February 2015

CLN Clinical Laboratory News

An AACC Publication | Volume 41, Number 2

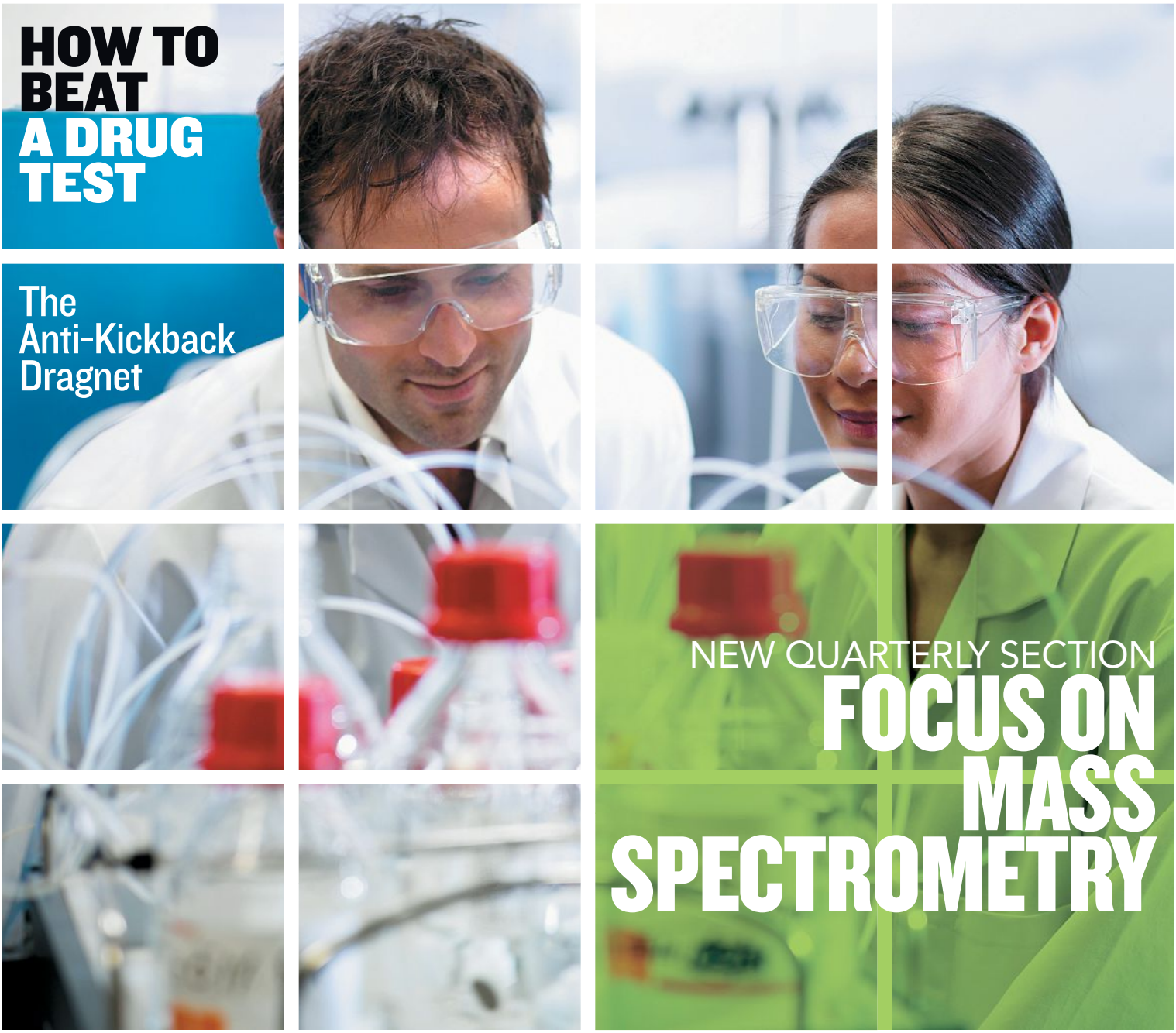
AGE OF DIABETES DIAGNOSIS

Older than 18 >75%

Younger than 18 <46%

PREVALENCE OF RESIDUAL C-PEPTIDE 3–5 YEARS LATER

PAGE 6



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Managing Editor Bill Malone
Senior Editor Genna Rollins
Communications Coordinator Christine DeLong
Contributors Amitava Dasgupta, PhD, DABCC, FACB

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Subscriptions
AACC
1850 K Street, NW, Suite 625
Washington, DC 20006
Phone: +1 202.857.0717 or +1 800.892.1400
Fax: +1 202.887.5093
E-mail: custserv@aacc.org

Editorial Correspondence
Bill Malone, Managing Editor
Clinical Laboratory News
1850 K Street, NW, Suite 625
Washington, DC 20006 USA
Phone: +1 202.835.8756 or +1 800.892.1400
Fax: +1 202.833.4568
E-mail: bmalone@aacc.org

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Clinical Laboratory News (ISSN 0161-9640) is the authoritative source for the clinical laboratorian.

Design and Production Management
McMURRY/TMG

Cover: Monty Rakusen/Glow Images

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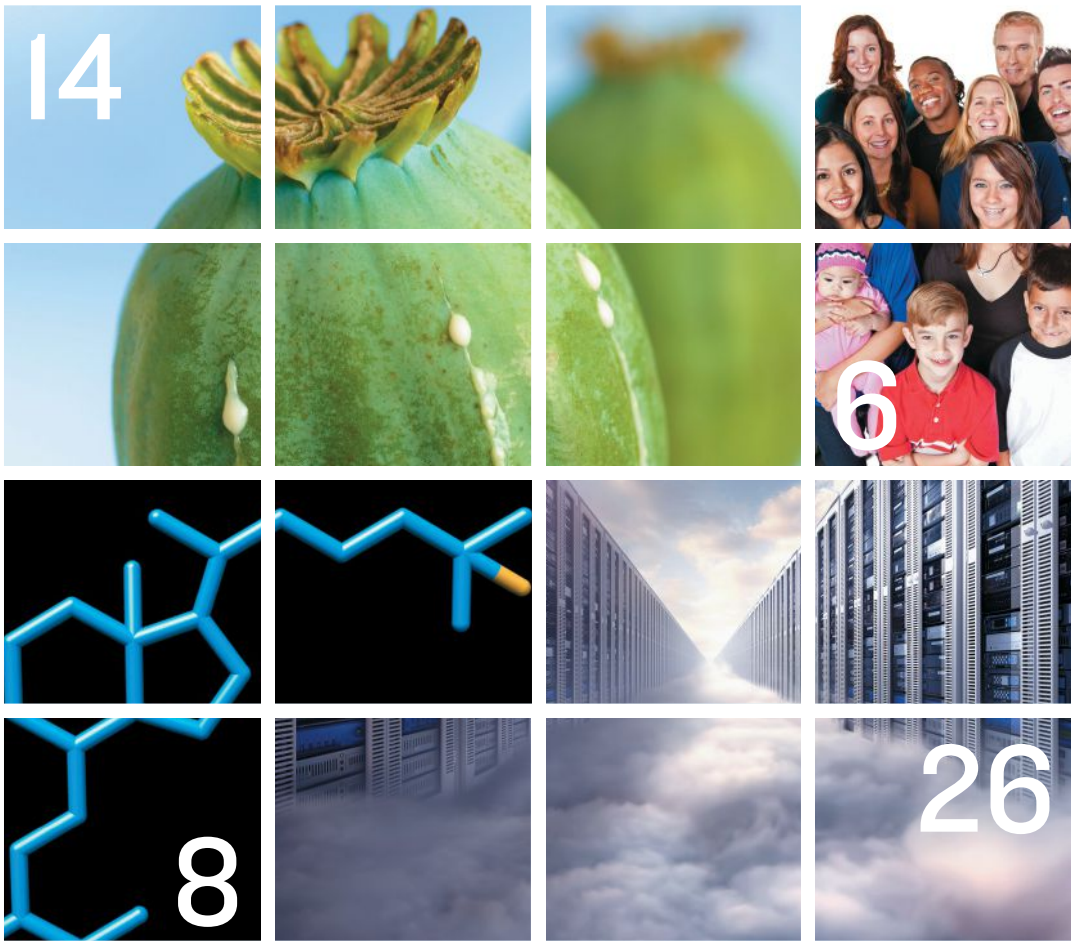

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One major advantage of PCT compared with other biomarkers is its early and rapid increase in response to bacterial infections and sepsis.
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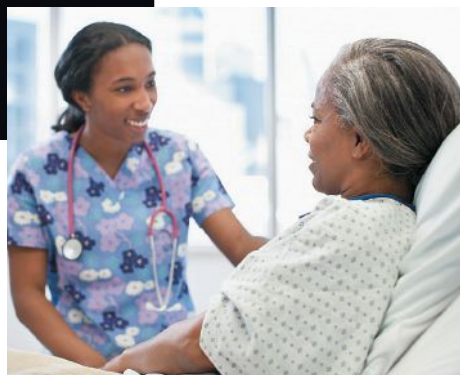
Federal Insider



Healthcare-Associated Infections Drop

Healthcare-acquired conditions declined by 17% over a 3-year period, according to a report released by the Department of Health and Human Services (HHS). The report estimates that 50,000 fewer patients died in hospitals and approximately $12 billion in healthcare costs were saved as a result of a reduction in hospital-acquired conditions from 2010 to 2013. This progress occurred during a period of concerted attention by hospitals throughout the country to reduce adverse events, due in part to provisions of the Affordable Care Act, such as Medicare payment incentives to improve the quality of care and the HHS Partnership for Patients initiative. Preliminary estimates show that in total, hospital patients experienced 1.3 million fewer hospital-acquired conditions from 2010 to 2013.

The data show that the most significant gains occurred in 2012 and 2013. According to preliminary estimates, in 2013 alone, almost 35,000 fewer patients died in hospitals, and approximately 800,000 fewer incidents of harm occurred, saving approximately $8 billion. Hospital-acquired conditions include adverse drug events, catheter-associated urinary tract infections, central line-associated bloodstream infections, pressure ulcers, and surgical site infections, among others.

OBAMA SIGNS NEWBORN SCREENING BILL

On December 18, President Obama signed the Newborn Screening Saves Lives Reauthorization Act of 2013, following concerted advocacy efforts by AACC and other organizations. The Senate passed the bill December 8 after months of delay were resolved by the addition of an amendment related to the privacy of newborn screening samples.

The act renews federal programs for the next 5 years that support state efforts to ensure that every newborn is tested for at least 31 conditions present at birth. The bill also reauthorizes federal programs that provide assistance to states to improve and expand their newborn screening programs, support parent and provider education, and ensure laboratory quality and surveillance for newborn screening programs through the Centers for Disease Control and Prevention. The act also will continue funding for research on treatments for conditions that early screening can detect.

In October, AACC held a briefing on Capitol Hill on why medical testing and especially the newborn screening legislation were crucial to improving children's health. As a follow up to this briefing, members of AACC's board of directors visited Senate offices in November to talk further about the critical role clinical tests play in ensuring that children receive the medical treatment they need. The association released a position statement in July that stresses the importance of identifying additional conditions for newborn screening beyond the core 29 conditions tested for in most states.

CDC DESIGNATES 35 U.S. HOSPITALS AS EBOLA TREATMENT CENTERS

State health officials have identified and designated 35 hospitals as Ebola treatment centers based on a collaborative decision with local health authorities and the hospital administration. The hospitals have the current capabilities, training, and resources to provide the complex treatment necessary to care for a person with Ebola while minimizing risk to healthcare workers, according to the Centers for Disease Control and Prevention (CDC).

More than 80% of returning travelers from Ebola-stricken countries live within 200 miles of an Ebola treatment center. During their active monitoring, state or local public health authorities communicate every day with potentially exposed individuals to check for symptoms and fever for the 21-day incubation period of the Ebola virus.

CDC also has been working with state and local public health officials to identify Ebola assessment hospitals that can serve as the point of referral for individuals being actively monitored and who develop symptoms compatible with Ebola. An assessment hospital would only care for a patient who might have Ebola during the time before a confirmed diagnosis is made, after which it would then transfer the patient to an Ebola treatment center. According to CDC, 15 states that have the majority of travelers now have plans in place to evaluate persons under investigation and provide care for up to 96 hours while testing can be arranged.

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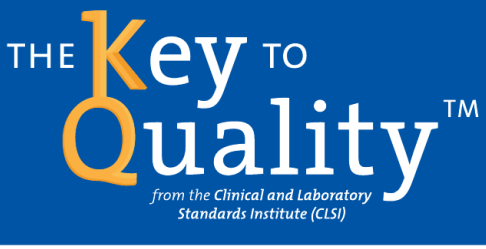

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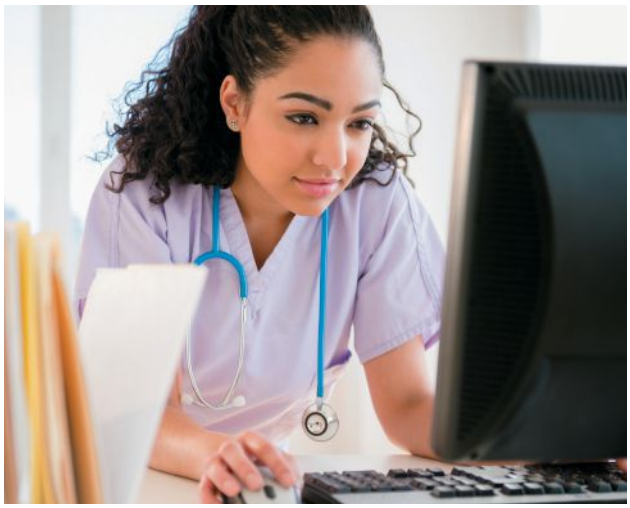
Bench Matters

Lab Formularies: The Time Is Now



Should hospital laboratorians develop formularies like our pharmacy colleagues did years ago? The impetus for pharmacies to develop formularies was to contain costs by having physicians order generic drugs rather than specialized drugs.

What is a laboratory formulary? In simple terms, it is a list of tests physicians can order. Why would a laboratory want to consider this approach? In the current healthcare environment, laboratorians need to reconsider their roles in healthcare organizations. However, since there are no generic laboratory tests, the impetus for a laboratory formulary cannot be the same as it was for the pharmacy.

Cheryl R. Caskey, MA, MLS

A major laboratory incentive to develop a formulary should be to limit inappropriate ordering of expensive molecular, genomic, and other esoteric tests. Lab formularies also hold the line on:

- Utilizing diagnostic tests suboptimally—i.e., ordering an old test when a newer, better test is available;
- Ordering tests found valuable in research but without extensive clinical validation or no documentation of improved diagnostic value versus existing tests; and
- Adding a new, improved test to a panel without replacing an existing panel test.

Many feel developing laboratory formularies would defy the laboratory professional stereotype. But consider this: hospital pharmacies once were treated as a back office function that just supplied drugs and gave doctors what they wanted—not unlike many laboratories today. Over time, pharmacies developed formularies in collaboration with physicians and now pharmacists influence drug orders. Today laboratorians can and should be making a difference in optimal laboratory test ordering.

Laboratory professionals should be fully engaged in test ordering; we need to be able to question test requests, suggest appropriate tests, and cancel inappropriate tests. All members of the lab staff have valuable contributions to make to this effort.

GETTING STARTED

So how does one start the process of developing a lab formulary? There's no single magic step—a multi-pronged approach is the only way to go; however, collaboration is essential. Clinicians, laboratory professionals at all levels, clinically engaged pathologists, and the laboratory director must be involved.

Education of all parties is another must. Everyone involved must understand how the test cycle works, know the roadblocks in the cycle, and how to overcome them. Physicians in particular also need a core knowledge of and competency in ordering tests correctly.

Beyond these broad considerations laboratorians have some particular responsibilities in influencing test ordering and working towards a formulary. One important action would be redesigning the lab requisition. Is the general requisition current and with limited esoteric tests? Tests should be organized by disease state or order patterns, and test bundling should be minimized. The lab also needs to establish a process to review new tests requested—they should only be ordered when data indicates their utility.

Another step along the way to developing a formulary is to review existing standing orders and consult with physicians to see how they are used in clinical practice. In addition, labs should set up an approval and cancel test process for certain tests to ensure that they are ordered only when medically necessary. The same applies to send-out tests. Labs also need to take a look at how they report results, adding information as necessary to assist physicians with clinical interpretation.

A LOOK AT DIFFERENT MODELS

Laboratories are just starting to implement formularies, and there is not an

Jamie Grill/Glow Images

extensive literature about this topic. One model involves a tiered approach in which one tier of tests is available to all physicians, a second tier is open to subspecialists, and a third tier—while not impossible to order—requires committee review/approval.

Other hospitals rely on their computerized physician order entry (CPOE) and electronic health record systems to hold the line on test utilization. After educating physicians, these institutions implement electronic-based barriers to ordering certain tests, with their main strategy being not having tests on the menu that should not be there, and reducing the overall inpatient test menu. These hospitals also use CPOE and lab information systems to monitor utilization trends closely for problems and to spend a lot of time with specialty groups regarding those problem areas. CPOE systems also have been used like an electronic gatekeeper, to prohibit certain test orders unless required pre-requisite test results indicate the need for a particular test.

Another strategy relies on using financial cutoffs for referral testing. This approach counts on the laboratory to be active in test utilization starting with the referral menu. For it to work well, laboratorians have to be educated to communicate more effectively with physicians. The goal is not to lean heavily on physicians about ordering unnecessary tests, but rather to ask how a particular test will influence patient management. If an in-house, less expensive test is available then the lab provides ordering options.

Some organizations have taken the tack of combining CPOE-guided test selection, one-on-one education with physicians, and cost limits for send-out tests. The focus here is on perfecting CPOE order sets so that test selections are offered based on patient symptoms, episodes, or upon admission with certain diagnosis codes. Only appropriate tests appear online for the specific scenario. These online order sets emanate from evidence-based best practices. Of course, physicians still can access the full test menu, but it is harder than normal to do.

The fact is, Medicare and other payers are increasingly targeting over-utilization and there is a need for laboratories to curb costs. Forward-thinking laboratorians will take advantage of this environment and make the laboratory part of the solution.

Cheryl R. Caskey, MA, MLS, is compliance officer at Omega Diagnostics in Shreveport, Louisiana.
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The Sample

Prevalence of Residual C-peptide Levels Higher With Later Age of Type I Diabetes On-Set, Regardless of Duration of Disease

A study examining the prevalence of detectable C-peptide in type 1 diabetes found evidence of residual insulin secretion in nearly one-third of individuals 3 years or longer after their type 1 diabetes diagnosis (Diabetes Care 2014; doi:10.2337/dc14-1952). While this finding is consistent with other studies, the authors took pains to overcome limitations in these prior efforts, which looked at only selected or small cohorts, or individuals with limited duration of diabetes. Accordingly, the authors' findings "[set] the stage for greater understanding of the heterogeneity of disease within these groupings."

The authors tested the frequency of residual insulin secretion in 919 individuals with type 1 diabetes at 28 sites participating in the T1D Exchange Clinic Network, which includes a network of more than 70 clinics, a clinic registry with data from at least 26,000 people with type 1 diabetes, a biobank collection of biosamples, and Glu, a patient-caregiver online community.

Study participants all had had type 1 diabetes for at least 3 years and had been diagnosed when they were between 6 months to 46 years old. The researchers stratified participants into subgroups based on their age at diagnosis and duration of disease.

All participants with detectable C-peptide level at baseline were invited to undergo mixed meal tolerance testing (MMTT) to measure stimulated C-peptide. In addition, up to 10 participants in each subgroup with undetectable non-fasting C-peptide levels were asked to undergo MMTT as a control group. Samples with C-peptide ≥0.017 nmol/L were considered detectable.

The prevalence of patients with detectable nonfasting C-peptide declined with duration of type 1 diabetes, but was consistently higher when patients developed diabetes at age 18 or older. When the researchers included both diagnosis age and diabetes duration in their regression models, each factor was independently associated with detectable C-peptide. Overall, more than three-quarters of participants diagnosed when they were older than age 18 had residual C-peptide levels 3–5 years after diagnosis, in comparison to 46% of those diagnosed when they were younger than age 18.

The researchers concluded that while using a non-fasting random blood draw to measure C-peptide is "a reasonable but not exact measure," they suggested there remains a need for MMTT in the context of clinical trial outcome evaluations. With the gradient of residual C-peptide levels between adult- and pediatric-onset disease, the authors also suggest that "important differences in the biological process of type 1 diabetes" might be at work in these two groups. The data also "reinforce the inadvisability of using C-peptide alone to differentiate between type 1 diabetes and other forms of diabetes."

HDL PROTEINS SAA AND SP-B INDEPENDENTLY ASSOCIATED WITH CARDIAC EVENTS AND ALL-CAUSE MORTALITY IN PATIENTS WITH DIABETIC KIDNEY DISEASE

In patients with diabetes on hemodialysis, the high-density lipoprotein cholesterol (HDL-C) proteins, serum amyloid A (SAA) and surfactant protein B (SP-B), are associated with cardiac events and all-cause mortality, independent of HDL-C (Clin J Am Soc Nephrol 2014; doi:10.2215/CJN.06560714). The findings

Steve Debenport/iStock

suggest that remodeling of the HDL proteome contributes to the increased risk of cardiovascular events and mortality in patients with kidney disease, according to the authors.

The authors conducted the study because of emerging lines of investigation about HDL-C. First, research now shows that overall, the HDL-C level alone is not enough to estimate the cardioprotective function of HDL-C. In addition, emerging evidence suggests the renal function moderates the effect of HDL-C and that atheroprotective HDL particles "may be rendered dysfunctional" in the context of chronic kidney disease.

The investigators conducted a post hoc analysis of the 4D Study, a multicenter trial of more than 1,200 patients with type 2 diabetes. The researchers developed an enzyme-linked immuno assay and used it to measure SP-B and SAA in archived samples from baseline visits for the 4D Study. They evaluated 10 end-points in patients ranging from composite of cardiac death, nonfatal myocardial infarction or stroke, to non-cardiovascular disease mortality.

The authors found that high concentrations of SAA were significantly and positively associated with risk of cardiac events, and that high concentrations of SP-B were significantly associated with all-cause mortality. Adjustment for HDL-C did not affect these associations, according to the authors.

FOUR MICRORNAS ASSOCIATED WITH HEART TRANSPLANT REJECTION

Four microRNAs discriminate "with a very high accuracy" between patients with heart transplant rejection and those without (Eur Heart J 2014; 35:3194–202). This differentiation occurs in both the tissue and serum, suggesting the microRNAs potentially could serve as non-invasive biomarkers of heart transplant rejection.

The investigators conducted the study because the gold standard for detecting acute heart transplant rejection is repeated endomyocardial biopsy, an invasive procedure with rare but potentially serious complications, discomfort for patients, and considerable costs. Emerging research suggests that microRNAs "may play a critical role" in regulating immune cell development and in modulating innate and adaptive immune responses.

The authors evaluated 113 heart transplant recipients, including 30 with biopsy-proven rejection matched to controls without rejection. They compared expression of 14 microRNAs in heart tissue and in serum. Of these, seven were differentially expressed in heart tissue, four of which also were differentially expressed in serum and correlated with tissue expression. These four microRNAs—miR-10a, miR-31, miR-92a, and miR-155—are associated with inflammatory burden in endothelial cells, inflammatory pathways, cardiomyocytes/interstitial cells, and endothelial cells, respectively.

DELAYED EXTRACTION INFLUENCES DNA INTEGRITY

Results from the first pan-European SPIDIA DNA external quality assessment (EQA) indicate that blood sample storage and DNA extraction procedures influence genomic DNA (gDNA) integrity and that PCR-based testing can yield different results if pre-analytical procedures are not standardized (Clin Chim Acta 2015; 440:205–10). SPIDIA was a 4-year project funded by the European Commission to develop quality guidelines and tools for molecular diagnostics and to standardize the related pre-analytical process. SPIDIA included implementation of an EQA to look at collection, transport and processing of blood samples for RNA- and DNA-based analyses.

In this report of the SPIDIA EQA, the SPIDIA laboratory in Florence, Italy looked specifically at the role of gDNA fragmentation in EQA samples on pre-analytical factors and on the results of a multiplex PCR test.

Participating laboratories extracted DNA from a SPIDIA EQA-provided blood sample without restrictions on sample storage temperature or time. After DNA extraction, the labs sent back DNA samples to the SPIDIA laboratory. The great majority of participants stored the sample before extraction and returned it to SPIDIA at 4 degrees Centigrade.

In an evaluation of high molecular weight (HMW) DNA by pulsed field gel electrophoresis, the SPIDIA lab found that HMW DNA integrity showed high variability "probably reflecting the influence of some pre-analytical factors, such as DNA extraction procedures and/or time-interval from block collection to DNA isolation." The SPIDIA lab also discovered a "relevant discrepancy" in values from samples extracted within 6 days, compared with those extracted between 6–10 days and after 10 days.

Additionally, the SPIDIA lab looked at the influence of gDNA integrity on a downstream multiplex PCR test. They found that short amplicons were not influenced by DNA integrity, resulting in the same number of successful PCRs independently of lab performance. However, with longer amplicons higher than 1500bp, high fragmented samples "tend to systematically have a lower number of successful PCRs compared to those classified as control (high gDNA integrity)."

Take Two: Gearing Up for the Next Vitamin D Commutability Study

BY JOHANNA E. CAMARA, PHD, ANDREW N. HOOFNAGLE, PHD, GRAHAM D. CARTER, PHD, AND CHRISTOPHER T. SEMPOS, PHD

As intense interest in vitamin D has boosted demand for testing, many labs have developed their own methods, even as manufacturers introduce new immunoassays for this growing market. However, vitamin D standardization is not complete and some assays can still disagree, potentially confusing clinicians and limiting the utility of these assays for patient care. Since 2010, the Vitamin D Standardization Program (VDSP), part of the National Institutes of Health Office of Dietary Supplements (ODS), and other stakeholders have made significant strides in promoting the standardization of total 25-hydroxyvitamin D [25(OH)D] in order to improve clinical decision-making and inform clinical and public health practice (*3*,*4*).

However, critical work remains before laboratories, clinicians, and patients can have full confidence that 25(OH)D measurements will be comparable over time, location, and laboratory procedure on an international scale. Now, VDSP and its partners are launching a study that aims to ensure the performance of reference materials by demonstrating commutability. Commutability means that reference materials behave like patient samples when tested in a clinical or research laboratory using a specific laboratory's procedure. As such, gauging commutability requires examining as many commercially available laboratory procedures and laboratory-developed procedures as possible. This article describes the new VDSP Commutability 2 Study and explains how assay manufacturers and individual laboratories can participate.

Study Design

VDSP and international collaborators already have developed the essential building blocks of standardization: reference measurement procedures (RMPs) and standard reference materials (SRMs) that together set a gold standard for aligning the results of different assays and methods. Developed by the National Institute of Standards and Technology (NIST) and Ghent University, the RMPs have improved the standardization for assay calibration, and are approved by the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

Similarly, the groundwork for implementing these standards has been laid by the Centers for Disease Control and Prevention's (CDC) Vitamin D Standardization Certification Program (VDSCP); the College of American Pathologists (CAP) Accuracy-Based Vitamin D Survey (ABVD Survey); and the Vitamin D External Quality Assessment Scheme (DEQAS) (*4*). NIST's SRMs are used as trueness controls, and the CAP and DEQAS PT/EQA programs establish and monitor an individual clinical or research laboratory's traceability to the NIST and Ghent RMPs.

NIST and the National Institutes of Health Office of Dietary Supplements, in collaboration with CAP and DEQAS, are coordinating this international study to test the commutability of NIST vitamin D SRMs and CAP/DEQAS test materials. VDSP also has worked closely with AACC and with the International Federation of Clinical Chemistry and Laboratory Medicine on the design of the study.

The overall design of VDSP Commutability 2 follows the Clinical and Laboratory Standards Institute (CLSI) guidelines EP14-A3 and EP30-A (*5*,*6*). The sample sets will consist of 50 healthy donor samples with native total 25(OH)D levels ranging from 5 nmol/L to 150 nmol/L (2 ng/mL to 60 ng/mL), as well as the serum-based NIST SRMs which have been value-assigned for 25(OH)D. In addition, PT/EQA materials from DEQAS and CAP will be included. The donor samples will be collected and prepared by Solomon Park Research Laboratories according to CLSI C37-A guidelines (*7*).

Donor sample sets will be blinded to participants. NIST SRMs in this study will include SRM 972a Vitamin D Metabolites in Frozen Human Serum (4 levels of material) and SRM 2973 Vitamin D Metabolites in Frozen Human Serum

Mari DeMarco, PhD

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2. Huh WK, Ault KA, Chelmow D, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: Interim clinical guidance. *Gynecol Onc.* 2015 Jan. doi: http://dx.doi.org/10.1016/j.ygyno.2014.12.022 [Epub ahead of print].

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(High Level, one level of material). In addition, a yet undetermined number of DEQAS and CAP samples are expected to be included in the test materials.

VDSP will provide participants with a run order protocol that will require the analysis in duplicate on a single day of all donor and test samples. This is intended to minimize the effects that may confound commutability assessment, such as lot-to-lot variability of calibrators and reagents. Target values for samples will be assigned by the NIST ID-LC-MS/MS RMP (*8*). While commutability will be based on the total 25(OH)D value, additional vitamin D metabolites such as 3-epi-25(OH)D3 and 24,25$(OH)_2$D3 will also likely be value-assigned by NIST ID-LC-MS/MS methods to investigate correlation with any trends seen in the data. Statistical assessment of the data will be conducted based on CLSI EP14-A3 and EP30-A guidelines (See Figure). To assess commutability under normal measurement conditions, only the first result from clinical laboratories will be considered, similar to typical patient sample analysis.

During the participant recruitment phase, VDSP will distribute to interested parties a questionnaire on assay and platform system, assay performance data (% coefficient of variation [CV]), as well as verification that laboratories agree to be identified in eventual summary publications. The utility of a previous VDSP commutability study was limited by how few laboratories agreed to publish their data.

FOR MORE INFORMATION ABOUT THE STUDY AND TO LET US KNOW IF YOU ARE INTERESTED IN PARTICIPATING, PLEASE CONTACT US AT:

VDSP@MAIL.NIH.GOV

Benefits for Participants

VDSP Commutability 2 Study is being designed to benefit all parties involved. All participants—including manufacturers, clinical, and research laboratories—will have access to a set of 50 single donor serum samples free-of-charge and will contribute directly to an international effort to standardize vitamin D measurements. VDSP will provide participants with a final report listing the NIST target values for all donor serum samples as well as the SRM and PT/EQA test materials. Reports will also include an evaluation of commutability and intra-batch bias versus NIST target values.

First and foremost, the agencies and programs providing study test samples, including NIST, CAP, and DEQAS, will better understand the commutability of their current SRMs and PT/EQA materials with a variety of different measurement assays and platforms. With this knowledge, the stakeholders in this partnership will work to improve current materials and provide information on existing gaps in metabolite concentration ranges. Such improvement in SRM and PT/EQA tools promotes the standardized measurement of total 25(OH)D worldwide by clinical and research laboratories.

Finally, for assay manufacturers, the results of this study will provide valuable data needed to recommend SRMs and PT/EQA programs to their customers. Results for particular samples may alert manufacturers—or those who are evaluating their in-house developed assays—to gaps in assay performance, such as identifying unanticipated cross-reactive molecules.

How to Get Involved

The focus of the study will be on commercially available assay platforms, and the goal is for all manufacturers to participate. Assays that are in development at the time of the study will also be considered. In addition, we welcome the participation of clinical and research laboratories using commercially available assay platforms, national or subnational nutrition surveys regardless of assay platform, and clinical/research laboratories with in-house developed assays. All laboratories wishing to participate must meet the minimum VDSP performance guideline of CV ≤ 10%. An additional requirement is that participating laboratories must agree prior to the study that their results—including the identification of assay platform and the laboratory of analysis—will be included, as appropriate, in papers published about the study. The number of participants will be limited; selected by NIST and ODS to balance coverage of the different assays and regions of the world.

For more information about the study and to let us know if you are interested in participating, please contact us at: vdsp@mail.nih.gov.

We look forward to working with you on this important commutability study.

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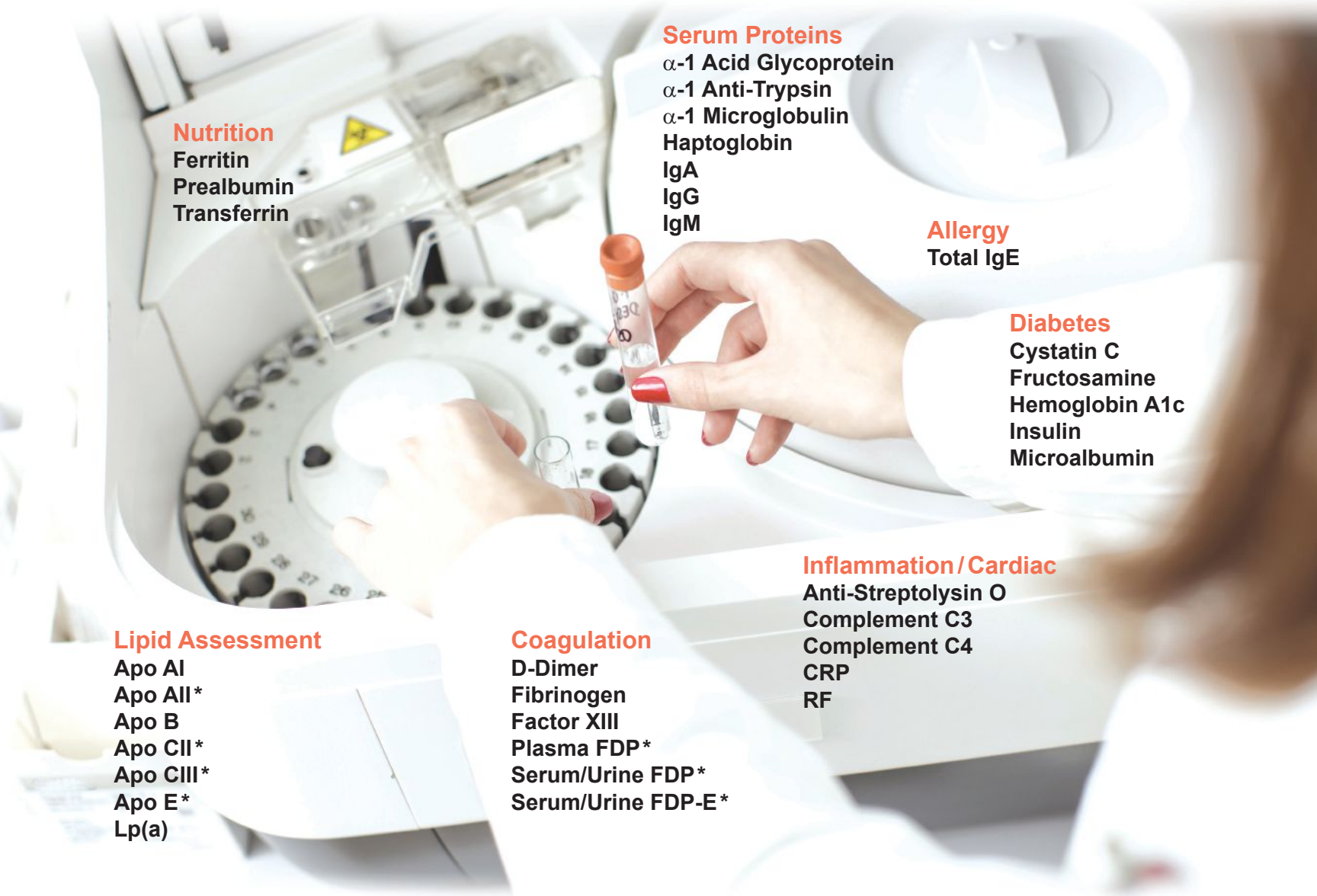

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TRENDS IN ANTI-KICKBACK LITIGATION

WHAT CLINICAL LABORATORIES SHOULD KNOW

Thomas Barwick/Getty Images

BY DAVID SCHER, JD, AND R. SCOTT OSWALD, JD

The prototypical kickback scheme is not difficult to spot: one physician pays another for patient referrals. This is a clear violation of the federal Anti-Kickback Statute, 42 U.S. Code § 1320a–7b (AKS), that anyone with even a passing understanding of the law could easily identify. What becomes more problematic for physicians, employees, and clinical laboratories is where the referring physician is not provided cash in exchange for a referral. Critically, some labs erroneously believe they are playing it safe by offering physicians free or discounted services, among other arrangements. But according to the Department of Justice, these practices still implicate the prohibitions on providing payments in exchange for referrals.

Guidance From the Government

The government's AKS guidelines are complicated and, though there are safe harbor provisions available under the AKS, the government will not hesitate to prosecute those entities it feels are operating even on the fringes of guidelines from the Centers for Medicare and Medicaid Services (CMS).

To help guide physicians and laboratories through the statutory web of the AKS, the Department of Health and Human Services' (HHS) Office of Inspector General (OIG) issued a "Special Fraud Alert" on June 25, 2014. In this document, OIG emphasized that "providing free or below-market goods or services to a physician who is a source of referrals, or paying such a physician more than fair market value for his or her services, could constitute illegal remuneration under the anti-kickback statute."

The consequences for engaging in any violative conduct can be incredibly damaging to a lab. HHS makes clear that if a lab, pursuant to a kickback scheme, submits a claim for a test or procedure that is not medically necessary, it can give rise to liability under the False Claims Act, 31 U.S.C. § 3729 (FCA). This seems an obvious result. But what is less obvious—and perhaps counterintuitive—is that, even if the services are, in fact, medically necessary, a lab can still be found to have submitted a false claim under the FCA. This is because any claim that has been submitted by a lab pursuant to a kickback scheme is inherently a false claim under the law. The government's reasoning is that, but for the kickback scheme, the patient would not have been provided the service and the government would not have made any payment.

Under the Spotlight

The most recent fraud alert bulletin from OIG highlights two kinds of arrangements in particular that are causes for concern. The first is blood specimen collection, processing, and packaging arrangements. For example, a lab may pay its physician clients for everything from collecting and centrifuging specimens, to packing them for transport. OIG notes that "payments under Specimen Processing Arrangements typically are made on a per-specimen or per-patient-encounter basis and often are associated with expensive or specialized tests." Since physicians can bill Medicare themselves for processing and packaging specimens for transport, OIG views such arrangements as suspect (See Box).

> **"Some labs believe** they are playing it safe by offering free or discounted services."

A second area OIG points to is registry payments, more commonly called observational outcomes databases, wherein a lab pays physicians to collect and submit patient data for research purposes. According to OIG, such arrangements "may be reasonable in certain limited circumstances,"

but they can also induce physicians to order unnecessary tests for the purpose of the research, among other problems. Warning signs that a registry arrangement runs afoul of the AKS include, among other characteristics, recommending that physicians order tests with a stated frequency or order multiple medically unnecessary tests; paying physicians on a per-patient or other volume basis; paying physicians at above fair-market value; or covering under the registry arrangement only those tests for which the lab has patents or that it performs exclusively.

What This Means for Labs and Their Employees

The takeaways from the OIG's guidance are clear. Labs need to closely scrutinize any arrangements with referring physicians. If the lab provides services to physicians at a discount or pays a physician more than fair market value for his or her services, there is a good chance that the lab is exposing itself to liability. Case law also suggests that labs need to closely monitor other methods of remuneration being given to referring physicians. Payments in kind are no less scrutinized than payments in cash.

For employees, it is important to know that you are protected if you disclose concerns about the manner in which your lab is compensating referring physicians. The FCA contains an "anti-retaliation" provision that protects employees from being "discharged, demoted, suspended, threatened, harassed, or in any other manner discriminated against in the terms and conditions" in which those employees have tried to stop their employers from submitting false claims under the FCA and the AKS. Moreover, the FCA provides an incentive program for employees who disclose fraud against the government. This could lead to the whistleblowing employee receiving as much as 30% of any money wrongfully obtained by the employer and recouped by the government.

We also must acknowledge that the AKS and the FCA play important roles in protecting patients, employees, and the government from wrongdoing by healthcare providers. But given the complexity of the statutes, it is not difficult to conceive of a scenario in which a lab finds itself on the wrong side of the guidelines. It is paramount that both employers and employees speak with counsel that specialize in these areas if they have any sense that they may be noncompliant.

David Scher is an attorney who focuses his practice on qui tam and whistleblower retaliation cases in California, Maryland, Washington, D.C., and nationally. Mr. Scher is a frequent news commentator and has been interviewed by Forbes.com, Politico, and the Washington Post in all matters of employment law.

R. Scott Oswald is the managing principal of The Employment Law Group. Based in Washington, D.C., Mr. Oswald handles all manner of whistleblower cases, including those litigated under the False Claims Act.

SIGNS OF TROUBLE IN SPECIMEN PROCESSING DEALS WITH PHYSICIANS

The Office of the Inspector General for the Department of Health and Human Services outlines six characteristics of specimen processing arrangements that may be evidence of unlawful kickbacks.

- Payment exceeds fair market value for services actually rendered by the party receiving the payment.
- Payment is for services for which payment is also made by a third party, such as Medicare.
- Payment is made directly to the ordering physician rather than to the ordering physician's group practice, which may bear the cost of collecting and processing the specimen.
- Payment is made on a per-specimen basis for more than one specimen collected during a single patient encounter or on a per-test, per-patient, or other basis that takes into account the volume or value of referrals.
- Payment is offered on the condition that the physician order either a specified volume or type of tests or test panel, especially if the panel includes duplicative tests (e.g., two or more tests performed using different methodologies that are intended to provide the same clinical information), or tests that otherwise are not reasonable and necessary or reimbursable.
- Payment is made to the physician or the physician's group practice, despite the fact that the specimen processing is actually being performed by a phlebotomist placed in the physician's office by the laboratory or a third party.

HOW PEOPLE TRY TO BEAT DRUG TESTING

ISSUES WITH URINARY ADULTERANTS AND THEIR DETECTION

BY AMITAVA DASGUPTA, PHD, DABCC, FACB

Illicit drug abuse remains a serious public health issue. According to the 2013 National Survey on Drug Abuse and Health, an estimated 24.6 million Americans age 12 years and older were current illicit drug users—9.4% of the U.S. population. Marijuana was the most commonly abused illicit drug, followed by cocaine, heroin, and hallucinogens.

Federal guidelines define an adulterated specimen as a urine specimen containing either a substance that is not a normal constituent or an endogenous substance at a concentration that is not a normal physiological concentration. Pre-employment screening programs typically do not involve direct supervision of specimen collection, so employment candidates may attempt to cheat drug testing by adulterating specimens. This makes it essential for laboratories to identify pre-analytically any such adulterated specimens.

Ways of Cheating a Drug Test

Usually people try to cheat drug testing by three different ways: substituting their urine with synthetic urine or drug-free urine purchased from a clandestine source; drinking a commercially available product to flush out drugs; or adding an adulterant in vitro to the urine specimen after collection.

Synthetic urine is difficult to detect because it has similar pH, creatinine, and specific gravity to normal urine. Specific tests are needed to identify compounds that are normal constituents of human urine but not found in synthetic urine, such as cortisol.

Commercially available products that adulterate urine or flush out drugs can be classified under two broad categories. The first includes fluids or tablets that, along with drinking large amounts of water, dilute urine. Common products are Absolute Detox XXL drink, Absolute Carbo Drinks, Ready Clean Drug Detox Drink, Fast Flush Capsules, and Ready Clean Gel Capsules.

The second category of products is in vitro urinary adulterants that are added to urine after collection. Examples include Stealth (peroxidase and peroxide), Klear (nitrite), Clean ADD-IT-ive (glutaraldehyde) and Urine Luck (pyridinium chlorochromate [PCC]). In addition, iodine is a strong oxidizing agent and may potentially destroy abused drugs, especially marijuana metabolites (*2*). Research also indicates that papain with intrinsic ester hydrolysis ability could significantly reduce the concentration of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), a metabolite of marijuana, if added to the urine specimen in vitro (*3*).

Household Chemicals as Urinary Adulterants

Would-be drug test cheaters might try adulterating their specimens with household chemicals, but most can be detected by specimen integrity testing. Both collection sites and laboratories have at their disposal a number of mechanisms to detect potentially invalid specimens. The temperature, for instance, should be within 90.5–98.9°F. The specific gravity should be between 1.005–1.030, and pH should be between 4.0–10.0. The creatinine concentration should be 20–400 mg/dL. However, some drug testing laboratories consider a creatinine concentration of 15 mg/dL as the lower end cutoff. One common adulterant, sodium chloride, always produces a specific gravity greater than 1.035 if added at a concentration necessary to produce a false-negative result.

Unfortunately, specimen integrity testing doesn't detect all adulterants. For example, it won't pick up adulteration of urine with Visine eye drops, isopropanol, or other urinary adulterants. However, effective spot tests and special urine dipsticks are available (See Table 1).

Flushing, Detoxification Agents, and Diuretics

Flushing and detoxification agents are frequently advertised as effective means of passing drug tests. Many of these products contain caffeine or other diuretics to increase the output of urine, as well as sugar

and natural or artificial flavoring agents. The objective is to produce diluted urine so that concentrations of abused drugs and or metabolites fall below the recommended cutoff concentrations.

Cone et al. evaluated the effect of excess fluid ingestion on false-negative marijuana and cocaine urine test results by studying the ability of Naturally Clean Herbal Tea, goldenseal root, and hydrochlorothiazide to cause false negative results. Volunteers drank one gallon of water, herbal tea, or took hydrochlorothiazide 22 hours after smoking marijuana cigarettes or intranasal administration of cocaine. Their creatinine levels dropped below the cutoff 2 hours after intake of excessive fluid. Marijuana and cocaine metabolite levels (as measured by both enzyme multiplied immunoassay technique [EMIT] and fluorescence polarization immunoassay [FPIA]) decreased significantly and frequently switched from positive to negative in subjects after consuming 2 quarts of fluid. Even excess water was effective in diluting a urine specimen to cause false negative results, although herbal tea diluted urine faster compared to water alone (5).

Using Spot Tests

When specimen integrity testing cannot detect an adulterated specimen, laboratories can employ a variety of effective spot tests.

• Urine Luck

Wu et al. reported that the active ingredient of "Urine Luck" was PCC, a strong oxidizing agent, which at a concentration of 100 gm/L, caused significantly decreased response rate for all EMIT II drug screens, indicating the possibility of false-negative results. In contrast, for the Abbott Abuscreen test, only morphine and marijuana assays were affected, but a false-positive result was observed with the amphetamine assays. This adulteration of urine did not alter GC/MS confirmation of methamphetamine, benzoylecgonine, and phencyclidine, but apparent concentrations of opiates and THC-COOH were significantly reduced.

Wu et al. also described a simple spot test using 1,5-diphenylcarbazide in methanol (10 gm/L) to detect the presence of PCC in urine, in which a reddish purple color developed in the

T1 Household chemicals and drugs of abuse testing by immunoassays

HOUSEHOLD CHEMICALS	DETECTION BY SPECIMEN INTEGRITY TEST	DRUGS AFFECTED	COMMENTS
Sodium chloride	Increased specific gravity	Amphetamine, barbiturates, benzoylecgonine, cannabinoid (as marijuana metabolite), opiates, phencyclidine	False negative result with EMIT assay. It may also affect other immunoassays.
Vinegar	Reduced pH	Cannabinoid test	CEDIA assay is free from interference of vinegar.
Liquid hand soap	Increased pH	Cannabinoid, barbiturate, methaqualone, benzodiazepine	False negative test results with EMIT assay.
Detergents/Laundry soap	Increased pH	Cannabinoid, phencyclidine, barbiturates, amphetamines	Decreased levels with EMIT but at very high levels immunoassays by all manufacturers are affected.
Sodium bicarbonate	Increased pH	Opiates	Decreased levels with EMIT.
Denture cleaning tablet (sodium perborate)	Increased pH	Benzoylecgonine, MDMA, cannabinoid	False negative results with FPIA.
Sodium hypochlorite		Cannabinoids, benzodiazepines	False negative by EMIT.
Visine eye drops	Cannot be detected	Cannabinoid	Mostly affect cannabinoid test but can affect other tests.
Alcohol/Isopropanol	Cannot be detected	Methaqualone	Invalidate EMIT assay.

Previous: Peter Dazeley/Getty Images

presence of PCC (*6*). Moreover, adding a few drops of 3% household hydrogen peroxide solution to approximately 0.5 mL of urine specimen caused immediate development of a dark brown color and dark brown precipitate if PCC was present in the urine. As a strong oxidizing agent, PCC could also liberate iodine from potassium iodide solution in acidic medium (*7*). Notably, several other adulterants available online contain PCC.

• Nitrite Containing Agents

Products such as "Klear," which contains potassium nitrite, can cause interference in GC/MS confirmation of THC-COOH. However, a bisulfite step at the beginning of sample preparation can eliminate this problem (*8*).

Nitrite in urine may arise in vivo in patients receiving medications such as nitroglycerin, isosorbide dinitrate, and nitroprusside, or due to urinary tract infection. However, concentrations of nitrite usually are below 36 µg/mL in such specimens, while nitrite concentrations are 1,910–12,200 µg/mL in urine specimens adulterated with nitrite (*9*).

Nitrite can be easily detected by simple spot tests. Addition of a few drops of a nitrite-adulterated urine specimen to 0.5 mL of 1% potassium permanganate solution, followed by addition of a few drops of 2N hydrochloric acid, turned the pink permanganate solution colorless with effervescence. Another spot test to detect nitrite used 1% potassium iodide solution. Adding a few drops of nitrite adulterated urine to 0.5 mL of potassium iodide solution, followed by addition of a few drops of 2N hydrochloric acid, resulted in immediate release of iodine from the colorless potassium iodide solution. If any organic solvent, such as hexane, was added the iodine was readily transferred in the organic layer giving the layer a distinct color of iodine (*7*). Nitrite could also be detected by diazotizing sulfanilamide and coupling the product with N-(1-napthyl) ethylenediamine.

• Stealth

Stealth is an adulterant which consists of two vials, one containing a powder (peroxidase) and another vial containing a liquid (hydrogen peroxide), both added to the urine. Stealth is capable of invalidating immunoassay screening of THC-COOH, LSD, and opiates using both Roche ONLINE assays and Microgenics CEDIA assays if these drug or metabolites are present in modest concentrations (125–150% of cutoff values). In addition, GC/MS confirmation could be affected (*10*).

Valtier and Cody described a rapid spot test to detect the presence of Stealth in urine. Addition of 10 µL of urine to 50 µL of tetramethylbenzidine working solution, followed by addition of 500 µL of 0.1 M phosphate buffer solution caused the specimen to turn dark brown. Peroxidase activity could also be monitored by using a spectrophotometer (*11*). Our investigation showed that if a few drops of a urine specimen adulterated with Stealth were added to potassium dichromate followed by a few drops of 2N hydrochloric acid, a deep blue color developed immediately, which usually faded with time.

• Glutaraldehyde

Glutaraldehyde containing products were one of the first that appeared in the market to invalidate drugs of abuse testing. Glutaraldehyde solutions are also available in hospitals and clinics as a cleaning agent. Glutaraldehyde at a concentration of 0.75% volume could lead to false-negative screening results for a cannabinoid test using the EMIT II drugs of abuse screen. Amphetamine, methadone, benzodiazepine, opiate, and cocaine metabolite tests are also affected at glutaraldehyde concentration between 1 and 2% using EMIT II immunoassays.

Wu et al. described a simple fluorometric method for the detection of glutaraldehyde in urine. When 0.5 mL of urine was heated with 1.0 mL of 7.7 mmol/L potassium dihydrogen phosphate (pH 3.0) saturated with diethyl-thiobarbituric acid for 1 hour at 96–98°C in a heating block, a yellow green fluorophore developed if glutaraldehyde was present. Shaking the specimen with n-butanol resulted in the transfer of this adduct to the organic layer which could be viewed under long wavelength UV light. Glutaraldehyde in urine can also be estimated using a fluorometer (*14*).

• Zinc Sulfate: A New Urinary Adulterant

Although not widely used, zinc sulfate is an effective urinary adulterant that could invalidate all drug tests using EMIT assay. Currently there is no suitable method for detecting zinc sulfate in adulterated urine. Therefore, two rapid spot tests to detect the presence of zinc sulfate in urine were developed. Addition of 3–4 drops of 1N sodium hydroxide solution to approximately 1 mL of urine containing zinc sulfate resulted in formation of a white precipitate, which was soluble in excess sodium hydroxide. In the second spot test, addition of 3–4 drops of 1% sodium chromate solution to 1 mL of urine containing zinc sulfate followed by addition of 4–5 drops of 1N sodium hydroxide led to formation of a yellow precipitate (zinc chromate) (*15*).

Testing Urine Specimens for Adulterants

Specially designed urine dipsticks such as AdultaCheck 4, AdultaCheck 6, or Intect 7 can be used to detect many adulterants in urine. AdultaCheck 6 detects creatinine, oxidants, nitrite, glutaraldehyde, pH, and chromate. The Intect 7 test strip for checking adulteration in urine is composed of seven different pads to test for creatinine, nitrite, glutaraldehyde, pH, specific gravity, bleach, and PCC.

Guidelines from the Substance Abuse and Mental Health Services Administration require additional tests for urine specimens with abnormal physical characteristics or ones that show characteristics of an adulterated specimen during initial screening or confirmatory tests. A pH less than 3 or more than 11, and nitrite concentrations greater than 500 mg/mL indicate the presence of adulterants. A nitrite colorimetric test or a general oxidant colorimetric test should be performed to identify nitrite.

The presence of chromium (VI) in a urine specimen also is indicative of adulteration at a cutoff concentration of 50 mg/mL. The presence of chromium in a urine specimen could

be confirmed by a chromium colorimetric test or a general test for the presence of oxidant. A confirmatory test should be performed using multi-wavelength spectrophotometry, ion chromatography, atomic absorption spectrophotometry, capillary electrophoresis, or inductively coupled plasma mass spectrometry.

Elemental halogens, such as pure bromine or iodine, can also be used as adulterants. The presence of these halogens should be confirmed by a halogen colorimetric test or a general test for the presence of oxidants. Confirmatory tests may employ multi-wavelength spectrophotometry, ion chromatography, atomic absorption spectrophotometer, capillary electrophoresis, or inductively coupled plasma mass spectrometry.

To detect glutaraldehyde, laboratories should use a general aldehyde test or the characteristic immunoassay response in one or more drug immunoassay tests for initial screening. Similarly, the presence of PCC should be confirmed using a general test for the presence of oxidant and a GC/MS confirmatory test. Finally, surfactant should be verified by using a surfactant colorimetric test with a greater than or equal to 100 mg/mL dodecyl benzene sulfonate equivalent cutoff.

Conclusion

It is essential for laboratories to detect adulterated urine in the pre-analytical step, as many adulterants invalidate immunoassay screening tests. Although routine specimen integrity tests can detect most of the household adulterants except Visine eye drops and alcohol/isopropanol, adulterants containing strong oxidizing agents such as potassium nitrite, pyridinium chlorochromate, or Stealth require a different approach. Spot tests, specially designed urine dipsticks, as well as more analytically sophisticated methods such as chromatographic methods, are available in the toxicology laboratory to identify these adulterants. If a urine specimen is adulterated it must be documented and reported, but no further testing is necessary.

IT IS ESSENTIAL FOR LABORATORIES TO DETECT ADULTERATED URINE IN THE PRE-ANALYTICAL STEP, AS MANY ADULTERANTS INVALIDATE IMMUNOASSAY SCREENING TESTS.

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Amitava Dasgupta, PhD, DABCC, FACB, is a professor of pathology and laboratory medicine at the University of Texas-Houston Medical School. He is the medical director of laboratory services of TIRR-Memorial Hospital as well as director of clinical chemistry and point-of-care testing at Memorial-Hermann Hospital in Houston. He has published 209 peer-reviewed papers in various journals and is on the editorial board of five journals.

+EMAIL: Amitava.Dasgupta@uth.tmc.edu

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Regulatory Roundup

DiaDexus Receives Clearance for Test That Predicts Risk of Heart Disease

The Food and Drug Administration (FDA) has cleared a new screening test from the company diaDexus that predicts a patient's risk of future coronary heart disease events, such as heart attacks. FDA cleared the test for use in all adults with no history of heart disease, but studies submitted by diaDexus and reviewed by FDA show that the test is better at discerning this risk in women, and particularly in black women.

Known as the PLAC Test for Lp-PLA2, this new assay measures the activity of lipoprotein-associated phospholipase A2 (Lp-PLA2) in a patient's blood.

Lp-PLA2 is a biological marker for vascular inflammation, a condition associated with the buildup of plaque in arteries that supply blood to the heart. Patients with test results that show Lp-PLA2 activity greater than the level of 225 nmol/min/mL are at increased risk for a coronary heart disease event, while patients with test results below this level are at decreased risk.

The FDA requested data analyses of additional subgroups, including black women, which showed that black women experienced a higher jump in the rate of CHD events compared to other participants when Lp-PLA2 levels were higher than 225 nmol/min/mL. As a result, the test's labeling contains separate performance data for black women, black men, white women, and white men.

FDA APPROVES FIRST TEST TO CONFIRM HUMAN T-CELL LYMPHOTROPIC VIRUS-I/II ANTIBODIES

The Food and Drug Administration (FDA) approved MP Diagnostics HTLV Blot 2.4, the first FDA-licensed supplemental test for Human T-cell Lymphotropic Virus-I/II (HTLV-I/II). This test is intended for use as an additional, more specific test for human serum or plasma specimens that have previously tested positive on an FDA-licensed HTLV-I/II blood donor screening test.

The MP Diagnostics HTLV Blot 2.4 is a qualitative enzyme immunoassay test intended to confirm infection with HTLV and to differentiate between HTLV-I and HTLV-II. The MP Diagnostics HTLV Blot 2.4 provides blood establishments with additional information to convey to the donor, confirming HTLV infection and determining which virus type is causing the infection.

FDA GRANTS CLIA WAIVER FOR ALERE DETERMINE HIV-1/2 AG/AB COMBO TEST

The Food and Drug Administration (FDA) has granted CLIA waiver for the Alere Determine HIV-1/2 Ag/Ab Combo test. Until now, the test has been available for sale in the United States to health facilities and laboratories licensed to conduct tests of moderate complexity. With this approval, the test will now be available for use in physician offices, clinics, and other public health settings.

FDA approved the Determine HIV-1/2 Ag/Ab Combo in August 2013 as the first fourth-generation, rapid point-of-care test that detects both HIV-1/2 antibodies and free HIV-1 p24 antigen. Due to its capability to detect p24 antigen, which can appear in only days after infection and before the HIV antibody is detectable, fourth-generation tests may detect HIV infection earlier in the course of the disease.

FIRST CLIA WAIVER FOR SYPHILIS TEST

The U.S. Food and Drug Administration (FDA) recently granted the first-ever CLIA waiver for a rapid screening test for syphilis, the Syphilis Health Check test. The test is a qualitative rapid membrane immunochromatographic assay for the detection of *Treponema pallidum* antibodies in human whole blood, serum, or plasma.

The test is performed by obtaining a sample of whole blood from a finger stick. Results are available in as little as 12 minutes. All positive tests should be followed up with further syphilis serological laboratory testing and clinical evaluation before final diagnosis, according to FDA.

FDA first cleared the Syphilis Health Check test in 2011 and categorized it under CLIA as moderate- and high-complexity. The test is manufactured by VEDA LAB of Alencon, France for Diagnostics Direct. It is also distributed by Trinity Biotech USA.

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BY ALAN ROCKWOOD, PHD, DABCC

The FUTURE of Clinical Mass Spectrometry

How a Prized Researcher's Tool Has Gained Its Footing in Laboratory Medicine

As a mass spectrometrist who has migrated into the realm of clinical chemistry, I frequently tell my clinical chemistry colleagues that mass spectrometrists want to take over the world. Although an exaggeration, this statement is seldom challenged, probably in recognition of the many positive ways that mass spectrometry already has influenced the clinical laboratory and the practice of medicine, with the number of new applications growing rapidly. But before looking to the future of clinical mass spectrometry, it is useful first to consider briefly the historical context of mass spectrometry in the clinical lab.

Small Molecules, Big Results

In what I consider the Neolithic Age of clinical mass spectrometry, there were relatively few applications, many of them toxicology assays using gas chromatography-mass spectrometry (GC-MS). However, the field really began to flower when it entered its own Bronze Age, about a decade to 15 years ago, as the large clinical laboratories began to adopt liquid chromatography-tandem mass spectrometry (LC-MS/MS), primarily using reverse phase LC at conventional flow rates and triple quadrupole tandem mass spectrometers. The targeted analytes were almost invariably small molecules, and there were inter-related drivers for adoption. In some cases, such as the immunosuppressant sirolimus, no approved immunoassay was available, so laboratories turned to other technologies, such as LC-MS/MS.

Clinical chemists soon began developing multi-analyte panels using a single method, such as immunosuppressant panels and steroid panels. Perhaps the most notable early examples of multi-analyte methods are tandem mass spectrometry methods for acyl carnitines and amino acids for expanded newborn screening. Unlike most of the tandem mass spectrometry-based methods for the clinical lab, these expanded newborn screening applications typically use flow-injection with no chromatographic separation and are semi-quantitative or qualitative in nature.

Cost reduction also has driven adoption of mass spectrometry. The experience in our laboratory, however, is that this sometimes is an illusory goal. For example, most mass spectrometry methods require labor-intensive—and hence costly—sample preparation. Other factors include limited sample counts over which to amortize fixed expenses, and the fact that expertise for maintenance and assay development may be in short supply. These factors disproportionally affect smaller labs. However, even at a smaller lab it can be cost-effective to bring a mass spectrometry-based assay in-house to avoid send out costs.

The shift to mass spectrometry is often justifiable by factors other than cost savings, such as improved assay quality. This boost in quality is exemplified by testosterone. It became evident that traditional immunoassays were unreliable for measuring testosterone in patients with low endogenous concentrations, such as in women and children. The greater specificity of LC-MS/MS enabled specimens from this group of patients to be analyzed more reliably than before.

The Iron Age: Proteins and Peptides

The analysis of proteins and peptides is becoming important as clinical mass spectrometry enters what I consider its Iron Age. Although not the first example, thyroglobulin by LC-MS/MS is probably the most notable, and most of the major laboratories either already are or are planning to offer this testing. Similarly, the move toward protein and peptide analysis is no surprise. Mass spectrometry already is a dominant technology in the field of proteomics research, which has been fruitful in pointing the way toward mass spectrometry-based techniques and technologies that can be applied to existing protein biomarkers, such as thyroglobulin.

At the present time, LC-MS/MS using triple quadrupole mass spectrometers dominates the field of clinical mass spectrometry, and it is probably fair to say that most of the instruments in use are not necessarily at the extreme cutting edge of technology. There are good reasons for this. Although, as mass spectrometrists, we may be interested in pushing to the limits of the technology, as clinical chemists, we are responsible for developing robust methods suitable for patient care. This latter requirement can be somewhat at odds with early adoption of the latest technology, or even early adoption of the most recent generation of instruments using an established technology. Indeed, in my own lab we have experienced the pain of prematurely adopting the latest and greatest technology on more than one occasion. To do our jobs well, we must lean toward innovation but adopt new technologies at an appropriate time.

What's Next?

Many laboratorians are likely aware that bacterial identification by mass spectrometry is on an exponential growth path. While still in its infancy, we should expect both the range of applications and technologies used to expand in the future.

Another developing and intriguing technology is imaging of tissue sections by mass spectrometry. Although the time needed to acquire images of high spatial resolution may be a barrier to widespread adoption, the potentially higher degree of information content of mass spectrometry compared to traditional stain-based histology would make it an extremely powerful technique.

Functional assays also represent an exciting area. Early examples include a renin activity assay using mass spectrometric detection of angiotensin I and a kidney function assay using mass spectrometric detection of certain iodinated compounds.

Time-of-flight (TOF) mass spectrometry seems ripe for adoption, both as a single stage mass analyzer and as a quadrupole time-of-flight (Q-TOF) hybrid tandem mass spectrometer. As a single stage mass spectrometer, TOF MS has the advantage of detecting the full mass spectrum simultaneously, a significant advantage for multi-analyte analysis. Some laboratories already use TOF for multi-analyte drug screens.

Q-TOF, being a type of tandem mass spectrometer, retains the high selectivity of tandem mass spectrometry but further enhances selectivity through greater mass accuracy and resolution of the TOF-based second mass analyzer. In addition, Q-TOF produces the full product ion spectrum, enhancing information content. These advantages are offset somewhat by lower sensitivity of any given MS/MS transition. Orbitrap instruments, either as single stage mass spectrometers or as a component within a hybrid tandem mass spectrometer, share many of the features of TOF and Q-TOF instruments with even higher resolution and mass accuracy.

Ion mobility-related separations (in conjunction with mass spectrometry) are beginning to be used in clinical laboratories, providing an additional dimension of selectivity. Other trends in the integration of separation science with mass spectrometry include ultrahigh pressure chromatography, low-flow chromatography, multidimensional online chromatography, and practical capillary electrophoresis interfaces to mass spectrometers.

Looking further into the future, we can expect the trend toward increasing automation ultimately to result in the introduction of fully automated clinical analyzers using mass spectrometry-based detection, at which point mass spectrometry will become widely used by smaller clinical laboratories. In the interim, an intermediate degree of user-friendly automation might make mass spectrometry more suitable for smaller laboratories.

Another trend that so far has largely escaped the attention of clinical chemists is the development of transportable, luggable, or even handheld mass spectrometers for remote sensing. An ultimate end point of these developments could be a point-of-care clinical mass spectrometer.

Mass spectrometry also has inherent multi-analyte capabilities. This leads some researchers to consider developing the "everything analyzer" for medical applications. Aside from the daunting technical hurdles of such a project, one must also realize that this would require a paradigm shift in the way physicians order and use laboratory tests, so it may be best to think of this possibility in terms of the far future.

The field now awaits the next generations of instruments, information technology, and vendor support that will move mass spectrometry into the mainstream. This will be enabled by automation of sample preparation and data analysis, integration with existing automated platforms, Food and Drug Administration clearance/approval, commercially available reagent kits and calibrators, and seamless communication with existing electronic systems. Indeed the future for mass spectrometry in the clinical lab is a bright one, and we can expect progress toward a wider diversity of technologies employed, a wider range of applications, and a larger and more diverse user base.

In future issues of this special section of *CLN*, articles will tackle many of the topics raised above, as well as other practical aspects of mass spectrometry in the clinical laboratory.

INTRODUCING *CLN*'S FOCUS ON MASS SPECTROMETRY

Judy Stone, PhD, MT(ASCP), DABCC

With this issue, *CLN* launches a new quarterly special section on clinical mass spectrometry. The goal of the editorial board for this section is to present practical information, accessible resources, and timely perspective on the use of mass spectrometry in clinical diagnostics. Authors will include industry representatives with technical expertise, but more often our contributors will be those with invaluable production experience from laboratories reporting dozens to thousands of patient results per day using mass spectrometry. We welcome your feedback about this issue and suggestions for future topics is encouraged. **+EMAIL:** jastone@ucsd.edu

MASS SPECTROMETRY

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WITH DEBORAH FRENCH, PHD, DABCC

IMPLEMENTING Mass Spectrometry in the Clinical Lab

The first of a two-part Q&A

Q What was the level of expertise with LC-MS/MS in your laboratory before you purchased an instrument?

A When we brought liquid chromatography-tandem mass spectrometry (LC-MS/MS) testing into our lab, only I had direct experience in mass spectrometry. I had been working with LC-MS/MS for approximately 2 years and had developed an assay for 25-hydroxyvitamin D and another for opioid and opiate analysis.

In our section, we have a senior supervisor, a supervisor, two specialists, and the medical technologists. To date, we have trained the supervisor, two specialists, and one medical technologist to run the LC-MS/MS system—it is not a bench that everyone rotates on like the other benches. During training, I explained the components of the system and how they function, the maintenance schedule, and some of the unique qualities of this technology.

I emphasized that LC-MS/MS requires high-quality solvents and reagents, the necessity of adding an internal standard immediately and accurately to each sample, and some intricacies of the software—by far the biggest challenge to a new user. During training, the technologists observed me complete each procedure, and then I observed them until we were all comfortable that each person was ready to work alone. I underscored the fact that it is very difficult to break the instrument, acknowledging that it can be a very intimidating piece of equipment.

Q How did you justify your LC-MS/MS purchase?

A There were two main reasons for bringing LC-MS/MS into our lab: to improve turnaround time and to save money, as we were sending a large number of samples to reference laboratories. The most challenging part of preparing a return on investment (ROI) calculation was determining everything that was required to set up the lab. Many factors were not obvious, such as electrical work, ducts for proper venting, and reconfiguring existing cabinets. The most helpful resources in this situation were the vendors' site planning guides. Talking to the service engineers who actually install the instruments was also invaluable. I would caution others not to overlook the service contract charge covering both the LC and the MS in the cost calculations, as it is significant.

It was also challenging to estimate the cost of the reagents and consumables for each assay we planned on developing, without knowing exactly which LC method and what type of extraction we would perform. Our approach was to review the literature to identify common methods, as well as talk with applications specialists from the MS companies.

Our administration required that we obtain quotes from three vendors of comparable LC-MS/MS systems, including the service contract, as well as a written justification for choosing the instrument that we picked.

The most challenging part of preparing a return on investment calculation was determining everything that was required to set up the lab.

Q What was your process for selecting an instrument?

A First, we developed a list of questions to ask each of the four vendors under consideration, including all the technical requirements such as space, venting, electrical, as well as questions about the sensitivity for the analytes we wanted to measure—specifically at that time 25-hydroxyvitamin D, total testosterone, and estradiol. We also asked about service in our area—how quickly would they guarantee a service engineer would be on-site if we had a problem—as well as references of other clinical laboratories using their instruments with whom we could speak.

The second step was narrowing the choice from four to two vendors. We sent each vendor serum samples from pediatric patients so that they had to extract these samples and then quantify the total testosterone in them. We picked pediatric samples since they were the samples that we were going to run on the LC-MS/MS assay when we developed it in our lab.

To be honest, making the final choice between these two vendors was one of the hardest decisions I've had to make. Not much separated the two instruments as far as sensitivity was concerned, and the overall list of pros and cons for each instrument/vendor combination was different, but comparable. It took a wise friend to sit me down before I made my decision and say, "whatever instrument you pick, you'll make it work."

In the end, we purchased the instrument from the vendor with whom I had the most experience. We knew what kind of service we could expect, and that the applications specialists were very hands-on and experienced with the assays we planned to develop. Now, 4 years on I'm happy to say I do not regret my decision.

Q How did installation compare to an automated chemistry analyzer?

A In our laboratory, we had to install new electrical outlets (220 volt, 30 amp circuits) and install new ducts to vent the instrument. We also decided to buy a nitrogen generator bench and have the air compressor separate to keep the noise in the lab to a minimum. Placing the air compressor in another room required copper piping to pump the air from the air compressor to the nitrogen generator bench. We also had to get cabinets removed from the floor in order to fit the MS and the nitrogen generator bench, and from above the bench to make space for the LC system.

Importantly, these instruments are large and heavy, so we discovered that it is important to know how to get them in your lab. Is the door frame large enough? Are pallets and pallet jacks allowed in your building (they are not in ours)? If not, who is going to volunteer to lift the mass spec without dropping it? The instrument also took up more space than we had imagined. Not just the footprint, but space for carrying out sample preparation and storing calibrators and quality control materials. In addition, a multi-tube vortex, or a piece of dry-down equipment, each need space and add other requirements, such as a nitrogen supply and fume hood.

Finally, interfacing the LC-MS/MS to a laboratory information system can be costly and tedious. We are very lucky to have great information technology support, so we did not need to purchase an interface. A word of caution: the cost of interfacing should be included in an ROI calculation.

Overall, the process took far longer than I expected. From the time we started to look at systems to our LC-MS/MS installation took over a year; developing and clinically validating the first assay took nearly another year. However, 4 years after purchase, I'm pleased to report that our laboratory administration and physicians are very satisfied with our LC-MS/MS results, and we've met our goals for reducing send-out costs and turnaround time. I'll describe our experience with method development and validation in the next issue of *CLN*'s Focus on Mass Spectrometry.

BY YAN VICTORIA ZHANG, PHD, DABCC

PASSIONATE About Mass Spectrometry?

AACC Launches New Mass Spectrometry and Separation Sciences Division

The new AACC Mass Spectrometry and Separation Sciences Division (MSSS) fulfills a previously unmet need in AACC by providing a formal space for clinical professionals, industry scientists, and regulatory leaders to exchange ideas and best practices in the fields of mass spectrometry and advanced separation sciences. I am honored to be the first chair of this division, and am grateful to the many AACC members who helped us get to this stage.

The MSSS Division is committed to bringing value to its members and seeks to contribute to our medical community in several targeted ways. First, the division will serve as a leading resource for mass spectrometry, chromatography, and other advanced separations sciences for AACC members. We will also work to promote the translation of novel diagnostic methods and mass spectrometry assays into clinical and other accredited laboratories. Another critical aim is to foster collaboration among AACC divisions and other entities through joint programs and outreach. The division also plans to develop educational programs and publications that enhance understanding and highlight applications of mass spectrometry and separation sciences. Finally, the MSSS Division will enable AACC members to engage national and international laboratory organizations, diagnostics and technology manufacturers, and regulatory agencies that share our common interests.

The division has been hard at work planning a variety of activities for 2015 that include educational programs for the AACC Annual Meeting & Clinical Lab Expo, local section meetings, a stand-alone conference, and collaborations with other professional organizations. In addition to our division meeting during the annual meeting, we also plan to offer two poster awards and participate in the poster walk event.

Creation of this new division is timely considering FDA's proposed oversight of laboratory-developed tests. We plan workshops and discussion groups to follow up on these developments. We will keep members informed of our progress through emails, the division website, AACC Artery—a new online community for AACC members—and our newsletter.

I would like to take this opportunity to recognize the leaders of the division for their hard work and commitment to getting the MSSS Division off the ground. Steven Cotton, PhD, Yusheng Zhu, PhD, and Brent Dixon, PhD, exhibited remarkable dedication under an ambitious timeline, and AACC Past President Steven Wong, PhD, and AACC Division Management Chair Saeed Jortani, PhD, provided invaluable guidance and support.

I hope many AACC members will join us to grow our division. I look forward to working with friends and colleagues from academia, industry, and regulatory agencies to enhance the awareness, visibility, and applications of mass spectrometry and advanced separation sciences in clinical diagnostics. With your help, we will build the MSSS Division into a leading resource for laboratory medicine to improve patient care.

Industry Playbook

Claritas and NextCODE Health Collaborate on Diagnosis of Rare Childhood Diseases

Claritas Genomics and NextCODE Health have entered a partnership to expand the use of genomic sequencing for the diagnosis and treatment of rare childhood diseases. Affiliated with Boston Children's Hospital, Claritas is a CLIA-certified clinical laboratory that serves the DNA-based diagnostic testing needs of children's hospitals that every year admit hundreds of thousands of patients with genetic disorders. NextCODE's integrated clinical and research platform will enable Claritas to speed the delivery of genetic test results for more children and support building a seamless connection between research and clinical care. **Claritas also plans to use NextCODE's database architecture to build a centralized, cloud-based variant database that will help healthcare providers and researchers make challenging diagnoses and discover new disease genes.**

"One of the early challenges physicians and children's families face is understanding the information in genetic tests and what it means for their child's health and care," says Patrice Milos, CEO of Claritas. "NextCODE's system supports identification of known mutations, enables us to rapidly hone in on novel ones, and visualize them on screen, linking the sequencing information to our information on the child's clinical condition."

ROCHE BUYS NON-INVASIVE PRENATAL TESTING SERVICE PROVIDER

Roche has acquired Ariosa Diagnostics, a molecular diagnostics testing service provider that offers through its CLIA laboratory a non-invasive prenatal testing service using cell-free DNA (cfDNA) technology. Ariosa's proprietary Harmony Prenatal Test is a blood test that can be performed as early as the 10th week of pregnancy, and has been validated in clinical studies involving more than 22,000 women of all ages and risk categories. By evaluating cfDNA found in maternal blood, the test assesses the risk of trisomy 21, or Down syndrome, with a false positive rate of less than 0.1%, as well as trisomies 13 and 18, which can also lead to severe genetic conditions.

"Circulating cfDNA has the promise of providing early diagnostic information through a simple blood test in many important segments, including pregnancy, cancer, and transplantation, aligning with our strategy in personalized healthcare and commitment to setting new standards of care," said Roland Diggelmann, chief operating officer of Roche Diagnostics Division.

JOHNS HOPKINS LICENSES PGS TECHNOLOGY TO GOOD START GENETICS

Good Start Genetics, a commercial-stage molecular information company, has entered into an exclusive license agreement with Johns Hopkins University for the Fast Aneuploidy Screening Test-Sequencing System (FAST-SeqS) invented by Bert Vogelstein, MD, Ken Kinzler, PhD, Nickolas Papadopoulos, PhD, and MD-PhD candidate Isaac Kinde of Johns Hopkins. FAST-SeqS is a preimplantation genetic screening (PGS) method that enables embryos to be screened for chromosomal abnormalities prior to implantation in an in vitro fertilization setting. This increases the potential of transferring an embryo with the correct number of chromosomes and potentially increases pregnancy rates. Specifically, FAST-SeqS counts the number of chromosomes in an embryo by using a single primer pair to select and amplify distinct sections of the genome that occur on every chromosome. Existing PGS methods also assess chromosome copy number, but are costly and often require lengthy turnaround times for results. This new approach

Chris Clor/Glow Images

might help reduce costs associated with PGS and make this testing more accessible to a wider range of patients, and it might also have applications beyond aneuploidy screening.

THERMO FISHER, SAMSUNG TO DEVELOP NEW POINT-OF-CARE TESTS

Thermo Fisher Scientific and Samsung Electronics are collaborating to answer the healthcare industry's demand for better point-of-care (POC) diagnostics that facilitate more rapid diagnosis and treatment. Together, the two companies will design, develop, and market new POC solutions for a broad range of uses, including the detection of sepsis, drugs of abuse, and therapeutic drug monitoring, as well as the detection of cardiac problems and women's health conditions.

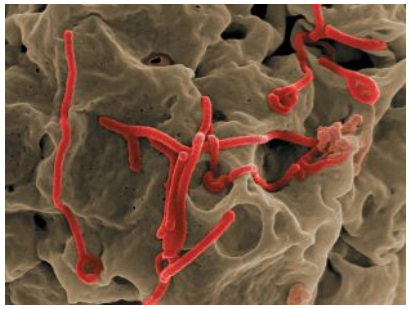
"Samsung has developed a compelling and innovative suite of point-of-care platforms," said Marc Tremblay, PhD, president of Thermo Fisher's clinical diagnostics business. "We look forward to working with Samsung to add some of our leading biomarkers and assays to their platforms to create a truly differentiated testing menu."

AGILENT, BAYLOR COLLEGE OF MEDICINE OPEN NEW MASS SPECTROMETRY RESEARCH CENTER

Agilent Technologies and Baylor College of Medicine in Houston have joined forces to advance research and training in the field of metabolomics by opening the Agilent Technologies Mass Spectrometry Center of Excellence as part of the Alkek Center for Molecular Discovery and the Baylor College of Medicine Core Laboratory in the college's department of molecular and cellular biology. Agilent is equipping the new center with two systems configured for metabolomics: an Agilent 6495 triple quadrupole liquid chromatography/mass spectrometry (LC/MS) system and an Agilent 6550 iFunnel quadrupole time-of-flight LC/MS system with a switchable GC APCI interface. Baylor and Agilent will use the equipment collaboratively to analyze samples, conduct research, and train students.

"The growing significance of metabolomics, not only in life science research but in many application spaces, has resulted in the need for more analytical capabilities," said Arun Sreekumar, PhD, co-director of the Alkek Center. "Baylor and Agilent have identified several areas of mutual interest—metabolomics, lipidomics, clinical research, disease research—where we believe we can make real progress together."

IDT, UBIQUITOME COLLABORATE ON RAPID EBOLA FIELD TEST



Courtesy of NIAID

Integrated DNA Technologies (IDT) and Ubiquitome have entered a partnership to develop a rapid test that can diagnose Ebola in the field. Named the Ubiquitome Freedom4 Real-Time RT-PCR Ebola Virus assay, this test is designed for Ubiquitome's handheld real-time PCR device, the Freedom4. This platform can run on battery power alone for up to 6 hours, is housed in durable aluminum casing, and includes laser-based optical detection. IDT will also leverage its PrimeTime qPCR assay platform to develop this rapid Ebola test.

"The Ubiquitome Freedom4 Real-Time RT-PCR Ebola Virus assay will allow rapid, accurate field testing of Ebola virus disease," said Paul Pickering, CEO of Ubiquitome. "This is important because regions affected by this disease are often far from an established laboratory."

The research and development organization Battelle will conduct validation of the Ubiquitome Freedom4 Real-Time RT-PCR Ebola Virus assay.

ATRECA, JANSSEN BIOTECH TO INVESTIGATE AUTOIMMUNE DISORDERS

Atreca has teamed with Janssen Biotech to apply Atreca's Immune Repertoire Capture technology to autoimmune disease. The goal of the collaboration, facilitated by the Johnson & Johnson Innovation Center in California, is to detail the molecular mechanisms underlying autoimmune diseases and define patient subgroups with distinct disease biology to inform better treatment. Immune Repertoire Capture technology employs proprietary single-cell analysis to deliver full-length, natively paired antibody and T-cell receptor repertoires along with the levels of co-expressed genes that reveal cell subtype and phenotype. These data reveal the activity of the immune system and enable identification of the molecular targets of an immune response. Applied to human disease, Immune Repertoire Capture has the potential to serve as an engine for the discovery and development of novel therapeutics, vaccines, and diagnostics.

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Ask The Expert

Do We Need Procalcitonin for Sepsis?

EXPERT
Sutirtha Chakraborty, MBBS, MD, FACB

Why should procalcitonin testing be a part of the sepsis management protocol?

A: Globally, sepsis and its complications are a major cause of acute illness and death. The American College of Chest Physicians and Society of Critical Care Medicine defined sepsis as systemic inflammatory response caused by infection. However, the major challenge remains, how can we prove there is an infection? Culture best identifies it, but only in about 30% of patients with sepsis. False positivity of cultures further complicates the situation. Clinical signs of sepsis—including fever, tachycardia, and leucocytosis—are non-specific and overlap with signs of systemic inflammatory response syndromes (SIRS) of non-infectious origin, making detection of sepsis a clinical challenge. As a result, delay in diagnosis and treatment of sepsis is responsible for increased mortality.

In order to prove the presence of bacterial infection, serum biomarkers like procalcitonin (PCT) are considered useful. Biochemically, PCT is the prohormone of the hormone calcitonin, released into the circulation in response to bacterial infection. PCT is the best-studied sepsis biomarker for clinical use. Among all sepsis markers, only PCT has achieved universal use throughout developed countries in the last decade.

One major advantage of PCT compared with other biomarkers is its early and rapid increase in response to bacterial infections and sepsis. High PCT concentrations are commonly found in bacterial infection, in contrast to much lower levels in viral infection. However, even though PCT is virtually undetectable (less than 0.1 ng/mL) in healthy individuals, elevated serum PCT concentrations are not always specific for sepsis. Many studies have linked elevated PCT to SIRS, localized bacterial infection, autoimmune disease, burns, severe trauma, surgery, pancreatitis, as well as viral, parasitic, and fungal infections.

Despite these challenges, PCT has some other obvious clinical advantages: improved accuracy of early clinical sepsis diagnosis, utility for assessing effectiveness of sepsis treatment, and a role in antibiotic stewardship. For respiratory tract infection in intensive care unit patients who have sepsis and post-operative infections, randomized-controlled studies have shown the efficacy of using PCT algorithms to guide antibiotic decisions. PCT-guided antibiotic therapy leads to significant reduction in the length of antibiotic therapy. However, serial PCT measurements are needed in order to judiciously use PCT in assessing therapeutic effectiveness and antibiotic stewardship. Surviving Sepsis Campaign: International Guidelines (2012) suggests that PCT measurements can be used for sepsis diagnosis and to discontinue antibiotic therapy in patients who initially seem septic, but have no subsequent evidence of infection.

PCT has a very high negative-predictive value as a marker of bacterial infection, making it useful to rule out sepsis in emergency department and critical care settings. Nevertheless, falsely low PCT levels can be seen during the early course or localized state of an infection. As such, one critical area for further research is highly sensitive PCT assays that allow monitoring of subtle changes of PCT at very low concentrations. This will increase the sensitivity of the test and thus the safety of patients. PCT has the ability to be the troponin of bacterial sepsis provided such highly sensitive assays can be developed.

Like any biomarker, PCT is not perfect and has some significant limitations. It is an expensive test to run, significantly more than C-reactive protein, blood counts, and other assays. It is not, therefore, the single definitive test for sepsis diagnosis, but rather must be interpreted in context of medical history, physical examination, microbiological assessment, and other relevant laboratory parameters. Nevertheless, PCT use has the evidence base of several high quality large clinical trials making it one of the strongest contenders of the sepsis biomarker arena.

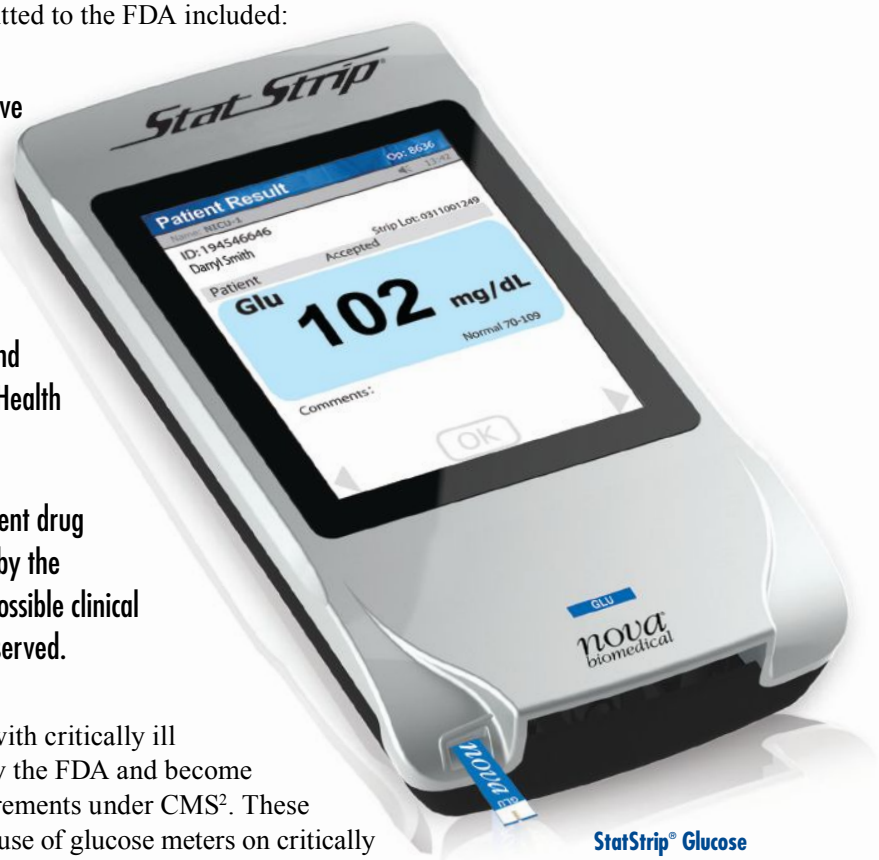
Sutirtha Chakraborty, MBBS, MD, FACB, is the chief consultant in the Department of Clinical Biochemistry at Peerless Hospital & B K Roy Research Center, Kolkata, India.
+EMAIL: sutirtha@peerlesshospital.com

The only glucose meter cleared by the U.S. FDA for use with critically ill patients

In the last several years an unacceptably high number of adverse patient events and more than 16 deaths[1] have been traced to the use of glucose meters in hospitals in the U.S. The FDA has just announced that it now requires hospital meters to be designed for and tested on critically ill patients in order to be cleared for use in these patient populations. To date, only one meter, the Nova StatStrip Glucose Hospital Meter System has been found to be accurate enough to obtain this new FDA clearance.

StatStrip Glucose has been designed specifically to be free of clinical interferences that can be present in critically ill patients. The proof data submitted to the FDA included:

- 1,698 individual critical care patients from five university medical centers had StatStrip Glucose results paired with an IDMS traceable laboratory glucose reference method.
- Data from multiple intensive care settings representing 19 medical condition categories and 257 subcategories as designated by the World Health Organization were included.
- Over 8,000 medications representing 33 parent drug classes and 134 drug subclasses as designated by the United States Pharmacopeia were studied for possible clinical interferences; no clinical interferences were observed.



StatStrip® Glucose Hospital Meter System

All other glucose meters currently in use with critically ill patients are now classified as "off-label" by the FDA and become subject to "high complexity testing" requirements under CMS[2]. These requirements are so stringent that off label use of glucose meters on critically ill patients is not a practical alternative. Testing would not be performed by nurses, only by individuals degreed in laboratory medical technology.

1. DIABETES CARE, VOLUME 33, Number 4, April 2010.
2. Centers for Medicare & Medicaid Services.

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