food and Drug Administration (FDA) for Overa, the successor to Vermillion’s Ova1 test for determining ovarian cancer risk in conjunction with independent clinical and imaging assessment prior to planned surgery for a woman with a pelvic mass. Overa retains three of Ova’s five biomarkers, while replacing two with human epididymis protein 4 and follicle stimulating hormone. The aim of this change is to improve specificity and reduce the need for physicians to determine menopausal status when interpreting the test. While other ovarian cancer tests such as CA 125, Risk of Ovarian Malignancy Algorithm, and Ova1 require patient reported or physician determination of menopausal status, Overa uses a single cutoff of 5, irrespective of menopause. This is intended to simplify both interpretation of the result and patient counseling. The test is run on a single Roche cobas 6000 platform, and like Ova1, stratifies patients into low- or high-risk of malignancy on a scale of zero to 10.
Many new clinical applications of mass spectrometry (MS) are evolving without the benefit of appropriate reimbursement policy from either the Centers for Medicare and Medicaid Services (CMS) or commercial payers. At the same time, in reaction to the overutilization of urine drug testing in pain management, payers have made draconian cuts to reimbursement for testing by traditional chromatography-MS (LC-MS) methods. Clinical laboratories now are at a point where we need new, more precise procedure codes not only to describe MS technology but also to enable payers to set appropriate reimbursement rates.

It is not unusual for reimbursement and codes to lag behind adoption of new technology. This lag can even benefit laboratories when existing payment rates are generous compared to the cost of the new technology. However, it can also lead to overutilization based on the attractive profit margins and lack of clear coverage policy. This has been the case with urine-based drug testing in patients taking opiates for pain management.

**Issues With LC-MS Drug Testing**

Prior to 2014, laboratories were paid about $20 per reported drug for quantitative urine drug tests performed by LC-MS methods. This meant that a large, physician-specified panel of tests could yield significant reimbursement. Aggressive promotion of such panels by reference laboratories and the growth of physician office labs performing these very profitable panels led to overutilization—and in many cases medically unnecessary testing. CMS and other payers have responded to the dramatic rise in testing by imposing such severe cuts that smaller providers may no longer be able to offer LC-MS–based drug testing services, especially in rural areas where such services are needed most. With new Healthcare Common Procedure Coding System (HCPCS) codes created by CMS and Current Procedural Terminology (CPT) codes from the American Medical Association (AMA) more accurately describing drug testing procedures, restrictive coverage policy is limiting the number of analytes tested for as well as the frequency of testing.

As payers work to determine final payment levels, providers and the in vitro diagnostics industry continue to debate exactly how testing should be reported. They remain undecided on whether to use CPT codes or HCPCS codes, as well as on how to set payment at a level that provides fair reimbursement without limiting patient access by forcing out of the market smaller laboratories or those specializing in drug testing. An overriding concern is that changes in both coding and reimbursement could imperil future clinical MS applications that have no relationship to drug testing.

**Reimbursement for New and Existing Assays**

Coding and reimbursement systems should accurately identify what is done so that payers can assign appropriate payment. Current CPT codes for individual analytes generally satisfy this criteria and pose no problem when the analyte is measured by LC-MS rather than other technology such as immunoassay. Most analyte-specific codes apply to any method, meaning payment is method-independent.

However, when labs use new methods such as MS to measure analytes not currently listed on the CMS Clinical Lab Fee Schedule—meaning the method does not have analyte-specific codes—they use method codes until analyte-specific codes become available. This is the case when labs use LC-MS to measure complex protein panels, specific genes, or emerging infectious organisms such as Zika virus. Importantly, this is not the case for new urine drug tests: labs report these using CMS HCPCS codes or drug class-specific CPT codes that include “not otherwise specified” drugs.

For example, if a laboratory uses LC-MS to measure vitamin D, coding
and payment are straightforward. The CPT code for 25-hydroxyvitamin D (82306), like most analyte-specific codes, does not prescribe the method to be used, and thus is applicable to any method the laboratory has properly validated. CMS reimbursement for CPT code 82306 is $40.33 regardless of method. Similarly, immunosuppressants have analyte-specific codes with the same reimbursement irrespective of method.

Alternately, if a laboratory offers a "vitamin panel" including the quantitative measurement of vitamins C, D, E, B-12, and K by LC-MS, the five separate existing CPT codes describing each vitamin would be reported and paid individually since no code exists for a "vitamin panel." The current total CMS reimbursement for the five vitamins in this panel would be $112.24 regardless of whether a lab measured all of the components in a single LC-MS procedure.

Prior to 2016, there were six separate CPT codes available to report new LC-MS or MS-only assays without analyte-specific CPT codes. Effective January 1, 2016, only two CPT codes are available for such procedures: one for chromatography-MS methods (82542 Column chromatography, includes mass spectrometry, if performed, non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen) and one for MS only (83789, Mass spectrometry and tandem mass spectrometry, non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen). These codes can only be used for non-drug assays, and must importantly, include the term “each specimen,” which limits each code to only one unit of service per patient sample—even if the lab tests different specimen types (i.e. urine and serum).

Previously, labs could use LC-MS codes for “each unique combination of stationary and mobile phase,” allowing multiple units of service for complex situations. Since all of the prior codes were paid the same amount ($24.80 during 2015), the two revised codes were automatically set at the same rate for 2016 since they were considered revised rather than new codes, and consequently not eligible for repricing on the CMS fee schedule. The result is that even the most complex LC-MS or pure MS procedures are currently limited to a payment of $25.60 unless performed as an analyte-specific test already identified by a unique CPT code.

Stakeholders have asked CMS to reconsider these as new codes. If the agency accepts this reconsideration request, stakeholders will be allowed to comment on appropriate payment levels for the two remaining codes, hopefully convincing CMS to either increase reimbursement or adopt more descriptive HCPCS codes.

Possible Future Relief From PAMA

The Protecting Access to Medicare Act (PAMA) has potential to bring some relief, but the regulations will also mean that laboratories will need to change the way they think about achieving appropriate reimbursement levels for any new assay. Since PAMA eventually will require CMS to set reimbursement at the median of commercial rates for each test, payment amounts for new and unique assays including complex esoteric MS procedures will depend on convincing commercial payers of the clinical value of the test. Both coverage and payment level will be based primarily on whether the test results affect patient diagnosis or treatment and thereby improve outcomes and reduce overall healthcare costs.

PAMA also requires that CMS create test-specific codes to report all Food and Drug Administration-cleared tests as well as laboratory-developed tests. This expanded set of codes will be used to bill commercial payers. But until CMS is able to calculate a payment level based on these commercial payments, the agency will continue to set payment using existing cross-walking and gap-filling methods. However, CMS’s initial payment levels will be temporary and may increase significantly if commercial payers prove willing to pay more based on the demonstrated clinical value of a new assay or technology.

To illustrate, let’s assume a laboratory develops a proprietary LC-MS assay for a panel of proteins that guides cancer treatment. This assay is shown to significantly improve outcomes by an average of $10,000 per patient because it enables doctors to stop ordering ineffective chemotherapy treatments. As a result, commercial payers agree to pay $2,000 for this test. CMS subsequently will pay the same amount, even if the agency initially set the payment at $25.80 based on cross-walking payment to the existing 82542 code.

In summary, during the next few years all parties involved in developing new MS-based clinical assays will have to carefully monitor reimbursement versus costs and communicate effectively to commercial payers the value proposition for new tests. At the same time, all laboratories and MS suppliers will have to make sure that properly descriptive procedure codes are available either from CMS or AMA.

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Parallel to the progress in next-generation and whole genome sequencing, major developments in mass spectrometry (MS) and bioinformatics are now offering complementary data for the drive toward precision medicine. This signals the dawning of what we call next-generation clinical MS. An influential 2012 Institute of Medicine report outlined the forces behind this advancement in MS. Titled The Evolution of Translational Omics: Lessons Learned and the Path Forward, this document characterized molecular disciplines to include genomics, proteomics, metabolomics, and other omics biomarkers. Further, a 2012 National Institutes of Health (NIH) announcement about new metabolomics programs described the metabolome as the sum of all metabolites in an organism—both endogenous and exogenous—at any given moment. These expression markers are essential to providing clues in disease etiology.

Metabolomic and proteomic technologies include many innovative MS applications, both with and without liquid chromatography (LC) or gas chromatography. A game-changer in microbiology has been clinical application of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF). MALDI-TOF’s ease of sample preparation and rapid, automated analysis and informatics have set the standard for new MS clinical applications.

Next generation MS will deliver faster turnaround times and increased sensitivity and specificity. These technologies will be easier for laboratories to apply with improved automation, and eventually, integrated online automation within clinical core laboratories. Online automation will use cloud computing to enable result and data harmonization, and data portability.

Next Generation MS in Action
At AACC’s 5th Annual Mass Spectrometry and Separation Sciences for Laboratory Medicine Conference, held October 1–2, 2015, distinguished speakers offered a glimpse into next-generation clinical MS. In the opening address, Hans Maurer, PhD, a professor of pharmacology and toxicology and head of the department of experimental and clinical toxicology at Saarland University in Germany, focused on the use of high resolution mass spectrometry (HRMS) in toxicology. Current HRMS technologies such as time-of-flight and Orbitrap for accurate mass detection bring greater accuracy to drug screening, drug metabolism studies, and drug quantitation and monitoring. HRMS also enables laboratories to use smaller samples with less preparation time, easier MS optimization, higher sensitivity and selectivity, and a full scan analysis that delivers retrospective interpretation and more comprehensive quantitation.

AACC President-Elect Michael Bennett, PhD, a professor of pathology at the University of Pennsylvania and director of the clinical chemistry and metabolic disease laboratory at The Children’s Hospital of Philadelphia, discussed using MS to assess metabolic disorders for newborn screening. Bennett explored how MS is improving screening and diagnosis for certain diseases, such as ornithine transcarbamylase deficiency. He also explained how targeted organic acid profiling is being used to diagnose a variety of metabolic disorders, with complicating issues related to secondary metabolites from the microbiome and xenobiotics.

Brent Dixon, PhD, chief scientist at Physician’s Choice Laboratory Services, illustrated how pain management is being transformed through use of complementary pharmacogenomics data and conventional toxicology testing. For example, cytochrome P450 allele analysis can affect an individual’s pharmacokinetics and inform drug dosing for warfarin, methadone, and clopidogrel.

Another emerging technology profiled during the conference was tissue imaging using MS. Richard Caprioli, PhD, the Stanford Moore Chair in Biochemistry and Director of the Mass Spectrometry Research Center at Vanderbilt University School of Medicine in Nashville, described how, using a tissue biopsy and laser ablation, an advanced pixel array offers images at single mass-to-charge values. Laboratories might perform this technique using single cells or even whole animal sections, such as a mouse kidney. Researchers have used MS-based tissue imaging to study the effect of diabetes on renal glomerulus, to perform histology-directed analysis from frozen section and paraffin embedded tissue, and to diagnose malignant melanoma. Caprioli’s lab is advancing clinical
application of this technique with a new pathology interface. He predicted that high-throughput imaging MS will be complementary to other imaging technologies.

Another example of next-generation MS is single cell proteomics. Sean Bendall, PhD, an assistant professor of pathology at Stanford University School of Medicine in California, noted that single cell analysis by mass cytology will present many opportunities for diagnostics. For example, this technique might be used for profiling immune system response and predicting a patient’s recovery after surgery. Other potential applications include identifying phenotype versus function in diseases such as acute myeloid leukemia.

Ultimately, the velocity of next-generation MS will depend on the ability of laboratory medicine professionals to translate emerging biomarkers from bench to bedside. Yan Victoria Zhang, PhD, director of the clinical mass spectrometry and toxicology lab and associate professor of pathology and laboratory medicine at the University of Rochester in New York, shared her experience translating omics biomarkers. On this front, laboratories face many challenges, including the complexity of the human proteome, lack of coherent research pipelines, and lack of standardization in sample collection. In moving biomarkers from bench to bedside, mass spectrometists have made integral contributions using MALDI and electro-spray platforms. Candidate biomarkers have to be validated extensively, and laboratorians must ensure sample quality by dealing with pre-analytic issues as well as intra- and inter-individual variability.

As next-generation MS advances, the contributions of MS in proteomics must be married to those of genomics. At the conference, Henry Rodriguez, PhD, director of the office of cancer clinical proteomics research at the National Cancer Institute (NCI), gave examples of how this is occurring with regard to colorectal, ovarian, and breast cancer. One group leading the proteogenomics effort is NCI’s Clinical Proteomic Tumor Analysis Consortium. This consortium works to identify proteins that derive from alterations in cancer genomes. These proteins could lead to new assays and new insights into cancer biology. In ovarian cancer, for example, deep proteomic analysis has identified biomarkers that correlate with survival.

In addition to these conference presentations, the January 2016 special issue of AACC’s Clinical Chemistry journal focused on cutting-edge MS. Interestingly, one of the issue co-editors, Graham Cooks, PhD, is researching miniaturized MS using three-dimensional printing. This issue of Clinical Chemistry also highlighted pre-analytic advances in paper spray and dry blood spot analyses.

Another on-going development is the use of alternate samples such as oral fluid for drug screening and confirmation. The Substance Abuse and Mental Health Services Administration has published a draft document about the use of oral fluid as a complement to current urine drug testing for the workplace. If this program were to be approved, MS, especially LC-MS/MS, would be vital in offering drug confirmation.

Advancing the Next Generation AACC’s Mass Spectrometry and Separation Science (MSSS) Division is dedicated to building a community that advances next-generation clinical MS. This community will include contributions from laboratory scientists with clinical, basic research, and corporate research and development backgrounds. We aim to educate all laboratory medicine professionals—from bench-level scientists to laboratory directors. In September 2016, the AACC/MSSS: Mass Spectrometry and Separation Sciences for Laboratory Medicine conference will focus on some of the key areas explored during the 2016 conference. It also will emphasize educational outreach to both clinical and anatomic pathology. In addition, AACC’s MSSS division will be hosting hands-on workshops for pathology residents and laboratory scientists during the upcoming 68th AACC Annual Scientific Meeting & Clinical Lab Expo in Philadelphia.

As next-generation MS progresses, simply knowing all the technical ins- and-outs won’t be enough. Laboratory medicine professionals also must stay engaged with regulators and payers. In fact, the Food and Drug Administration is hosting a workshop on May 2 titled Mass Spectrometry in the Clinic: Regulatory Considerations Surrounding Validation of Liquid Chromatography-Mass Spectrometry Based Devices. This event will cover analytical and clinical study designs and considerations for validation and use of LC/MS-based tests. Since MS-based tests are mostly regarded as laboratory-developed tests, this workshop could have important implications for future MS assays. Zhang will offer public comments on behalf of AACC at this workshop.

Harmonization of clinical and translational assays also will be important as next-generation MS matures. Harmonization and other goals for next-generation MS will require collaboration among laboratorians in government agencies such as NCI, other NIH Institutes, and the Centers for Disease Control and Prevention, to name a few. Growing news coverage of pharmacogenomics and personalized medicine shows the public is gaining a better awareness of and appreciation for new disciplines related to laboratory medicine. Now is the time for laboratorians to keep our commitment to patient care and patient safety by driving the next steps in clinical MS testing.

Ultimately, the velocity of next-generation MS will depend on the ability of laboratory medicine professionals to translate emerging biomarkers from bench to bedside.
Partnership Aims to Expand Access to NGS Newborn Screening Panels in Canada

Newborn Screening Ontario (NSO) has partnered with Tute Genomics and UNIConnect to establish an informatics infrastructure for NSO’s new next-generation sequencing (NGS) testing panels. NSO operates the highest sample volume laboratory in Canada for molecular testing, and is also the most comprehensive newborn screening program in the country. The Ontario Ministry of Health and Long Term Care have contracted NSO to offer NGS panels and a multiple ligation-dependent probe amplification assay for the diagnostic confirmation of a variety of congenital disorders. The move is the latest in a series of efforts by the Ontario government aimed at increasing genetic testing capacity in Ontario and lowering costs for tests that are currently performed largely outside Canada.

NSO initially aims to prove the clinical utility of its NGS panels by offering confirmatory diagnostic testing for the subset of newborns who screen positive for congenital disorders using traditional biochemical testing. Once the clinical utility of the NGS panels is confirmed, NSO hopes to expand diagnostic testing to all newborns who screen positive, and in the future may explore the possibility of NGS as a screening test for all of the babies born annually in Ontario.

To enable the delivery of these services, Tute Genomics will deploy its cloud-based informatics and genome interpretation platform in conjunction with partner UNIConnect’s Precision Molecular Diagnostics laboratory information management system. NSO will use the combined solution to manage bioinformatics pipelines in support of all NGS data generated, enabling the organization to identify clinically actionable variants and generate reports from raw sequencing data in an efficient manner.

ZIKA RUO ASSAYS, REAGENTS SURGE IN RESPONSE TO GROWING EPIDEMIC

With Zika infections on the rise, numerous companies have launched research use only (RUO) tests for the virus. The MultiFlex Mosquito-borne Panel developed by Luminex partner GenArraytion is now available as a multi-analyte RUO assay that can detect multiple disease agents, including Zika, and Luminex has partnered with the University of Sao Paulo, Brazil to validate it. Thermo Fisher has begun offering the Euroimmun Ag, an RUO test kit for the serological detection of Zika that can differentiate between the Zika, dengue, and chikungunya viruses. Additionally, Siemens has released the Versant kPCR Zika 1.0 assay, an RUO genetic test for Zika that runs on the Siemens Versant kPCR molecular system or other commercially available PCR systems.

Like many of these companies, Siemens took the RUO route to speed the availability of its assay for critical research. “We hope the Siemens Healthcare assay will help researchers identify the Zika virus, assist in the development of a vaccine, and ultimately help find a cure,” said Fernando Beils, head of Siemens Healthcare, Molecular Diagnostics. “Our aim is to help researchers fight the Zika outbreak in any way we can.”

Aalto Bio Reagents has also jumped on the Zika bandwagon and created the first mouse monoclonal antibodies to Zika NS1 protein and envelope protein to support the development of Zika diagnostic assays.

CENTER FOR INFECTIOUS DISEASE RESEARCH, SATVI DEVELOP TEST FOR TB RISK

The Center for Infectious Disease Research and the University of Cape Town’s South African Tuberculosis Vaccine Initiative (SATVI)
have developed a blood test that is intended to predict whether a latent *Mycobacterium tuberculosis* infection is likely to develop into active tuberculosis disease (TB). Findings published in *The Lancet* show that this test can identify, with a 10–20% false positive rate the majority of individuals who will progress to active TB more than 1 year before the disease manifests.

To develop the test, scientists studied RNA expression patterns in blood samples obtained from a study of more than 6,000 teenagers infected with *Mycobacterium tuberculosis* from South Africa, who were followed for more than 2 years to identify which individuals progressed to active TB disease. They identified RNA biomarkers that predicted disease progression that were then confirmed using samples obtained from a study of 4,500 adults. The organizations believe that the test could eventually be developed into a diagnostic for large-scale efforts to screen and preventatively treat the disease. They now plan to evaluate the test in a clinical trial to determine if targeted therapy can halt the development of TB in at-risk individuals.

**IBM, NEW YORK GENOME CENTER TO BUILD GENETIC DATABASE FOR CANCER RESEARCH**

The New York Genome Center and IBM plan to use IBM’s Watson—a technology platform that analyzes big data—to create a comprehensive and open repository of genetic data to accelerate cancer research. The two organizations will work together to build the capacity to house contributed data, train Watson’s cognitive computing capabilities for genomic analysis, and enable the Center’s member institutions and other research collaborators to sequence and analyze DNA and RNA from patients’ tumors.

In the first phase of the project, the two organizations will examine genetic information from 200 cancer patients to compare how different types of sequencing might impact possible treatment options—examining whole genome and whole exome sequencing as well as clinical panels currently in wide use. Sequencing and clinical data will be fed into Watson to accelerate and focus reviews of massive amounts of medical evidence to help identify existing drugs that might target patients’ cancer-causing mutations. Clinically relevant insights will then be returned to each patient’s physician to potentially support the patient’s treatment decisions.

**INSILIXA, DNA SOFTWARE COLLABORATE ON INFECTIOUS DISEASES PANELS**

InSilixa and DNA Software have teamed to develop infectious diseases assays for InSilixa’s sample-to-answer complementary metal-oxide-semiconductor (CMOS) biochip platform. The proprietary CMOS biochip technology enables rapid detection, quantification, and genotyping of viruses and bacteria in clinical samples while simultaneously identifying their drug resistance profiles using a highly-multiplexed targeted mutation detection technique. The first generation of InSilixa’s products will focus on molecular diagnostics applications in near-patient and point-of-care settings, including analyzing seasonal respiratory infection outbreaks, rapidly detecting multiple-drug resistant bacteria in urgent care settings, and detecting, quantifying, and performing drug resistance genotyping of HIV in blood samples. Under the terms of the agreement, DNA Software will provide multiplexed PCR designs, algorithms for signature sequence identification, ThermoBlast for scanning oligonucleotides against collections of genomic sequences, and PCR design software.

**CHOP, SEVEN BRIDGES PARTNER ON DATA PLATFORM FOR PEDIATRIC ONCOLOGY**

The Children’s Hospital of Philadelphia (CHOP) and Seven Bridges, a biomedical data analysis company, have jointly developed Cavatica, a new cloud-based environment for securely storing, sharing, and analyzing large volumes of pediatric cancer patient genomics data. Cavatica will support CHOP’s commitment to the White House Precision Medicine Initiative through the newly launched Center for Data Driven Discovery in Biomedicine, which is located at CHOP. The Cavatica system is designed to aid researchers in using a growing body of curated pediatric genomic data to make discoveries that will help doctors match cancer therapies more precisely to individual children. To start, Cavatica will support the data sharing and large-volume genomic analyses needs of two integrated consortia: the Children’s Brain Tumor Tissue Consortium and Pacific Pediatric Neuro-Oncology Consortium, both of which are dedicated to the study of childhood brain tumors.
Investigating Hyponatremia

EXPERT
Ibrahim A. Hashim, PhD, DABCC, FACB

How is hyponatremia diagnosed?
A: Labs measure serum sodium with an ion-selective electrode (ISE) using one of two approaches: the indirect method, which involves diluting the sample prior to measurement, and the direct method, which uses neat, undiluted sample. Although newer technologies require much smaller sample volumes, an estimated 83% of laboratories still measure sodium levels using an indirect methodology.

Overall, serum osmolality and urinary sodium measurements are integral to the diagnosis and management of hyponatremia. Also required are clinical assessment and additional laboratory investigations that may include measuring urine osmolality, serum aldosterone, cortisol, and natriuretic peptide levels.

What methods are used to determine serum osmolality?
Serum osmolality is measured using either freezing point depression or vapor pressure techniques. Sodium is the predominant extracellular solute and major contributor to serum osmolality, while other constituents include glucose and urea. Several formulas are available for calculating serum osmolality, but this one is the most widely used: osmolality = 2 x [Sodium] + [Glucose]/18 + [Urea]/2.8.

What does low serum osmolality indicate?
Potential causes of hyponatremia associated with low osmolality (hypotonicity) and excess extracellular fluid include heart failure, liver cirrhosis, and renal impairment. In patients with heart failure, B-type natriuretic peptides will be increased and urinary sodium will be > 20 mmol/L. Urinary sodium will be low in patients with liver cirrhosis, whereas patients with reduced renal function will also exhibit high urinary sodium because the kidneys aren’t properly reabsorbing it.

Hyponatremia, low osmolality, and normal fluid levels can also indicate renal impairment, as well as the syndrome of inappropriate antidiuretic hormone secretion, adrenal insufficiency, and hypothyroidism. Each of these conditions is characterized by normal or low urea, low creatinine and urea/creatinine ratio, low uric acid, and a urinary sodium ≥ 20–30 mmol/L.

Potential causes of hyponatremia, low osmolality, and fluid loss include gastrointestinal disorders, diuretic therapy, and exercise. Another culprit is cerebral salt loss following a subarachnoid hemorrhage, head injury, or neurological procedures. Typical lab values with these conditions include elevated urea, elevated creatinine and urea/creatinine ratio, elevated uric acid, and a reduced urinary sodium < 20–30 mmol/L.

What does normal serum osmolality indicate?
Hyponatremia in patients with normal osmolality (isotonicity) suggests the presence of pseudohyponatremia, for which corrective action is not required. This can be confirmed by testing for elevated lipids and/or proteins.

When lipids and/or proteins are present in plasma in increased concentrations, this can decrease the percentage of plasma water in the blood. This does not impact direct methods for measuring sodium. However, with indirect methods that apply a dilution step prior to sample analysis, this can lead to falsely low sodium results.

What does high serum osmolality indicate?
Hyperglycemia can cause apparent hyponatremia, and may result in osmotic diuresis leading to high osmolality (hypertonicity). High glucose drives water from the intracellular to the extracellular space, diluting sodium concentration. In such a case, measured sodium levels can be corrected by adding 1.6 to 2.4 mmol of sodium for every 100 mg/dL of glucose above 100 mg/dL. High osmolality can also suggest the presence of other solutes such as mannitol.

What leads to a false hyponatremia result?
In addition to pseudohyponatremia, spurious hyponatremia can occur if a sample is contaminated with intravenous fluid containing sodium at less than half that of serum, such as in dextrose infusion. A simple repeat collection often resolves the issue.

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