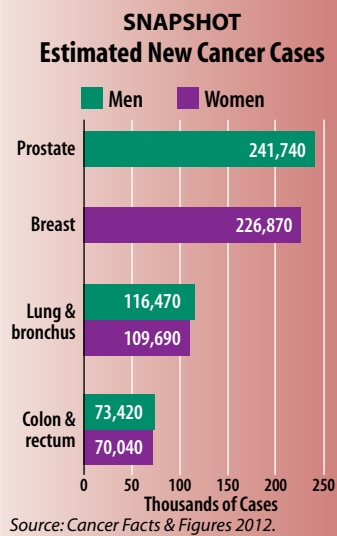


CANCER DEATH RATES CONTINUE TO DROP

A new report released by the American Cancer Society (ACS) found that since 1999 cancer death rates in the U.S. have continued to decline in both men and women, as well as among all racial and ethnic groups, with the exception of American Indians and Alaskan Natives.

From 1990 through 2008, the most recent year for which data are available, overall cancer death rates decreased by about 23% in men and 15% in women. That means that more than 1 million deaths from cancer were avoided.

The organization's latest data paint a positive picture, revealing that death rates continue to decline for lung, colon, breast and prostate cancer, which are responsible for the most cancer deaths. Over the past decade, however, the figures show a slight increase in people developing some less common cancers, including pancreas, liver, thyroid, and kidney cancers. The report



sites the increasing prevalence of obesity as a possible reason for the spike. In addition, oropharyngeal cancers, which are linked to human papilloma-virus infection, are on the rise.

Lung cancer is responsible for the largest drop in death rates for men, down 40%, while breast cancer deaths have declined by 34% for women.

African American and Latino men saw the most dramatic drop in death rates, with respective declines of 2.4% and 2.3% annually. However, African American men still have a 15% higher cancer incidence and 33% higher death rate compared with Caucasian men. African American women have a 6% lower cancer incidence rate compared with Caucasian women but have a 16% higher death rate.

Even with this good news, ACS predicts a total of 1,638,910 new cancer cases and 577,190 deaths from cancer will occur in the U.S. this year.

The full report is available from CA: A Cancer Journal for Clinicians doi:10.3322/caac.20138.

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The Quest for Pediatric Reference Ranges

Why the National Children's Study Promises Answers

BY BILL MALONE

When Congress authorized the National Children's Health Study (NCS) in 2000, laboratorians immediately saw the potential for pediatric lab medicine. A longitudinal study that will follow 100,000 children across the country from before birth to age 21, NCS will collect the rarest of samples: blood from a diverse, healthy population of young children. A growing collection of such samples seemed like the ideal starting point for work on pediatric reference ranges, long a cause for concern among pediatricians and laboratorians because of the dearth of quality data, especially for children under 3 years of age.

Now, AACC is taking the lead for the lab community by funding the first pediatric reference range studies on the early samples available from NCS subjects. After working with NCS in an advisory role for 6 years, members of the AACC Pediatric Reference Range Committee (PRRC) are beginning two pilot studies that will establish age-related reference ranges for steroid hormones and amino acids.

Ultimately, the committee aims to draw in other stakeholders to continue these studies and take full advantage of what the NCS has to offer, according to chair Michael Bennett, PhD, FRCPath. "I've been a



See **Reference Ranges**, continued on page 3

New Paradigms for Hepatitis C Virus Treatment

Will HCV Nucleic Acid Testing be a Must for All Clinical Labs?

BY GENNA ROLLINS

Blockbuster drugs that singularly transform management of a disease are rare, but two new protease inhibitors designed to treat hepatitis C virus (HCV) have the potential to do just that. The medications, boceprevir and telaprevir, both approved by the Food and Drug Administration (FDA) in May 2011, are the first direct acting agents for treatment of HCV to receive a nod from the agency. In clinical trials leading to their approval, both demonstrated sustained virologic response rates of 65–70% in the most common but difficult-to-treat HCV genotype 1, which compares with 40–45% in established therapies. Experts say these landmark drugs are merely the opening salvo in a new offensive against HCV, one that could radically change treatment of the disease, as well as the type and volume of lab tests needed.

"These new drugs absolutely changed the landscape. In the past, the gold standard was dual peginterferon-ribavirin therapy, but the response rates were less-than-stellar and the side-effects somewhat difficult. When you combine that with a significant duration of therapy, it hasn't been a perfect environment for treatment, although that was all we had," observed Rick Pesano, MD, PhD, medical director of infectious diseases at Quest Diagnostics. "Now, with the direct acting

See **HCV Treatment**, continued on page 5

IN THIS ISSUE

3 AACC Welcomes
EVP Vukovljak

8 Lab 2012
Toxic Alcohol
Poisonings

11 Guidelines: Glucose
Testing for All
Hospitalized Patients

12 Regulatory Profiles

13 Industry Profiles

14 Diagnostic Profiles

15 News from the FDA

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NCS Is Boon for Pediatric Lab Med

Reference Ranges, continued from page 1

pediatric clinical chemist all of my career, and throughout that time, we've always struggled with reference ranges," he said. "One of the problems that we've always had in children's hospitals is that you don't have healthy children in the hospital, so you can't comfortably use that data. With a large cohort of healthy children from across the country, the National Children's Study offers laboratorians a unique opportunity to develop good reference ranges and also to understand better how the biomarkers that we measure in our labs reflect normal childhood development." Bennett is director of the Michael J. Palmieri Metabolic Laboratory at Children's Hospital of Philadelphia and professor of pathology and laboratory medicine at the University of Pennsylvania Perelman School of Medicine.

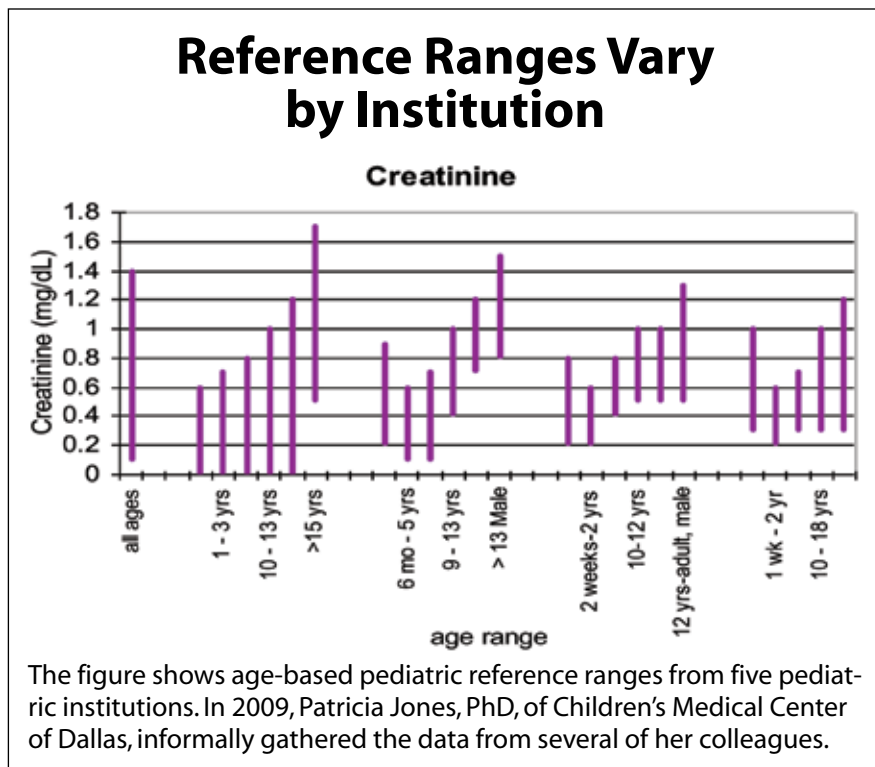
A New Chance to Tackle an Old Problem

Although published data exists for pediatric reference ranges, so far no study has proven complete enough or robust enough to establish widely recognized standards for lab medicine, noted Patricia Jones, PhD, the primary investigator for the AACC-funded studies. "Pediatric reference ranges are really a wide open area. Even institutions using the same instruments have different reference ranges. It's a subject that we all recognize needs a lot of work." Jones is clinical director of the chemistry and metabolic disease labs at Children's Medical Center of Dallas and professor of pathology and medical laboratory science at UT Southwestern Medical Center. When she surveyed her colleagues in other pediatric institutions on reference ranges for common analytes, like electrolytes and glucose,

she discovered that the ranges varied from lab to lab (See Box, right). This weakness of reference ranges poses real consequences for patients, making interpretation difficult for some tests and potentially leading to inappropriate treatment decisions or misdiagnoses, she added.

Other researchers, both in the U.S. and internationally, are addressing pediatric reference ranges, but so far cannot match the size and scope of NCS (CLN 2009;35(9)). For example, Children's Health Improvement through Laboratory Diagnostics (CHILDx), sponsored by ARUP Laboratories and the University of Utah Department of Pathology, has recruited thousands of subjects to establish reference ranges for more than 100 analytes in children 7 to 17 and 45 analytes in children age 6 months to 7 years. However, all of the subjects are local to Utah and primarily Caucasian, limiting how CHILDx findings can be applied elsewhere.

The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has ambitious goals as well. The project aims to establish a comprehensive database of reference ranges for Canadian children from birth to 18 years. However, the great cost and difficulty of obtaining samples from young, healthy children has slowed progress, said Vijaylaxmi Grey, PhD, a CALIPER co-investigator and past member of the PRRC. "In CALIPER, we are collecting our own samples, which is a huge endeavor," she said. "While we have completed some pilot studies, funding has been a major issue in getting it off the ground." Only recently did CALIPER receive funding for 4 years from the Canadian Institutes of Health Research. Grey is director of



pediatric clinical biochemistry in the Hamilton Regional Laboratory Program and a professor of pathology and molecular medicine at McMaster University in Hamilton, Ontario.

Grey pointed out that the most difficult samples to collect are those of neonates—the age group that now makes up the bulk of available samples from the NCS. "This is one of the major advantages of the NCS," she said. "It is still a challenge for us in CALIPER because it's such a difficult group to gain access to," (See Box, p. 4).

With all the difficulties of sample collection already taken care of, and the potential for a diverse cohort spanning the nation, NCS looked like the perfect opening for the lab community to make progress on pediatric reference ranges, Bennett said. Initially, the committee focused on helping NCS collect high-quality samples, as well as recommendations for which analytes should be routinely analyzed as part of the main study protocol. With the study planning to follow subjects for 21 years, the committee advised on storage conditions of samples and traceability of assays to keep up with new technologies of the future. "Traceability is a really important issue because when we adopt methods in the future, if the numbers are different it could certainly change interpretation of the outcomes data

and compromise the study," Bennett said.

Grey concurred and noted that traceability is also a major priority for CALIPER as well. "Field methods certainly must be traceable to some sort of gold standard methodology that defines the unit of measurement," she said.

A major success for the PRRC was convincing NCS to collect dried blood spots in addition to serum, Bennett emphasized. "This is very significant because, with the technologies that we're seeing, this is a medium for measuring a large number of potentially useful biomarkers," Bennett said. "We recommended they do this across the board not only because these samples store more easily, but as technology improves, even though the samples are tiny, it's possible to get quite a lot of information out of them."

Getting Started

After spending several years helping ensure that NCS would collect samples suitable for the kinds of research needed in pediatric reference ranges, in 2011 the PRRC decided it was time to take the next step and launch two supplemental methodological studies. They hope to establish a base of knowledge from working closely with NCS that can be a jumping-off point for future efforts,

See **Reference Ranges**, continued on page 4

AACC Welcomes New EVP Lana Vukovljak



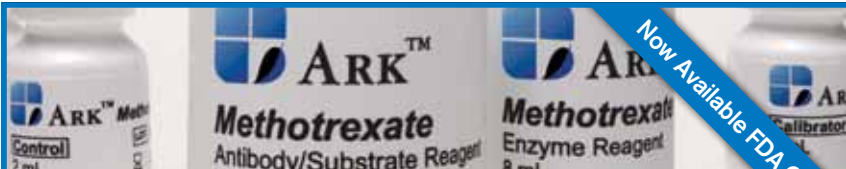
Lana Vukovljak will assume the leadership of AACC at the end of this month, becoming the executive vice president of the association. She succeeds Richard Flaherty, who retired at the end of December after 20 years of distinguished service to the organization.

"The AACC leadership is looking forward to the next phase in our association's life. The organization is very strong right now, but we need to grow and change to stay that way," said Greg Miller, AACC President. "We feel that with Ms. Vukovljak's skills, she can help us meet

ever changing challenges in laboratory medicine. We view this as a very exciting time for the AACC."

Ms. Vukovljak comes to AACC from the American Association of Diabetes Educators (AADE) where she served as chief executive officer. During her tenure with AADE, Vukovljak guided the board of directors through a revision in their governance model, improved business intelligence, increased the value of membership through strategic initiatives, and developed diversified products and services to meet members' needs. She also increased recognition and collaboration with federal and other non-profit or for-profit organizations that resulted in changes to program accreditation and reimbursement for diabetes education services by the Centers for Medicare and Medicaid Services. Prior to joining AADE, she held senior leadership positions in several organizations in the areas of adult education and continuing education.

"I am very excited about joining AACC," said Vukovljak. "I look forward to working with its volunteers and staff in leading the organization as it explores opportunities for expansion and sustainable growth."



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Pilot Studies Lay Groundwork for Future

Reference Ranges, continued from page 3

according to Bennett. “Basically we designed these studies as a proof of principle. We wanted to develop in-depth knowledge about how the samples and data would be accessed,” he said. “Being a federally funded study, NCS has very strict rules for accessing the samples. We also chose biomarkers that we knew going forward would help impact the understanding of normal childhood development.”

Jones’s study focuses on reference ranges for four steroid hormones: 17-hydroxy-progesterone, androstenedione, testosterone, and aldosterone, using liquid chromatography tandem mass spectrometry (LC-MS/MS). While more will be available in the future, Jones ended up with approximately 300 samples, which NCS indicated was the most they could afford to share due to the very early nature of the NCS program. Jones selected 17-hydroxy progesterone and androstenedione because, among other reasons, the tests are frequently ordered for children with congenital adrenal hyperplasia. “We have a pretty high volume of these test that come through our lab, so we had already brought them in house for a tandem mass spectrometer,” Jones said. “This is also one of those areas where reference ranges, particularly in the first months of life, are very, very important, yet we just don’t have a lot of information from normal children because it’s so difficult to get samples from newborns.” As the primary investigator, Jones is also responsible for securing the data associated with the NCS samples, a major undertaking considering the stringent requirements of a federally funded study on children.

Jones is sharing the samples with Dennis Dietzen, PhD, who will develop reference ranges for 35 amino acids. Dietzen is medical director of the core laboratory at St. Louis Children’s Hospital and an associate professor of pediatrics at the Washington University School of Medicine in St. Louis. “Amino acids are flags for many different things,” Dietzen said. “From dietary intake to energy utilization, there is a wealth of information in the pattern of amino acids,” Dietzen will also use LC-MS/MS, employing a technique developed in his lab that can analyze dozens of amino acids quantitatively in a 20-minute run. Prior techniques took 2 to 3 hours. Using LC-MS/MS was a strategic choice that ties into why the PRRC advised NCS to collect dried blood spots in the first place, Jones said. “Obviously, technology will likely change considerably in the next 20 years of the National Children’s Study, and the nice thing about tandem mass spectrometry is that if you’re starting with good, traceable standards, then the results of your assay should still make sense in 10 or 20 years,” she said.

However, due to the fact that most labs use serum for these analytes, both studies will also have to examine results in comparison to other methods. “In the process of developing the assay for dried blood spots, we will develop it against a serum assay and determine how we can relate results back,” Jones said.

The seed money provided by AACC will enable both studies to be complete within

about a year. After that, the PRRC will encourage others in the lab community to pick up where they left off and keep these and other projects going as NCS moves forward. “A clear advantage for the NCS cohort is that we can measure the biological variation occurring within the same group of children over time,” Dietzen said. “So, for example, if we track amino acids from birth all the way through age 21, we will also learn an enormous amount about normal developmental changes that happen in those amino acid profiles.”

Looking for Support

With these initial studies underway, the PRRC is also ramping up efforts to build awareness and spark new collaborations with other stakeholders. For example, in vitro diagnostics manufacturers could either request NCS samples to produce pediatric reference ranges when developing new assays, or provide funding to a third party that would perform the studies. Several companies could also band together and donate reagents to a researcher to generate the data. In either case, the PRRC stands ready to offer help based on its experience

with the current pilot studies.

So far, the feedback from companies has been positive, Dietzen said. “Those diagnostic manufacturers that we’ve talked to really believe that providing a complete interpretive picture for their assay is the right thing to do,” he said. “This is just good business practice for a manufacturer. Even though pediatric samples constitute a relatively small fraction of the diagnostic market, the ability to provide accurate pediatric reference ranges can appeal to a company based on the fact that they want labs to use their equipment and reagents appropriately in every segment of the population.”

The PRRC also wants to work with commercial labs that perform testing for those analytes that are already a part of NCS research. In order to mine this data to develop reference ranges, these labs would likely need to develop separate study proposals with NCS for the new research.

As a knowledge broker and coordinator, the members of the PRRC are eager to hear from those that would like learn more about working with NCS, Jones said. “My hope is to help facilitate other people doing this type of study. It’s an area that needs so much work, and I’m confident that there are other people out there who want to be a part of it.”

CLN

Establishing Pediatric Reference Ranges

Absent a comprehensive database of data from healthy children such as the National Children’s Study plans to generate, labs have had to make do with limited numbers of samples from hospitalized children.

Although standards for developing pediatric reference ranges have been published by Clinical and Laboratory Standards Institute (CLSI) and other sources, they require samples from healthy children, said Patricia Jones, PhD. “One of the biggest problems with pediatric reference ranges is that according to the method for setting reference ranges, you have to use 120 samples at each age from healthy individuals, and that’s impossible for a pediatric institution,” she said. “As a result, labs have to set their reference ranges the best way they can, and that doesn’t always involve healthy children. Should you turn around and use those reference ranges on healthy children? That’s anyone’s guess and there is a lot of debate about it.” Jones is clinical director of the chemistry and metabolic disease labs at Children’s Medical Center of Dallas and professor of pathology and medical laboratory science at UT Southwestern Medical Center.

One method many labs use is to take an entire hospitalized population and remove outliers. However, despite the availability of published statistical methods to make this work, data from sick children is still not the same as that from healthy children, commented Vijaylaxmi Grey, PhD. “I think there is a limitation to using hospitalized data,” she said. “If you look at the use of banked data for adults, much more wellness testing is done. In children, however, testing is more judicious—you only test when they’re ill, so the percentage of normal values in a children’s hospital is much less. I don’t think it should be ruled out as an approach, but I don’t think it’s going to be the same as what we’ve been able to do for adults. A reference range is by definition from a healthy, normal person.” Grey is director of pediatric clinical biochemistry in the Hamilton Regional Laboratory Program and professor of pathology and molecular medicine at McMaster University in Hamilton, Ontario.

Importantly, there is a big difference between establishing reference ranges—for which experts recommend the 120 samples from healthy individuals—and verifying published reference ranges for use at a particular institution, Jones noted. For the latter purpose, the lab can use as few as 20 samples, according to the third edition of the CLSI guideline “Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline (CLSI C28-A3).”

Regimens Challenge Clinicians, Patients

HCV Treatment, continued from page 1

agents, we've not only significantly upped the cure rate, but also shortened the duration of therapy. These drugs have absolutely changed the topography of therapy, and it's not over. They're the wave of the future."

An Imperfect Standard of Care

The standard of care for HCV treatment since the early 1990s has been interferon, aimed at boosting the immune system rather than attacking the virus outright. By the late 1990s, ribavirin was added to the mix, increasing sustained virologic response rates and decreasing relapse rates. A few years later, long-acting pegylated forms of interferon came along, but the peginterferon/ribavirin combination maxed out at about a 45% overall sustained response rate.

Yet the need to find better treatments is great. HCV accounts for about 45% of all liver disease and is the leading cause of cirrhosis, liver failure, and liver transplantation. An estimated 3.9 million people—more than 1% of the U.S. population—carry the virus, but perhaps as many as 80% are unaware of it. The virus simmers in the background for years, eventually causing significant liver damage. Even so, most patients either opt not to pursue therapy at all, or drop-out after starting due to its rigor: weekly injections for nearly a year and a plethora of some annoying, and other potentially serious, side-effects.

Strict New Regimens

Enter boceprevir and telaprevir. Both compounds inhibit the HCV NS3/4A serine protease, effectively preventing cleavage of the HCV polyprotein chain and curtailing replication of the virus. The drugs were approved for treatment-naïve patients, or those with unsuccessful prior treatment, including failed or relapsed responses. In Phase 3 clinical trials, sustained virologic response rates were as high as 96% for certain subjects on boceprevir, and 89% for telaprevir, with overall response rates of 70–75%.

Based on these findings, FDA approved both drugs to be taken in combination with peginterferon/ribavirin, according to strict drug dosage and viral load testing schedules under a response-guided approach to therapy that determines duration of treatment (See Table, p.7). Findings from clinical trials suggest that about half of patients will need only 28 weeks of therapy, whereas others whose previous treatment failed or who have slower virologic responses will stay on regimen for a full 48 weeks. The bottom line with either drug is that monitoring virologic response via nucleic acid testing is essential.

"HCV rapidly mutates and virtually all our patients will develop resistance to the current protease inhibitors, if those protease inhibitors are used as monotherapy. This is why we use them in combination with peginterferon/ribavirin. The protease inhibitor rapidly brings down HCV RNA and then the effects of peginterferon/ribavirin eradicate the virus in a high percentage of patients, leading to sustained virologic response and cure," said Mitchell Shiffman, MD, medical director of the Liver Institute of Virginia at Bon Secours

Virginia Health System in Newport News. "Breakthrough primarily occurs due to resistance to interferon. If the interferon isn't working and can't eradicate HCV, resistance to the protease inhibitor can emerge. So measuring HCV RNA during treatment is critical." Shiffman recently participated in an AACC webinar involving current issues in hepatitis testing.

Boceprevir requires a 4-week lead-in with dual peginterferon/ribavirin therapy, followed by 24 weeks of triple boceprevir/peginterferon/ribavirin treatment. The package insert and American Association for the Study of Liver Diseases (AASLD) practice guidelines recommend HCV RNA viral load testing at weeks 8 and 24. Patients who have not been treated before and

who have undetectable HCV RNA levels at these two junctures may be considered for a shortened 28-week course of therapy. However, those who either had treatment before or detectable viral levels ≥ 100 IU/mL at week 8 but undetectable levels at week 24 would need a full 36 weeks of triple therapy, followed by combination peginterferon/ribavirin for 12 weeks, for a total 48 weeks' treatment.

In contrast, patients taking telaprevir do not have a 4-week lead-in with peginterferon/ribavirin. Instead, they immediately start 12 weeks of triple telaprevir/peginterferon/ribavirin therapy, with HCV-RNA viral testing at weeks 4 and 12, followed by at least 24 weeks of dual peginterferon/ribavirin therapy, for a total of 28 weeks' treatment. Those with undetectable viral loads at both weeks 4 and 12 would be eligible for the shortened 28-week regimen. How-

ever, those with detectable levels $< 1,000$ IU/mL at both time points would stay on dual therapy for an additional 36 weeks, for a total 48 weeks' therapy. Patients treated previously with only partial or no response also would stay on dual therapy for an additional 36 weeks, for 48 weeks in total.

With either drug, therapy must be stopped anytime viral loads exceed recommended thresholds, > 100 IU/mL in the case of boceprevir and $> 1,000$ IU/mL for telaprevir. "We have to be vigilant about adhering to these rules because the continuation of a failing regimen has negative implications for the selection of further resistance and consequences that may be unfavorable for future courses of therapy," noted Raymond Chung, MD, chief of hepatology and vice chief of gastroenterology at Massachusetts General Hospital in Boston.

See **HCV Treatment**, continued on page 6

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Change Coming to HCV Testing

HCV Treatment, continued from page 5

Implementing HCV Nucleic Acid Testing

Given the importance of nucleic acid testing in assessing the success of the new regimens and both providers' and patients' renewed interest in pursuing HCV treatment, labs that have not yet invested in HCV RNA PCR assays might consider doing so, according to Chong-Gee Teo, MD, PhD, chief of the Centers for Disease Control and Prevention's viral hepatitis lab. "With the promise of faster and more accepted therapy with the protease inhibitors, there's a drive to go beyond antibody-based screening of HCV to identify patients who are actively infected," he explained. "Most labs right now test for antibodies to HCV and will stop there unless a physician requests confirmation that a patient's actively infected. So labs might now expect more demand for HCV RNA testing." Teo also pointed out that since nucleic acid testing requires specimen preparation and testing platforms different than those used in conventional antibody testing, labs that pursue the technology will need special expertise, equipment, and reagents to support the move.

Experts urged labs that either already offer or expect to implement HCV nucleic acid testing to review the telaprevir and

boceprevir package inserts to ensure that their technologies are sufficiently robust. "The requirements are very similar with some minor differences. The assay should have a lower limit of HCV RNA quantitation ≤ 25 IU/mL. But it also has to have a limit of HCV RNA detection of 10–15 IU/mL. That's a change," observed Pesano. "In the past, we've had some technologies for detecting HCV RNA that were not that sensitive. But the new required levels of quantitation and detection are needed in order for physicians to be comfortable taking patients off therapy and making sure they've succeeded in care. The lab technologies have moved in combination with those requirements."

Shiffman recommended that labs "use a very good, reliable PCR assay. The ones that are FDA-approved will reliably measure down to a level of 40 or 60 IU/mL, which is sufficient for the 100 IU/mL cutoff utilized for boceprevir treatment and clearly lower than the 1,000 IU/mL for telaprevir. What is unacceptable is to measure HCV RNA levels with a cutoff level of 650 IU/mL or higher in our current treatment paradigms."

Pesano also advised laboratorians to be prepared to discuss virologic response in the context of HCV RNA testing. "A ques-

tion I get on a regular basis is around clinicians' understanding of when patients' viral loads are adequately low for the physicians to be confident in moving to the next stage in therapy. Many people are learning the thresholds now, and depending on the test that's used, it can be confusing sometimes to clinicians between limit of detection and limit of quantitation. But these data points are imperative in monitoring HCV patients appropriately."

The Importance of Timely Turnaround

Hepatologists emphasized the importance of providing timely HCV RNA results so clinicians can adjust treatment regimens as necessary. "Our greatest need for the future is rapid virologic testing. Having these tests sent out with seven-to-14 day turnaround times challenges our ability to make decisions around therapy," said David Nelson, professor of medicine and associate dean for clinical research in the division of gastroenterology, hepatology, and nutrition at the University of Florida College of Medicine in Gainesville. "We're looking for resistance, and if you see a rapid increase in virus, you know that's resistance." AASLD guidelines define virologic breakthrough as >1 log increase in serum HCV RNA above nadir.

Echoing this sentiment, Chung relayed lessons he learned about the need for fast turnaround times. "We ran into a very practical issue, which was that the third-party payer wanted to see evidence that we were not violating the week four stop rule for telaprevir, meaning they needed to know that the HCV RNA level was below 1,000 IU/mL before approving a prescription renewal. So in some cases we were sweating it out to make sure they got that information. It's a multi-step process, and if not done in a timely way, the patient runs the risk of missing renewal of their medications, and jeopardizing the success of therapy. That's a highly practical consequence of slow turnaround times."

Those circumstances aside, Chung indicated that a few days' processing time is about the norm for most labs. "This is not a test that's run 18 hours per day every day. It's a batch decision on the part of molecular diagnostic labs as to when they have enough for the next run. Depending on the volume, that may be two or three times a week. Our turnaround time has been two or three days, and for most purposes, that's been acceptable."

Still, Nelson is pushing the envelope for speedier turnaround times. "In the future I'd like to have it so that a patient gives a blood sample and within half-an-hour I'd have a good idea of what their viral response has been and what their CBC is. Then I can be very efficient and appropriately change the course of therapy as needed without having to wait three days to a week. If we can get to the technology to point-of-service efficiency, I think we'll really improve care in this arena."

IL28B Testing: A Flash in the Pan?

Availability of boceprevir, telaprevir, and other protease and polymerase inhibitors in the pipeline is not only changing demand for HCV RNA testing but also other molecular diagnostic tests. The landmark discovery in 2009 that *IL28B* modulated interferon response prompted an immediate interest in testing for haplotypes of this

gene, an interest that will soon fade, said experts. "It's becoming less important in clinical algorithms or clinical decision-making with introduction of the new direct acting agents. With the next wave of treatments that are on the horizon, *IL28B* status is probably not going to be a factor at all in determining response," predicted Nelson.

By the same token, at least for now hepatologists report being more interested than ever before in HCV subtyping. "There's a consistent efficacy difference between genotype IA and IB with the new direct acting agents. Depending on the regimen used, IB appears to experience higher sustained response rates in comparison to IA. As such, knowledge of the subtype will factor into, at least in the early going, our prognostication about future sustained response rates," observed Chung. "As we get better with pan-genotypic coverage, those distinctions will start to dissipate. We're in a unique period right now when, with the current generation of protease inhibitors, we're seeing this discordance between IA and IB. It's not dramatic, but it's noteworthy." In Nelson's view, the difference between IA-IB response rates is substantial enough that he suggested labs unable to offer robust subtype analysis will be left in the dust.

Other Treatment Challenges

Aside from molecular diagnostic considerations, hepatologists emphasized that triple protease inhibitor/peginterferon/ribavirin therapy also is picking up demand for other lab tests, such as CBCs. Anemia, a well-known side-effect of peginterferon/ribavirin treatment, can be accentuated with either boceprevir or telaprevir. "It's proving to be very challenging and unusually severe in some cases," said Chung. "Not only have we had to dose-reduce ribavirin, but we've also employed erythropoietin-stimulating agents, and even needed to transfuse some patients. Unfortunately, despite assiduous monitoring, we've had to admit some patients for severe anemia."

Drug-drug interactions between boceprevir, telaprevir, and a host of other drugs is another consideration for managing patients on triple HCV therapy. The extent to which any lab testing will be beneficial in this realm remains to be seen, according to Nelson. "There are extensive drug-drug interactions around the *CYP450/CYP3A4* pathways, but currently we don't have data to say which SNP would be predictive of significant adverse events. Clearly there's a role for research in looking at pharmacogenomic associations, and hopefully that type of data is on the way."

Absent further guidance or evidence, extreme vigilance in this area will be the order of the day, according to Anna Lok, MD, Alice Lohrman Andrews research professor in hepatology, director of clinical hepatology, and professor of internal medicine at the University of Michigan Health System in Ann Arbor. "Very important, the physician needs to review all of the patient's medications prior to start of triple therapy, and some meds might need to be stopped or switched to others. It's also imperative that patients inform physicians if they are started on new meds during the course of treatment."

As enthusiastic as the hepatology community is about the availability of the new protease inhibitors, managing these treatments is so challenging that some provid-

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Patient History	WEEK						Clinical Action
	0	4	5	8	12	24	
boceprevir, peginterferon, ribavirin (B+P+R)							
	P+R Lead-in	B+P+R	HCV RNA	HCV RNA	HCV RNA	HCV RNA	
Previously untreated			UD		UD		Eligible for shortened 28-wk regimen
			D	D <100 IU/mL	UD	UD	Continue B+P+R to W36, then 12 wks' P+R
			D	D <100 IU/mL	D		Stop therapy
			D	D >100 IU/mL			Stop therapy
Previously treated	P+R Lead-in	B+P+R	UD		UD		Continue B+P+R to W36
			D	D <100 IU/mL	UD	UD	Continue B+P+R to W36, then 12 wks' P+R
			D	D <100 IU/mL	D		Stop therapy
			D	D >100 IU/mL			Stop therapy
telaprevir, peginterferon, ribavirin (T+P+R)							
	T+P+R		HCV RNA	HCV RNA		HCV RNA	
Previously untreated or prior relapse			U	U			T+P+R to W12, then eligible for shortened P+R to W24
			D <1,000 IU/mL	D <1,000 IU/mL	UD		T+P+R to W12, then P+R to W48
			D <1,000 IU/mL	D <1,000 IU/mL	D		Stop therapy
			D <1,000 IU/mL	D >1,000 IU/mL			Stop therapy
Prior partial or null responder	T+P+R		U	U			T+P+R to W12, then P+R to W48
			D <1,000 IU/mL	D <1,000 IU/mL	UD		T+P+R to W12, then P+R to W48
			D <1,000 IU/mL	D <1,000 IU/mL	D		Stop therapy
			D <1,000 IU/mL	D >1,000 IU/mL			Stop therapy

Legend: UD, undetectable; D, detectable; B+P+R, boceprevir, peginterferon, ribavirin; T+P+R, telaprevir, peginterferon, ribavirin; P+R, peginterferon, ribavirin

Sources: boceprevir and telaprevir package inserts; *Hepatology* 2011;54:1433-44; *Clin Infect Dis* 2012;54:96-104.

ers might back away from the regimens, according to Chung. "The complexity of the therapy has intensified manpower utilization, and in some ways, despite the promise, has narrowed the spectrum of providers who are equipped to be in the business of treating these patients," he said. "It's actually made many of us think that for most patients whose disease can afford to wait two, three, or four years until the next generation of direct acting agents are available, deferral may be the most rational step."

The Road Ahead

Even if therapy with boceprevir and telaprevir has some bumps and curves, experts see a much smoother course just down the road. For example, findings about a new polymerase inhibitor presented as an abstract at a recent AASLD meeting have taken the field by storm. The agent, PSI-7977, showed 100% virologic response rates when taken only with ribavirin or with three different peginterferon regimens. "This presentation left the group quiet without a comment for virtually a minute as everybody pondered these results," recalled Shiffman. "This leaves the door open for the first time now that we think we can cure HCV without peginterferon/ribavirin. We look forward to Phase III clinical trials starting some time in 2012." CLN

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Toxic Alcohols

Practical Challenges in Laboratory Diagnosis of Ingestions

MATTHEW D. KRASOWSKI, MD, PHD

With most attention today focused on drugs-of-abuse, toxic alcohol poisonings are often overlooked. Yet ethylene glycol, methanol, and isopropanol, which are found in products that are inexpensive and readily obtainable, can cause significant harm to individuals who ingest them (1). Toxic alcohol poisonings occur in accidental ingestions, self-harm attempts, or when isopropanol is used as a substitute for ethanol in drinks. Of the three compounds, ethylene glycol and methanol are particularly dangerous, mainly due to metabolites formed in the body that can cause severe organ damage.

Laboratory diagnosis of toxic alcohol ingestions presents several practical challenges (1,2). While most clinical laboratories can quickly and accurately quantitate plasma or serum ethanol levels using enzymatic assays on common chemistry analyzers, rapid assays for other alcohols have not been commercially available or have lacked specificity. The gold standard assay for determining toxic alcohol concentrations in plasma or serum is gas chromatography (GC), a labor-intensive technique not available in most clinical settings.

Many hospitals typically send out specimens for GC analysis, thereby precluding the 2–4 hour turnaround time for toxic alcohol analysis recommended by a National Academy of Clinical Biochemistry consensus panel (3). Clinical diagnosis can be challenging given that patients who have ingested toxic alcohols often present with non-specific signs and symptoms. They also come to the emergency department in an altered state of consciousness, or are semi-conscious, or even unconscious and unable to provide a reliable history (1,3).

Because toxic alcohol poisonings can

ing in children as well as pets. Individuals also sometimes ingest it deliberately in self-harm attempts. The body metabolizes ethylene glycol to glycolic acid and oxalic acid. The latter metabolite has the potential to cause severe renal injury by precipitating with calcium in the renal tubules.

Windshield fluid, a variety of cleaning solvents, canned cooking fuel, and even some liquid fuels for model railroad sets contain methanol (1). Although it has a relatively sweet odor, ingesting methanol leads to a burning, unpleasant taste, which likely explains why it is a less common cause of

bottles intended for medical purposes. Ingesting isopropanol is generally less serious clinically than ethylene glycol or methanol, as it is primarily metabolized to acetone. Individuals commonly experience gastrointestinal symptoms, including vomiting, that effectively limit the amount of the compound absorbed systemically.

In addition to specific organ damage caused by the metabolites of ethylene glycol and methanol, all the toxic alcohols also are capable of producing substantial respiratory and central nervous system (CNS) depression. These effects can be life-threatening, especially with large overdoses. In fact, toxic alcohol ingestion can be fatal due to respiratory failure, brain injury, or accidental trauma that occurs during intoxication. Furthermore, the fatality risk increases when individuals also ingest other CNS or respiratory depressants like ethanol, benzodiazepines, or opiates.

An additional compound sometimes classified as a toxic alcohol is propylene glycol (1). Although chemically similar to ethylene glycol and also used in some brands of automobile antifreeze, propylene glycol is generally much less toxic than ethylene glycol. A variety of products, including cosmetics, ointments, and some activated charcoal preparations, contain propylene glycol. It also is used as a diluent for intravenous preparations of poorly water-soluble drugs, including lorazepam, diazepam, and etomidate. Researchers have described toxicity resulting from overdoses of propylene glycol-containing antifreeze and repeated intravenous administrations of medications containing propylene glycol as the diluent. In particular, patients under extended sedation with lorazepam, such as intubated patients on mechanical ventilation, can suffer toxicity.

Table 1 summarizes the characteristics of the toxic alcohols.

Treatment of Toxic Alcohol Ingestion

Patients who have ingested ethylene glycol or methanol receive antidotal treatment with fomepizole (4-methylpyrazole) or ethanol (4,5). Both compounds prevent the first, rate-limiting enzymatic reaction catalyzed by alcohol dehydrogenase. Fomepizole is a competitive antagonist of alcohol dehydrogenase, while ethanol is a substrate for the enzyme that also competes with eth-

be fatal for patients, laboratories need to be prepared to meet the challenges of providing accurate diagnostic test results. This article will review the characteristics of toxic alcohols and focus on recent advances in their laboratory analysis.

Characteristics of the Toxic Alcohols

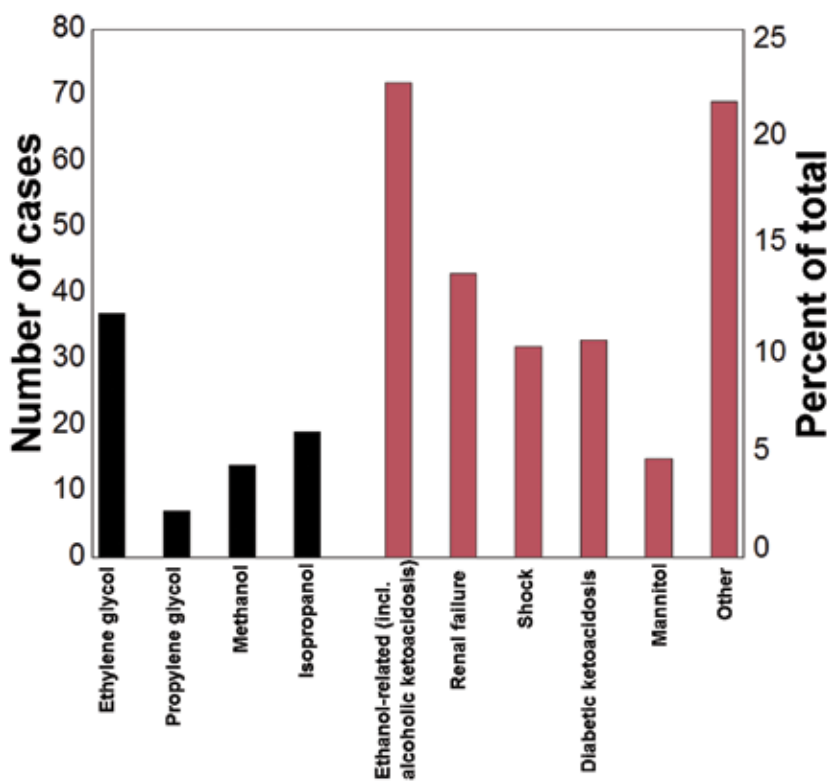
Ethylene glycol is a sweet-tasting compound found in most automobile antifreezes (1). The pleasant taste of ethylene glycol contributes to accidental poison-

ing than ethylene glycol. The body metabolizes methanol to formic acid, a toxic compound that can cause blindness as a result of permanent injury to the optic nerve.

Rubbing alcohol is the most common source of isopropanol. People sometimes mistakenly think that isopropanol is a safe substitute for ethanol. Others drink it in self-harm attempts, including in hospital settings, such as a delirious or suicidal patient who gains access to rubbing alcohol



Figure 1
Causes of Elevated Osmolal Gaps



This graph shows the causes of elevated osmolal gap from a retrospective study. The black bars indicate number of patients with toxic alcohol ingestion as the likely primary cause of the elevated osmolal gap. The red bars represent suspected primary causes of elevated osmolal gap in the absence of toxic alcohol ingestions.

Source: Reproduced from reference 10 (BioMed Central publisher).

ylene glycol and methanol. Fomepizole and ethanol act as antidotes by preventing the enzymatic conversions of ethylene glycol and methanol to toxic metabolites, allowing the kidneys to clear the toxic alcohols.

Treatment with fomepizole has steadily increased in the U.S. because it is easier to administer than intravenous ethanol. Furthermore, ethanol has the potential to further contribute to the patient's CNS and respiratory depression in cases of ethylene glycol and methanol ingestion. However, the high cost of fomepizole, more than \$1,000 per dose, presents another challenge, especially for hospitals that rarely encounter ethylene glycol or methanol ingestions. Some networks of hospitals now share the drug, alleviating the need for each to stock it individually.

Antidotal therapy with fomepizole or ethanol, however, may not be sufficient in patients who present more than several hours after ingestion or for those who have ingested very large amounts. Laboratory findings of patients with late-presenting ingestions include metabolic acidosis, increased anion gap, and glycolic acid (1,6). In either of these situations, patients should undergo renal dialysis to clear both parent drug and their metabolites (4,5). Although quite effective in managing toxic alcohol ingestion, renal dialysis is not a simple procedure and requires specialized personnel.

Laboratory Diagnosis of Toxic Alcohol Ingestion

While some patients have observable oxalate crystals, especially 8 hours or more after ingestion of ethylene glycol, examining urine for oxalate crystals should not be used a screening test for ingestion due to low sensitivity and specificity. The preferred method to detect and quantitate ethylene glycol, isopropanol, and methanol

is GC with flame ionization coupled with a technique called head space analysis that eliminates time-consuming liquid extractions (1,2). Laboratories also can measure

Table 1
Common Sources, Clinical Signs, and Analytical Methods for Alcohols

Alcohol	Common Sources	Osmolal Gap	Anion Gap	Analytical Methods
ethanol	alcoholic beverages	present	possible	enzymatic assay, GC
ethylene glycol	automobile antifreeze	present	present	GC, GC/MS, enzymatic assay
isopropanol	rubbing alcohol	present	absent	GC
methanol	windshield fluid, cooking fuels	present	present	GC
propylene glycol	activated charcoal, intravenous medications	present	possible	GC, GC/MS

Abbreviations: GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry

acetone, a metabolite of isopropanol, and glycolic acid, a metabolite of ethylene glycol, by GC. GC has high specificity for the toxic alcohols, with limits of detection of ≤ 10 mg/dL. Due to their higher volatility, ethanol, isopropanol, and methanol analysis requires a different procedure than ethylene glycol, which some laboratories analyze by gas chromatography/mass spectrometry (GC/MS).

The downside of GC and GC/MS is that both are labor-intensive and not automated. In hospitals that encounter toxic alcohol poisonings infrequently, clinical laboratory managers may find it difficult to justify performing GC in-house, especially when adding staff already is challenging. An additional consideration is that many toxic alcohol ingestions occur at night when laboratory staffing is often at minimum levels.

Consequently, diagnosis of toxic alcohol ingestion often depends on indirect laboratory findings, such as osmolal gap, acidosis, and anion gap.

An elevated osmolal gap is an early sign of toxic alcohol as well as propylene glycol ingestion, leading to the widespread use of this measurement as a screening test. Laboratories determine osmolality by measuring the freezing point depression of the patient's serum and estimating the osmolality contribution of the endogenous major contributors—sodium, blood urea nitrogen (BUN), and glucose—from an equation. By definition, the osmolal gap is the measured osmolality minus the estimated osmolality. Some equations also take into account the presence of serum ethanol.

Laboratorians should be aware that there is considerable debate about the use

Table 2
Laboratory Testing for Toxic Alcohol Ingestion

Laboratory Test or Calculated Parameter	Methodology	Clinical Value	Limitations
osmolal gap	calculated parameter	▶ high sensitivity for detecting toxic alcohol ingestions	▶ low specificity, especially for gaps <30 ▶ need to consider other causes of elevated gap
anion gap	calculated parameter	▶ easily calculated using common chemistry tests	▶ anion gap is late sign of ingestion ▶ sensitivity and specificity not well-defined for toxic alcohol ingestions
arterial blood gas	blood gas analyzer	▶ detect acidosis caused by ethylene glycol or methanol	acidosis is late sign of ingestion
ethylene glycol	GC or GC/MS	▶ gold standard for ethylene glycol detection/quantitation ▶ helps in determining when to stop antidote therapy or dialysis	▶ labor-intensive methodology ▶ not automatable.
ethylene glycol	enzymatic assay	▶ rapid determination of ethylene glycol on automated chemistry analyzer	▶ limited clinical experience ▶ requires user-defined parameters
glycolic acid	GC	▶ prognostic factor for ethylene glycol	▶ labor-intensive methodology ▶ not commonly performed even in labs with GC
isopropanol, methanol	GC	▶ gold standard method for isopropanol and methanol detection/quantitation ▶ can also detect acetone (metabolite of isopropanol) ▶ helps in determining when to stop antidote therapy or dialysis	▶ labor-intensive methodology ▶ not automatable
oxalate crystals, urine	urine microscopy	▶ seen in ethylene glycol ingestion	▶ unsuitable as screening test due to poor specificity and sensitivity ▶ late sign of ingestion

of osmolal gap to diagnose toxic alcohol ingestions, as well as a plethora of proposed empiric equations for estimating the contribution of sodium, BUN, glucose, and ethanol to serum osmolality (7,8). Table 2 summarizes the laboratory and calculated parameters used in the diagnosis of toxic alcohol ingestions.

An elevated osmolal gap, defined as $>10-15$, suggests the presence of additional osmotically active substances in blood. But differential diagnosis of an elevated osmolal gap is broad and also includes alcoholic ketoacidosis, which is seen in some patients following ethanol binges, diabetic ketoacidosis, mannitol infusion, renal failure, and shock. Alcoholic ketoacidosis also produces a substantial osmolal gap even in the absence of detectable plasma ethanol due to the formation of glycerol, acetone, and the acetone metabolites acetol and 1,2-propanediol in patients.

How Good is Osmolal Gap?

Despite the widespread clinical use of osmolal gap for the diagnosis of toxic alcohol ingestion, few studies have rigorously evaluated the diagnostic performance of this calculated parameter as a screening test. In one study, Lynd, et al., looked at retrospective data over a 6-year period from two tertiary care hospitals in Canada and found that osmolal gap was highly sensitive for toxic alcohol ingestion at a

cutoff of 10, with sensitivities approaching 1.0 for identifying toxic alcohol ingestions that required antidotal or renal dialysis therapy (9). The area under the curve (AUC) for receiver-operator characteristic (ROC) curve analysis range was 0.736–0.785, depending on the osmolality formula used. The researchers concluded that the main limitation of the measurement was lack of specificity, resulting in a positive predictive value of <0.5 , even for osmolal gaps up to 30.

Another group, Krasowski, et al., recently analyzed nearly 15 years of retrospective data from a U.S. academic medical center to ascertain the causes of elevated osmolal gaps (10). In this study, the hospital laboratory offered a testing panel with plasma sodium, BUN, glucose, osmolality, and ethanol. The laboratory used the tests to calculate osmolal gap and as part of the decision process for pursuing GC analysis of either a panel of methanol, isopropanol, ethanol, and acetone or a panel of ethylene and propylene glycol. The retrospective study revealed that recent heavy ethanol ingestion was the single most common cause of an osmolal gap >14 , with many of these cases having clinical histories and laboratory findings compatible with alcoholic ketoacidosis. The second most common cause was ingestion of toxic alcohols of any type. Other causes of elevated osmolal gap included diabetic ketoacidosis, renal failure,

shock, and recent mannitol infusion (Figure 1).

Many of the highest osmolal gaps occurred in patients ingesting toxic alcohols. For the patients who had elevated osmolal gaps resulting from some other cause, only 12% had an osmolal gap >30 on the initial laboratory work-up, 3% had >50 , and none had an osmolal gap >100 . In contrast, for patients who had a positive analysis for toxic alcohols by GC, 49% had an osmolal gap >30 on the initial laboratory work-up and 20% had an osmolal gap >50 .

Enzymatic Assay for Ethylene Glycol

Until recently, rapid and specific assays for alcohols other than ethanol were unavailable. Although colorimetric and enzymatic assays for ethylene glycol have been used in veterinary settings, lack of specificity for human use has been a problem. In particular, the assays produce false positives in samples containing propylene glycol and other related compounds, as well as 2,3-butanediol found in some chronic alcoholics. To meet the clinical need for a rapid and specific ethylene glycol assay, a research team recently developed an enzymatic assay based on a commercially available glycerol dehydrogenase assay (11).

The veterinary assay, marketed by Catachem, accurately quantitates ethylene glycol in serum or plasma samples that do not contain chemically similar compounds such as propylene glycol. While the assay fulfills the needs of veterinary settings in which animals that have ingested ethylene glycol would be unlikely to have interfering compounds in their blood, human samples may contain 2,3-butanediol from chronic ethanol use or propylene glycol from prior administration of activated charcoal or intravenous medications. The researchers successfully modified the parameters for the assay so that it would run on the Hitachi 917 and Olympus AU400 automated analyzers and achieved a specific method unaffected by interference from propylene glycol, 2,3-butanediol, or similar compounds. This finding holds promise for clinical laboratories interested in rapid triage of possible toxic alcohol ingestions.

Overcoming the Diagnostic Challenges

As long as toxic alcohols are available in cheap and easily obtainable products, emergency departments will likely continue to see patients who have ingested these compounds. Except for a small number of hospitals that have rapid access to GC or GC/MS analysis, clinicians are forced to diagnose toxic alcohol ingestions based on clinical history and indirect signs. While some laboratories use osmolal gap as a screening test due to its high sensitivity for detecting toxic alcohol ingestions, clinicians need to be aware that this test has poor specificity. Patients may also have laboratory findings of metabolic acidosis and anion gap, but these appear much later and their absence should not be used to rule-out ingestions.

The recent study by Juenke and colleagues (11) suggesting that a rapid and specific ethylene glycol enzymatic assay has clinical utility for human specimens is an exciting development for clinical laboratories. Even for hospitals that see few ethylene glycol ingestions, the availability of a rapid ethylene glycol assay may prove attractive given the expense of fomepizole and the invasiveness of dialysis therapy. Clearly, the

benefits of quickly ruling out ethylene glycol ingestion and focusing on other causes of altered mental status, especially in emergency departments that see large numbers of patients, is substantial.

However, clinical laboratories still need a rapid and specific clinical assay for methanol. Even though methanol ingestion is less common than ethylene glycol, a missed diagnosis could lead to patient harm. The ability to rapidly rule-out both ethylene glycol and methanol would be a further advance. **CLN**

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Panel Advises Blood Glucose Testing in All Hospitalized Patients

Are Glucose Meters Accurate Enough to do the Job?

BY GENNA ROLLINS

Amidst controversy about the pros and cons of tight glycemic control, an expert panel convened by The Endocrine Society is now recommending blood glucose testing for all patients on admission to a hospital (*J Clin Endocrinol Metab* 2012;97:16–38). The panel also proposed glycemic targets and focused on process and system improvements, with the goal of improving care for patients with hyperglycemia and diabetes. The guideline was intended to build upon a consensus statement on inpatient glycemic control issued jointly in 2009 by The Endocrine Society and American Diabetes Association, which focused more on critically ill patients (*Diabetes Care* 2009;32:1119–31).

The eight-member panel advised that all patients, whether diabetic or not, have laboratory-based blood glucose testing on admission. They also recommended bedside point-of-care testing (POCT) for at least 24–48 hours in patients without a history of diabetes but with blood glucose levels >140 mg/dL. In doing so, the panel cited observational studies that have found hyperglycemia in 32–38% of patients in community hospitals, but in whom a sizeable minority had no history of diabetes.

“Hyperglycemia is very common in hospitalized patients, particularly among those with cardiovascular disease, and is a harbinger of poor prognosis. Initial assessment of glucose levels in all inpatients provides clinicians with prognostically useful information,” said panel member Mikhail Kosiborod, MD, a cardiologist at Saint Luke’s Mid America Heart and Vascular Institute and associate professor of medicine at the University of Missouri-Kansas City. “It can help identify patients who should be screened for diabetes, and direct decisions in regard to the intensity of subsequent glucose monitoring and, if necessary, glucose-lowering treatment.”

Although the authors recommended bedside POCT for ongoing glycemic management using glucose meters that have demonstrated accuracy in acutely ill patients, they did not set specific analytical goals. The panel did warn that “the accuracy of most handheld glucose meters is far from optimal.” National Academy of Clinical Biochemistry (NACB) guidelines for laboratory analysis in the diagnosis and management of diabetes noted the lack of consensus on quality goals for glucose meters but suggested that meters should measure and report plasma glucose concentrations to facilitate comparison with assays performed in accredited laboratories (*Clin Chem* 2011;57:e1–47).

The chair of the NACB guidelines committee, David Sacks, MD, criticized the panel’s process and recommendations. “I was very disappointed that no lab person actually read these guidelines before they were published. It seems we could have provided useful input,” he observed. “For example, the panel said POCT has some advantages over lab venous glucose testing. I found that section very unpersuasive, and I don’t think it has advantages, other than results being available immediately. However, if the results aren’t accurate, then you

can make treatment and management errors.” Sacks is senior investigator and chief of clinical chemistry at the National Institutes of Health in Bethesda, Md.

NACB guidelines committee member, David Bruns, MD, shared Sacks’ concerns. “These recommendations reinforce the need to study glucose measuring devices used in hospital locations outside the ICU. The guidelines mention accuracy issues, but accuracy requires quantification to be meaningful. The question is, how accurate must the devices be?” Bruns is professor of pathology, director of clinical chemistry, and

associate director of the molecular diagnostics laboratory at the University of Virginia School of Medicine in Charlottesville.

The guideline calls for a pre-prandial glucose target <140 mg/dL and a random blood glucose <180 mg/dL for the majority of hospitalized patients who aren’t critically ill. On the flip side, the panel cautioned about the need to avoid hypoglycemia, and recommended that hospitals implement a standardized, nurse-initiated protocol for immediate intervention when a patient’s blood glucose level falls below 70 mg/dL. Sacks noted, however, that the guideline

does not mention well-documented performance issues with glucose meters in the hypoglycemic range.

In an effort to help diabetics manage their disease better and tease-out undiagnosed hyperglycemia, the panel recommended HbA1c testing in all hospitalized diabetics and others with glucose levels ≥140 mg/dL, if it has not been done within the preceding 2–3 months. The panel also suggested that hospital-wide protocols and systems are needed to effectively recognize and manage hyperglycemia in the hospital setting. **CLM**

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Keynote Presentation

Integration of Automated Biosensor Systems for Point-of-Use

Francis S. Ligler

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A highly regarded expert in biosensors and microfluidics, Dr. Ligler has published extensively and holds numerous patents. She is currently the Navy’s Senior Scientist for Biosensors and Biomaterials and the current Chair of the Bioengineering Section of the National Academy of Engineering. In her keynote address, Dr. Ligler will present her unique and lively perspective of optical biosensor technology as it applies to homeland security and clinical diagnostics.

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Essential Health Benefits Under Affordable Care Act in Flux

Even as the Supreme Court prepares to hear challenges to the Affordable Care Act next month, the department of Health and Human Services (HHS) is moving to give insurers and state governments more flexibility in the kind of benefits insurance exchanges must offer under the law. Physicians, hospitals, laboratories, and other healthcare stakeholders have monitored this area closely for signs as to which services will be paid for. According to a recent HHS announcement, stakeholders may get clues by looking to the largest plans already offered in their state.

By 2014, states must set up online markets for private coverage, with government assistance to help cover premiums for the tens of millions of uninsured Americans. If they do not, the federal government must step in and do it for them. However, how

HHS will handle rules for what kind of benefits must be offered—such as wellness screenings—have been mired in controversy, and a recent Institute of Medicine report did little to clarify HHS's course.

In response, HHS announced that states will have the flexibility to select an existing health plan to set the “benchmark” for the items and services included in the essential health benefits package. States would choose one of the following health insurance plans as a benchmark: one of the three largest small group plans in the state; one of the three largest state employee health plans; one of the three largest federal employee health plan options; or the largest HMO plan offered in the state's commercial market. Plans could modify coverage within a benefit category so long as they do not reduce the value of coverage.

The next big announcement in this area will be how HHS will limit cost sharing—a

sore spot for the lab community which has long fought copays for lab testing.

The HHS essential health benefits bulletin is available on the Centers for Medicare and Medicaid Services website, <http://ccio.cms.gov>.

CMS Selects 32 ACO Pioneers

The Centers for Medicare and Medicaid Services (CMS) has chosen 32 organizations as members of the agency's Pioneer Accountable Care Organization (ACO) program, a special ACO subcategory designed for healthcare organizations that have already proven they can offer highly coordinated care.

The aim is to learn from these early adopters of the ACO model with an emphasis on coordinating with private payers while cutting costs. Long-term, CMS hopes that these new payment models will allow organizations to move away from a payment system based on volume under the fee-for-service model, towards one where each ACO is paid based on the value of care it provides. Under the CMS Shared Savings Program, ACOs can get a cut of any money they save the Medicare program compared to a benchmark as long as they meet quality mileposts along the way.

CMS will allow pioneer ACOs to move more rapidly from the Shared Savings Program model to a population-based payment model—within 2 years if the ACO meets its quality goals. In a population-based payment model, some or all of the ACO's fee-for-service payments will be replaced by a prospective per-beneficiary monthly payment.

The final list of participating Pioneer ACOs and more information about the Pioneer ACO Model is available from the Center for Medicare and Medicaid Innovations website, <http://innovations.cms.gov/initiatives/aco/pioneer>.

CMS Launches In-Home Care Pilot

Up to 10,000 Medicare patients with chronic conditions will now be able to get most of the care they need at home under a new demonstration recently announced by the Centers for Medicare & Medicaid Services (CMS). The Indepen-

dence at Home Demonstration greatly expands the scope of in-home services available to chronically ill Medicare beneficiaries, including a complete range of primary care services, such as lab testing.

Under the pilot, medical practices led by physicians or nurse practitioners will provide primary care home visits tailored to the needs of beneficiaries with multiple chronic conditions and functional limitations. Participating healthcare providers will receive incentive payments if they reduce Medicare expenditures by providing high-quality care while reducing costs. CMS will use quality measures to ensure beneficiaries experience high quality care.

Medical practices eligible to participate in the demonstration must have physicians or nurse practitioners with experience delivering home-based primary care. Up to 50 practices will be selected and each must serve at least 200 Medicare fee-for-service beneficiaries with multiple chronic conditions and functional limitations. Practices in the demonstration will be responsible for coordinating patient care with other health and social service professionals.

More information is available on the CMS website, www.cms.gov.

Joint Commission Proposes Goal Focused on 'Overuse'

Proposed National Patient Safety Goal (NPSG) for 2013 aims to minimize the overuse of tests, treatments, and procedures to reduce risk to patients. According to the proposal, the Joint Commission defines overuse as the use of a health service in circumstances where the likelihood of benefit is negligible and, therefore, the patient faces only the risk of harm. Research has documented that overuse occurs with significant frequency in the U.S., the organization noted.

Hospitals would be allowed to select the test, treatment, or procedure they want to evaluate, or choose one of the following five topics: early induction of labor; insertion of tympanostomy tubes; red blood cell transfusions; percutaneous coronary interventions; and diagnostic ionizing radiation.

The proposal is available on the Joint Commission website, www.jointcommission.org.

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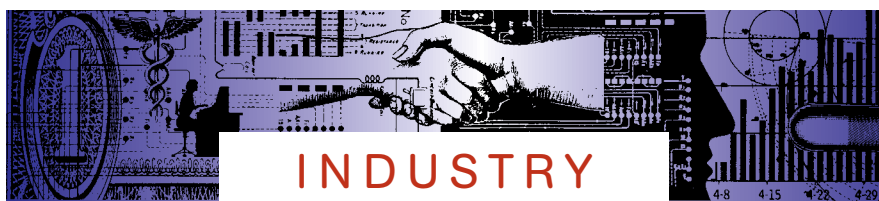
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INDUSTRY

Quest Diagnostics Purchases S.E.D. Labs

Albuquerque-based Lovelace Health System and Quest Diagnostics inked a definitive deal in which Lovelace's S.E.D. Medical Laboratories will become part of Quest. The agreement calls for Quest Diagnostics to acquire the assets of S.E.D. Medical Laboratories and manage inpatient labs for the four Lovelace hospitals, as well as serve Lovelace Health Plan members. "This agreement will provide S.E.D. and its medical and scientific staff with more access to innovation, new technology and capital resources," said Ron Stern, president and CEO of the Lovelace Health System. The deal also will significantly expand Quest Diagnostic's presence in New Mexico, where S.E.D. Medical Laboratories is based.

OvaGene Oncology Partners With Moffitt Cancer Center For microRNA-based Assays

OvaGene Oncology Inc. finalized a licensing and collaboration deal with the Tampa-based Moffitt Cancer Center, granting OvaGene exclusive worldwide rights to develop and commercialize proprietary microRNA-based assays that predict drug response for currently used cancer treatment drugs. The Moffitt Cancer Center developed the proprietary assays and validated them. "We are excited about our new partnership with OvaGene. It will enable us to accelerate our microRNA laboratory findings towards the clinic, as personalized medicine tools that may benefit patients in the near-term," said Johnathan Lancaster, MD, PhD, director of the Center for Women's Oncology at Moffitt Cancer Center.

Clariant and Acorn Forge Tumor Molecular Testing Partnership

Clariant and Acorn Research teamed up to focus on the molecular testing of tumor samples. The collaboration will combine Clariant's expertise in cancer diagnostics and Acorn's oncology network and advanced bioinformatics platform. The companies will work together to create standardized testing protocols across the Acorn network of oncology community practices and hospitals with treatment guidelines and clinical trial opportunities, in the hope of delivering individualized targeted treatments based on specific genetic markers.

Lineagen and Fast Forward Team On Blood-based Assays for MS

Lineagen, Inc., a molecular diagnostics company, and Fast Forward, LLC, a nonprofit subsidiary of the National Multiple Sclerosis Society, have partnered in an effort to fund the clinical development and

validation of a blood-based assay for MS. The goal of the collaboration is to develop a test to help clinicians diagnose MS, distinguish it from other neurological disorders, and provide the prognostic information needed to help guide treatment decisions. "This collaboration will enhance the development and validation of our proprietary gene and biomarker-based assay in MS.

The funding from Fast Forward will accelerate this clinical program, allowing the collaborative team to evaluate and affirm a broad number of biomarkers simultaneously, with the collective goal of delivering our best testing services to physicians and patients," said Michael S. Paul, PhD, president and CEO of Lineagen.

MinuteClinics, Axis-Shield Team To Offer POC HbA1c Tests

MinuteClinic, a division of CVS Caremark Corporation, and Axis-Shield have partnered to provide point-of-care HbA1c tests in 600 CVS pharmacies across

the nation. Under the terms of the deal, MinuteClinic will use Axis-Shield's Afinion analyzer in all 600 of its retail clinic locations. The fully automated analyzer will enable MinuteClinic's providers to gather a patient specimen and receive test results in 3 minutes or less. "We are pleased to be partnering with the largest provider of walk-in medical clinics to bring this testing directly to patients, enabling widespread access to routine hemoglobin A1c testing," said Ian Gilham, CEO of Axis-Shield. Providing HbA1c tests for patients with diabetes is part of MinuteClinic's business strategy to expand its services in chronic disease management.

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DIAGNOSTIC

Filtration Markers May Have Prognostic Value Independent of GFR

After adjustments for glomerular filtration rate (GFR), levels of creatinine, cystatin C, and β trace protein each remained directly associated with kidney failure but differed in their associations with mortality, according to a newly published study (J Am Soc Nephrol 2011 doi:10.1681/ASN.2011070663). The findings add to the body of knowledge about how these markers contribute prognostic information beyond that reflected in GFR.

The investigators analyzed data from the Modification of Diet in Renal Disease study, which included baseline levels for the markers from 816 participants with a median follow-up of 16.6 years. They measured GFR by urinary clearance of ^{125}I iothalamate and examined the association between the reciprocals of the biomarkers and ^{125}I iothalamate GFR. After adjustment for GFR in a Cox proportional hazards model and other statistical analyses, the authors found higher creatinine levels to be associated with increased risk for kidney failure but lower risk for all cause mortality. Higher levels of cystatin C and β trace protein were associated with higher risk for both kidney failure and all-cause mortality.

Based on their findings, the researchers called for future studies to report both associations with measured GFR and filtration markers in multivariable models assessing outcomes of chronic kidney disease and mortality.

Proteins Linked to Poor Outcomes in Idiopathic Pulmonary Fibrosis

University of Pittsburgh researchers have identified and independently validated that five proteins are associated with poor outcomes in patients with idiopathic pulmonary fibrosis (IPF) (Am J Respir Crit Care Med doi:10.1164/rccm.201101-00580C). Using a combination of protein markers and patient characteristics, they also derived a risk score that accurately distinguishes high- and low-mortality risk in IPF. This panel of proteins, along with three others the researchers previously identified, represents a “key step forward” in improving classification and management of IPF patients, according to the authors.

The investigators conducted the study because IPF has a variable course, and while clinical and physiological parameters have proven useful in monitoring disease, they have limited utility in predicting disease progression or IPF-related mortality. In addition, currently available medical therapies have little effect on IPF, so the authors hoped their findings might lay the groundwork for future drug studies.

The study involved 241 IPF patients, 140 in a derivation cohort and 101 in a validation cohort. The investigators analyzed

a total of 95 proteins, 92 using a multiplex bead-based immunoassay and three using an enzyme-linked immunosorbent assay. In all, 75 were detectable in plasma and five—MMP7, ICAM1, IL8, VCAM1, and S100A12—were found to be significantly associated with mortality and disease progression. The researchers similarly found in a validation cohort a significant association between these five markers and poor outcomes. In the latter, all five were predictive of significantly reduced survival when lung transplant was included as an event, but only two—ICAM1 and IL8—when transplant was excluded, and only ICAM1 predicted progression free survival.

The researchers also developed a personal clinical and molecular mortality index using both clinical parameters and protein concentrations in the derivation cohort, which was highly predictive of mortality in the validation cohort.

Three-Metabolite Signature Predictive of Progression to Alzheimer's disease

Finnish researchers have identified a three-metabolite signature that is predictive of progression from mild cognitive impairment (MCI) to Alzheimer's disease (AD) (Transl Psychiatry 2011 doi:10.1038/tp.2011.55). The findings could facilitate early diagnosis of AD and help identify new therapeutics for AD.

The PredictAD study involved 143 patients with diagnosed MCI, whose molecular signature was compared with both healthy controls and patients with AD. The researchers also identified patients with progressive (P-MCI) versus stable MCI. They used two different platforms to perform a broad metabolomics analysis at baseline and at follow-up. One involved ultra performance liquid chromatography-mass spectrometry (MS) to assess a global lipid signature, and the other used two-dimensional gas chromatography coupled to time-of-flight MS to assess small molecules such as amino acids, free fatty acids, ketoacids, and other organic acids. In all, 139 molecular lipids and 544 small polar metabolites were measured.

At baseline, AD patients had lower levels of ether phospholipids, phosphatidylcholines, sphingomyelins, and sterols. The researchers identified a three-metabolite signature associated with P-MCI and development of AD, including a lipid cluster containing predominantly phosphatidylcholines, a previously undescribed carboxylic acid, and 2,4-dihydroxybutanoic acid. The latter, which was the major contributor to the AD progression model, is a major component of cerebrospinal fluid (CSF), and also is found in plasma at concentrations nearly two orders of magnitude lower than in CSF. The findings primarily point to hypoxia, oxidative stress, and membrane lipid remodeling in progression to AD. The

authors called for validation of their findings in other studies.

Lower Post-Operative Hemoglobin Levels not Associated with Poorer Outcomes

A consortium of researchers representing 47 clinical sites found that in comparison to a restrictive strategy, a liberal transfusion strategy aimed at maintaining hemoglobin ≥ 10 g/dL did not reduce rates of death, inability to walk independently on 60-day follow-up, or in-hospital morbidity in elderly patients at high cardiovascular risk (N Engl J Med 2011;365:2453–62). The findings suggest that in post-operative patients who do not have symptoms of anemia or hemoglobin levels < 8 g/dL, it would be reasonable to withhold transfusion, even if they are elderly and have underlying cardiovascular disease (CVD) or risk factors.

The researchers conducted the study because even though post-operative and elderly patients frequently receive transfusions, the indications for this practice have not been studied thoroughly and remain controversial. The investigators sought to test the hypothesis that a higher threshold for blood transfusion would improve functional recovery and reduce morbidity and mortality, as compared to a more restrictive strategy.

The study involved 2,016 patients who were at least age 50 and had a history of or risk factors for CVD, and whose hemoglobin level was < 10 g/dL after hip-fracture surgery. Patients were randomly assigned to either a liberal transfusion strategy, with a hemoglobin threshold of 10 g/dL, or a restrictive strategy requiring symptoms of anemia or hemoglobin level < 8 g/dL for transfusion. Patients in the restrictive strategy group received 65% fewer units of blood than those in the liberal strategy group, and more than half of them did not receive any blood transfusion. Yet all primary and secondary outcomes between the two groups were not significantly different.

Tripled Clopidogrel Dosage in CYP2C19*2 Heterozygotes Lowers Platelet Reactivity

In patients with stable cardiovascular disease, tripling the maintenance dose of clopidogrel to 225 mg/day in *CYP2C19**2 heterozygotes resulted in levels of platelet reactivity similar to that achieved with the standard 75-mg dose in noncarriers (JAMA 2011;306:2221–28). However, in *CYP2C19**2 homozygotes, doses as high as 300 mg daily did not achieve comparable degrees of platelet inhibition. These findings help define how patients with different *CYP2C19* genotypes respond to different dosages of clopidogrel and may help guide further clinical studies.

Numerous studies have shown that in comparison to noncarriers, heterozygotes and homozygotes for loss-of-function *CYP2C19* alleles taking the standard 75-mg maintenance dose of clopidogrel have lower levels of the drug's active metabolite, reduced platelet inhibition, and higher rates of adverse cardiovascular events. However, data are needed to guide optimal treatment

in patients with loss-of-function *CYP2C19* alleles. This prompted the researchers to conduct a multicenter, randomized, double-blind trial under the hypothesis that increasing the maintenance dose of clopidogrel in *CYP2C19**2 allele carriers would reduce platelet reactivity.

*CYP2C19**2 heterozygotes taking 75 mg/day had significantly higher on-treatment platelet reactivity than did noncarriers, but doses up to 300 mg daily significantly reduced platelet reactivity, as measured by two different methods. In all, 52% of *CYP2C19**2 heterozygotes were clopidogrel nonresponders at a 75 mg dosage, but only 10% were at either 225 or 300 mg dosages. A daily dose of 225 mg reduced platelet reactivity in *CYP2C19**2 heterozygotes to levels seen in noncarriers taking a 75 mg dose. Similar reductions in platelet reactivity were not seen in *CYP2C19**2 homozygotes.

Fasting Serum Glucose Linked To Risk of Colorectal Cancer in Postmenopausal Women

In postmenopausal women, elevated fasting serum glucose, but not insulin or homeostasis model assessment-insulin resistance index (HOMA-IR), was associated with approximately a two-fold increased risk of colorectal cancer (Br J Cancer doi:10.1038/bjc.2011.512). The findings suggest that insulin resistance may confer an increased risk of colorectal cancer, although the biological mechanism has yet to be fully described.

Obesity, diabetes, and physical inactivity have consistently been associated with increased risk for colorectal cancer, but studies examining the association between the latter and circulating insulin and/or glucose levels have yielded conflicting results. This prompted a consortium of researchers to conduct a longitudinal study of a subsample of Women's Health Initiative clinical trial participants. The study involved 4,902 non-diabetics for whom there were baseline fasting serum insulin and glucose values. Fasting serum insulin and glucose tests also were performed during the median follow-up of 11.9 years.

In all, 81 cases of colorectal cancer developed during follow-up. These patients on average tended to be older, less physically active, and were more likely to be non-Hispanic whites. The researchers found baseline glucose levels to be positively associated with colorectal cancer and colon cancer risk. In both age- and multivariable-adjusted analyses, baseline insulin and HOMA-IR levels were not associated with colorectal cancer risk. However, in both analyses, baseline glucose levels were associated with risk: in the multivariable-adjusted analysis, the hazard ratio from highest to lowest tertile of glucose was 1.74. In just the subjects who developed colorectal cancer, the hazard ratio for highest tertile of glucose versus the lowest was 2.25, but insulin and HOMA-IR were not associated with risk.

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NEWS FROM THE FDA

BD Receives Clearance and CLIA Waiver for Influenza Test

Becton Dickinson received FDA clearance and a CLIA waiver for its new Veritor System for rapid detection of influenza A+B. The test system, which combines BD's nano detection particle and adaptive read technology, includes a digital read out of results and reports performance measured against polymerase chain reaction results.

Herceptin Test Approved

FDA approved Dako's companion diagnostic test for determining which breast cancer patients may benefit from treatment with Herceptin (trastuzumab). The firm's HER2 CISH pharmDx Kit measures copies of the *HER2* gene in formalin-fixed, paraffin-embedded breast cancer tissue specimens using a chromogenic in situ method.

Meridian Bio's *C. difficile* Test Cleared

Meridian Bioscience received FDA clearance to market its *Clostridium difficile* assay. The ImmunoCard *C. difficile* GDH assay detects the *C. difficile* common antigen glutamate dehydrogenase, which is present in both toxigenic and non-toxic strains of the bacteria. The test can be used to identify patients suspected of being infected with the bacterium.

Staphylococcus Test Gets Nod

FDA cleared Nanosphere's Verigene *Staphylococcus* Blood Culture Nucleic Acid Test for market. The BC-S test is designed to detect *Staphylococcus aureus*, *Staphylococcus epidermidis*, and the *mecA* gene, which confers resistance to the antibiotic methicillin/oxacillin. The test provides species and resistance detection from two types of gram-positive blood culture bottles in 2.5 hours, helping to guide appropriate antibiotic therapy for patients.

Clearance for Quidel's MDx Assays

Quidel received FDA clearance to market two of its molecular tests, one for detection of human metapneumovirus and the other for influenza A and B. The Quidel Molecular hMPV Assay and the Quidel Molecular Influenza A+B Assay are polymerase chain reaction-based tests designed to be used with the Applied Biosystems 7500 Fast DX thermocycler.



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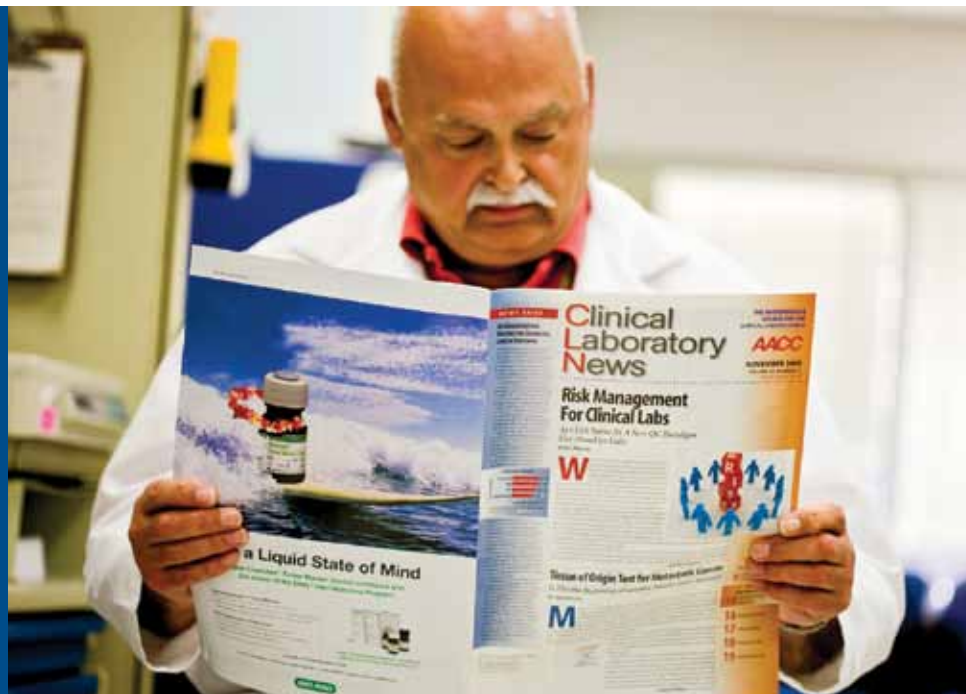
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