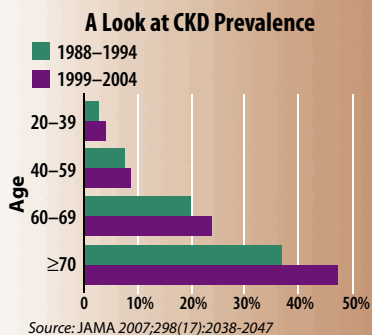


PATIENT AWARENESS TRAILS RISING CKD PREVALENCE

More than 13% of the U.S. population now has chronic kidney disease, a 3% increase over the last decade, yet most individuals with the disease remain unaware of it—even those with reduced kidney function and albuminuria, according to a new study published in the *Journal of the American Medical Association* (2007; 298(17): 2038–2047).

The researchers did a cross-sectional analysis of 1988–1994 and 1999–2004 NHANES data, comprised of a nationally representative sample of adults age 20 years and older, to determine the distribution of CKD stages and severity. Overall, the study found that prevalence of CKD stages 1 to 4 increased from 10% in 1988–1994 to 13.1% in 1999–2004, with corresponding increases in mild, moderate, and severely reduced eGFR, from 42.4% to 51.2%, 5.4% to 7.7%, and 0.21% to 0.35%, respectively. The trend was the same in all age groups (See Graph). In addition, prevalence of microalbuminuria increased from 7.1% to 8.2%, with a modest increase in macroalbuminuria that fell within the limits of random variation.



Demographic analysis of sex and racial groups showed increases in all categories. Prevalence among men increased from 8.2% to 11.1% and among women from 12.1% to 15%. For non-Hispanic whites, prevalence changed from 10.5% to 13.8%, for non-Hispanic Blacks from 10.2% to 11.7%, and among Mexican Americans 6.3% to 8%. The authors observed that despite adjusting their study for changes in the demographic makeup of the U.S. over the time period, differences remained substantial.

While higher prevalence of risk factors for CKD—diabetes, hypertension, and obesity—partially accounted for the overall increased prevalence of decreased eGFR, the increase in prevalence of albuminuria was entirely explained by these risk factors. Moreover, the researchers found that the proportion of those who said they knew they had weak or failing kidneys was very low, with only 42% of those at stage 4 reporting being aware of their disease.

Noting the particularly high prevalence of CKD in older populations and those with hypertension and diabetes, the researchers urge stakeholders to make CKD awareness more central to future public health planning.

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Outdated Lab Tests

Which Tests Should Be Considered Obsolete?

BY DEBORAH LEVENSON

A physician's order for a test that's no longer useful is a familiar scenario to most laboratorians. Even when newer testing strategies and assays have been proven superior to older ones and are included in clinical guidelines, some physicians cling to the older assays they have relied on in the past. Sometimes these less than useful tests remain in an institution's protocols or, occasionally, in government guidelines. But running these tests is a waste of time and money because they don't yield useful diagnostic information, say lab directors. In certain cases, an obsolete test may even impede an accurate diagnosis.

But convincing physicians not to order these tests—while difficult and time-consuming—is ultimately a worthy endeavor, agreed a group of laboratorians interviewed for this article, who emphasized their roles as consultants to physicians and partners in patient care. Explaining to physicians why a certain test is inappropriate is a unique responsibility of the lab, they noted. “We have several thousand tests that are commonly available today, and improvements upon those tests are pretty constant,” explained Brian Jackson, MD. “So there's lots of obsolescence going on. Physicians can't keep up with all of this on their own. Labs have to step up to educate them. I don't think anyone else can do it.”

CLN asked nine clinical chemists to identify lab tests that should no longer be used and queried readers about these tests' utility in an informal Internet survey (See Figure, p. 3). Tests that generated the most

See **Obsolete Tests**, continued on page 3



The New Vitamin D

What's Putting Fresh Emphasis on Lab Measurements?

BY GINA ROLLINS

Vitamin D is increasingly gaining recognition as a versatile agent that not only supports healthy bone formation and neuromuscular function but also plays a role in the development and progression of certain cancers, autoimmune disorders, and infectious diseases. Recent research has linked vitamin D to type 1 diabetes, multiple sclerosis, tuberculosis, and other conditions such as Alzheimer's disease and psoriasis. As evidence mounts on the therapeutic potential of vitamin D, clinicians will likely pay closer attention to patients' serum levels. Yet, questions remain about proper dietary amounts—both for the general population and disease treatment—as well as appropriate target levels of serum vitamin D, placing critical emphasis on the lab's role in providing accurate test results.

Indeed, the familiar vitamin D has earned a lot of attention lately, say those who study it. “When I entered the field we didn't know much about how vitamin D worked,” said Sylvia Christakos, PhD, professor of biochemistry and molecular biology at the University of Medicine and Dentistry of New Jersey in Newark. “Now we're at the point where it is being used therapeutically to affect disease states. For me, the next wave will be when we translate the new discoveries into curing disease.”

The renewed interest in vitamin D among researchers like Christakos originates from many different areas. For example, in the AIPC Study of Calcitriol Enhancing Taxotere Trial (ASCENT), treatment with the high-dose vitamin D metabolite calcitriol in combination with the chemotherapy drug docetaxel improved survival by 8 months

See **Vitamin D**, continued on page 6

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Some Lab Tests Won't Die

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discussion among the laboratorians included two cardiac markers, certain older thyroid tests, and qualitative pregnancy tests. While the lab directors wished that physicians would abandon particular outdated assays in general, they hesitated to call some tests obsolete because these assays are still useful in rare cases.

What's Really Obsolete?

Completely eliminating most tests that don't in general offer useful information isn't advisable, most of the lab directors agreed. They hesitated to label many tests of limited utility as obsolete. "For any of these tests, there are rare cases where you might really need them," explained Wes Schreiber, MD. "But those instances may be so few and far between that you can easily send the tests off to reference labs."

Whether a test is truly obsolete is rarely a black and white issue. "When you're talking about obsolete tests, there's definitely a continuum. It's difficult to say who should be the judge of what is completely useless, and individual laboratorians and pathologists are often uncomfortable making that call," Jackson explained. "Sometimes there's an unusual but valid use for a test. In general, though, labs are pretty conservative and most could stand to pull some tests off their menus." That's just the approach used by Nikola Baumann, PhD. "Calling a test 'obsolete' is a strong statement. In my lab, there are tests we don't perform, but might send out to a larger reference lab," she commented.

Cardiac Markers: What Can Go Away?

When asked about obsolete tests, many of the clinical chemists immediately thought of assays for cardiac markers: creatine kinase-MB (CKMB) and lactate dehydrogenase (LDH)/ LDH isoenzymes. While the laboratorians all told *CLN* they agree with recent National Academy for Clinical Biochemistry guidelines that identify troponin as the preferred marker for cardiac injury, most saw a continuing role for CKMB in assessing patients. They advised that LDH was no longer useful because both CKMB and troponin are more specific.

CKMB

Fred Apple, PhD: "Although troponin is clearly the superior marker, some labs in developing countries can't afford it. CKMB and total CK have roles as cost-effective options in places that can't afford troponin."

Catherine Hammett-Stabler, PhD: "There are places where troponin isn't readily available. You should certainly use troponin when available, but when it's not, CKMB is acceptable. I don't think you can get rid of CKMB any time soon in the U.S. because some cardiologists and emergency room (ER) physicians often aren't comfortable using a single troponin test, especially troponin T. But using troponin and CKMB together can be a problem. For example, patients with renal dysfunction sometimes have positive troponin T without a clear increase in CKMB. Some cardiologists and nephrologists think the situation could mean necrosis, but it may not be indicative of MI."

Nikola Baumann, PhD: "In our hospital,

physicians routinely order both CKMB and troponin I, which is unnecessary. Most of the time, CKMB will agree with the troponin I result for the purpose of diagnosing a myocardial infarction. But there may be times when the troponin I and the CKMB results do not agree. I always wonder how clinicians react to those discrepant results and whether they appreciate that troponin I is the more sensitive and specific test."

Wes Schreiber, MD: "Troponin has better sensitivity and specificity than CKMB, but CKMB is still a very good test. There's a place for CKMB in determining if a patient has had a second myocardial infarction (MI) following a recent MI. That's because CKMB drops more rapidly than troponin. Although CKMB isn't the preferred test to do when you suspect an MI, I'd be reluctant to take it off a lab test menu."

Nader Rifai, PhD: "Children's Hospital Boston uses troponin to identify minor myocardial injury resulting from a procedure or a drug's cardiotoxic effect. For this purpose, CKMB may be misleading, and troponin is clearly the way to go."

Bette Seamonds, PhD: "Troponin is really now the gold standard. A few hospital labs have quit offering CKMB. It's superfluous. I'm trying to convince our ER physicians and cardiologists that it should go. They argue that CKMB has a use in identifying renal patients with muscle damage."

Lawrence A. Kaplan, PhD: "We dropped CKMB from our testing menu at Bellevue Hospital (New York, N.Y.). It's rarely justified, so I rarely allowed it to happen. Some physicians argued that CKMB could size a myocardial infarction, but I never saw the data."

LDH/ LDH Isoenzymes

Lottie Goldsmith, BS, CLD: "The total enzymes, like LDH and AST, were used thirty to forty years ago to indicate coronary damage. Now we have tests that are so much better."

Catherine Hammett-Stabler, PhD: "Years ago LDH was a great cardiac marker for those MI patients who came in a long time after symptoms began. It was the last cardiac marker that got evaluated, and it stayed around for a long time. But with troponin, it's simply not needed now."

Wes Schreiber, MD: "Lactate dehydrogenase is very nonspecific. It's frequently abnormal because it is found in every organ, so as a cardiac marker, you shouldn't even think about it. LDH isoenzymes have been made outdated by troponin."

Nader Rifai, PhD: "I haven't seen that test for 20 years."

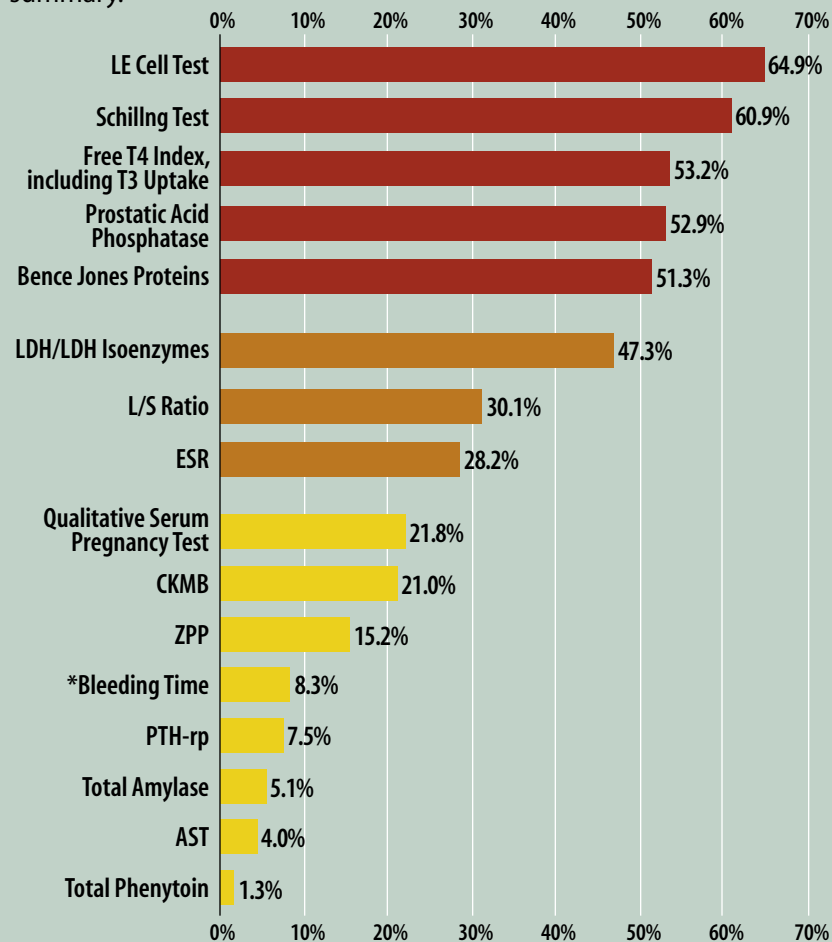
Is ESR Still Useful?

An inflammatory marker, erythrocyte sedimentation rate (ESR) is a nonspecific marker of inflammation that typically has been used in conjunction with other tests to help diagnose conditions associated with acute and chronic inflammation, including infections, cancers, and autoimmune diseases. The clinical chemists agreed that ESR, while quick and easy, is of limited use.

Bette Seamonds, PhD: "In the 1970s, ESR was part of a battery of inflammatory marker tests. Today, it's like voodoo when you have high-sensitivity CRP. Some protocols actually still call for it for stroke. But

CLN Survey: What Tests Do You Consider Obsolete?

CLN invited readers to participate in an informal survey regarding 16 tests identified as potentially obsolete. From a total of 467 responses, 386 from readers working in clinical settings were selected for this summary.*



*The survey did not specifically ask about bleeding time, but many participants noted it in response to a question asking them to suggest other tests they consider obsolete.

why do ESR in a stroke situation, when you want a rapid result? You can do CRP in minutes, while ESR takes an hour."

Catherine Hammett-Stabler, PhD: "For most inflammation, CRP and other markers are better. A couple of papers from 1999 do justify ESR to identify temporal arteritis, a condition related to stroke."

Nikola Baumann, PhD: "Everyone agrees it's completely nonspecific but it seems that few people want to get rid of it, and physicians continue to order it. An elevated ESR may reassure what the physician already suspects."

Wes Schreiber, MD: "ESR is very cheap and very easy, but it's also very nonspecific. Most people considered it obsolete ten years ago."

TSH Supplants Free Thyroxine (T4) Index and T3 Uptake

The group of lab directors generally agreed that the free T4 index and T3 uptake no longer belong in hospital labs. That's because the tests estimate hormone levels in the blood and have been replaced by the combination of more sensitive thyroid stimulating hormone (TSH) assays and free thyroxine assays that give direct measurements. However, they may have a valid place in reference labs because the free T4 index can be useful for a certain thyroid condition in newborns.

Brian Jackson, MD: "This is a pretty clear-cut case of something being really obsolete. The free T4 index is an estimate that requires a calculation. It became obsolete as soon as good free T4 assays became available."

Wes Schreiber, MD: "TSH is now the best test for thyroid function. T3 uptake was developed as an indirect way to estimate the amount of thyroxine-binding globulin, which affects the level of free thyroxine. Now, TSH can determine both hyperthyroidism and hypothyroidism very well."

Catherine Hammett-Stabler, PhD: "These are older tests developed to estimate free T4

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in serum. T3 uptake should be replaced by one of the free T4 assays, but I still have endocrinologists who are upset with me for getting rid of T3 uptake. Manufacturers would do the world a service by stopping production of T3 uptake assays.”

Lawrence A. Kaplan, PhD: “In most cases, I think it’s useless. But there are rare cases of newborns with low levels of thyroid binding globulin that T3 uptake can pick up. I made this test available through a reference laboratory just for these rare cases, though.”

Bette Seamonds, PhD: “I want to get rid of this test in the worst way. T3 uptake is one of God’s most useless tests. But it makes money. Half of my workload is outpatient, so I see a lot of requests for this test, although not as much as in the past.”

Why Use a Qualitative Serum Pregnancy Test?

While all of the experts agreed that quantitative serum pregnancy tests are far superior

to qualitative ones, most agreed that eliminating these urine tests from the ER would be difficult because some situations require a quick determination of pregnancy. Some of the laboratorians offered strategies for balancing accuracy and speed.

Nikola Baumann, PhD: “I could buy into the idea that qualitative pregnancy tests are obsolete in the central lab setting. We have good quantitative serum assays that have better sensitivity than the qualitative tests. However, I don’t think that point-of-care urine pregnancy tests will go away because the test is noninvasive, quick, and easy. In an ER setting, the qualitative urine test gives the fastest answer as to whether the patient is pregnant or not. In a STAT lab, serum assays could easily be validated for urine and used for the same purpose.”

Catherine Hammett-Stabler, PhD: “The qualitative test is a headache because it’s not foolproof. But you need it in some situations, such as if a woman needs x-rays.”

Bette Seamonds, PhD: “We have analyzers

for this test on urine in the ER. It’s hard to get accurate results manually because the timing means the test will likely be done incorrectly. The qualitative test done on whole blood is different story, if there’s not interference from hemolysis. I have an analyzer in the chemistry lab that gives results in 15 minutes. It’s the best option if the ER gets the sample to me quickly.”

Lawrence A. Kaplan, PhD: “This very rapid test is needed in the ER, although you could go another route. Get the serum in the laboratory and run it as a yes/no qualitative test.”

Lecithin/Sphingomyelin (L/S) Ratio: Still Useful?

Another controversial test is L/S ratio. A marker for fetal lung maturity that helps predict whether a baby will be born with respiratory distress syndrome, L/S ratio wasn’t considered useless by the laboratorians, but neither was it their first choice for this purpose.

Discussing the Tests

These nine clinical chemists shared their views on outdated tests.

Fred S. Apple, PhD

- ▶ Professor of Laboratory Medicine, Department of Laboratory Medicine and Pathology, University of Minnesota School of Medicine, Minneapolis, Minn.
- ▶ Medical Director of Clinical Laboratories and the Clinical Chemistry and Toxicology Laboratories at Hennepin County Medical Center, Minneapolis, Minn.



Lawrence A. Kaplan, PhD

- ▶ Consultant Clinical Chemist



Nikola Baumann, PhD

- ▶ Director of Clinical Chemistry, University of Illinois Medical Center at Chicago
- ▶ Assistant Professor, Pathology, University of Illinois at Chicago
- ▶ Member, *CLN* Board of Editors



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- ▶ Director, Clinical Chemistry, Children’s Hospital Boston (Mass.)
- ▶ Professor, Harvard Medical School, Boston, Mass.
- ▶ Editor, *Clinical Chemistry*



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- ▶ Co-Director, Transplant Laboratory, University of Miami Medical Center, Miami, Fla.



Bette Seamonds, PhD

- ▶ Clinical Chemist and Director of Point-of-Care Testing Services, Mercy Health Laboratories, Darby, Pa.



Catherine Hammett-Stabler, PhD

- ▶ Associate Director, Department of Pathology and Laboratory Medicine, University of North Carolina (UNC), Chapel Hill
- ▶ Associate Director of Core Laboratories, UNC Hospitals and Clinics



Wes Schreiber, MD

- ▶ Consultant Pathologist, Vancouver General Hospital, Vancouver, Canada
- ▶ Professor, Department of Pathology & Laboratory Medicine, The University of British Columbia, Vancouver, Canada
- ▶ Member, Editorial Review Board, Lab Tests Online, representing American Society for Clinical Pathology



Hammett-Stabler has received funding to study FLM and LDH isoenzymes in the past.

Brian Jackson, MD

- ▶ Medical Director of Infomatics, ARUP Laboratories, Salt Lake City, Utah
- ▶ Assistant Professor of Pathology (Clinical), University of Utah, Salt Lake City



Nikola Baumann, PhD: “Instead of L/S ratio, many labs perform the Fetal Lung Maturity (FLM) II (Abbott, Abbott Park, Ill.) test as the first-line test. If you get an intermediate or immature result using FLM II, one can do a secondary test. That could be the L/S ratio or lamellar body count, depending on the lab.”

Catherine Hammett-Stabler, PhD: “This test is very cumbersome. It takes hours to perform, it’s not very specific, and there are hundreds of variations on the original L/S ratio method. Lamellar body counts or FLM are better.”

Bette Seamonds, PhD: “When my hospital had maternity services, physicians who wanted L/S ratio had to speak to me directly. Instead, we used FLM in combination with fetal fibronectin (fFN). fFN gives a sense of whether the mom is high-risk for prematurity. Combined with FLM, it’s a pretty powerful tool.”

Changing Physician Ordering Habits

How should laboratorians convince physicians to use better tests? The group suggested careful explanations, educating interns, and reports that point out poor test choices to physicians.

Nader Rifai, PhD: “Remember who you are and what you are there for. You want to be a consultant, not a clerk. Contact whoever is ordering the test and explain why he or she should discontinue it in terms of good practice and cost restraint.”

Catherine Hammett-Stabler, PhD: “We have several ways these are handled. In many cases, our staff in Referral Testing does the initial investigation as to why the test was ordered and discusses the issue with the physicians. In other cases, the resident or fellow follows up with the ordering physician, discussing the testing choice and why another test is better. The most success in changing ordering habits comes from direct conversations. Sometimes a physician wants the test no matter what. In these cases, we call the results to the physician and talk about what the result doesn’t mean and steer him or her to another test. We may need two or three rounds with ‘repeat offenders.’”

Bette Seamonds, PhD: “I beat on my interns. I tell them what not to order. As a clinical chemist, I can raise them to a different level.”

Lawrence A. Kaplan, PhD: “It’s difficult to convince physicians to change, but it’s ultimately ‘doable.’ The most inflexible are the private practice physicians. In the hospitals, the doctors are under capitation. You tell an in-house physician, ‘Stop, you’re costing me too much money.’ When private practice doctors order tests, both they and the lab make money.”

Brian Jackson, MD: “Reference labs are in a difficult position to directly challenge physicians’ orders because we have no direct relationship with those physicians. On the other hand, academically-affiliated reference labs can be an educational resource for other laboratories. As part of ARUP’s Analyzing Test Ordering Patterns (ATOP) program, we take a broad look at our clients’ orders and provide reports that identify obsolete tests and other potentially misused tests. We also provide literature references the pathologists can take back to their physicians. Otherwise, reference labs are in a difficult position for changing what physicians order because we have no con-

tact with them.”

Wes Schreiber, MD: “As a rule, formal education programs have only short-term effects. The best tactic for changing physician behavior is to take the tests off the menu. Some of these tests should be discontinued altogether, while others should be available only by consultation with the lab.”

Other Outdated Lab Tests

While the group of lab directors most frequently identified old cardiac markers as outdated tests, they considered several other assays of very minimal use or obsolete. One is prostatic acid phosphatase, which has been replaced by PSA, a much more sensitive and specific marker of cancer. While two lab directors noted that some hospitals still use prostatic acid phosphatase in sexual assault workups, others pointed out that

PSA is just as useful for this purpose.

Zinc protoporphyrin (ZPP), abandoned as a lead poisoning test following CDC’s 1991 redefinition of lead poisoning to a cutoff level below what the assay can detect, is another test that is clearly unwarranted in most cases. However, some said ZPP could be useful in reference labs for spotting iron deficiency or whether high lead levels reflect a chronic rather than a single, acute exposure. Likewise, the lab directors agreed there is a place for parathyroid hormone-related peptide in reference labs for some tumors. The group roundly criticized the Schilling test as an outdated means of testing for vitamin B12 because it involves radioactivity.

In thinking about old tests and what has replaced them, many lab directors noted that assays are not only becoming more sophisti-

cated, but are also enhancing laboratorians’ role as partners in patient care. Fred Apple, PhD explained the factors driving these trends. “Assays are able to measure analytes at lower and lower concentrations, with improved precision, and are now directed at more tissue specific protein markers, with fewer false analytical positives. There’s been a shift toward measuring analytes in their more physiologically active form and towards molecular testing. We’re getting better at measuring low levels of biomarkers that are more organ-specific. We’ve moved away from qualitative tests to quantitative ones, from activity to mass measurements, from total to free fractions, and toward more physiologically active forms that better correlate with the pathophysiology of disease. It’s an exciting time in laboratory medicine.”

CLN

Experts Disagree on Optimal Levels

Vitamin D, from page 1

in patients with progressive metastatic prostate cancer. Even more far-reaching are the results of a recent meta-analysis that found a correlation between vitamin D supplementation and a 7 percent reduction in all-cause mortality.

A Tightly Regulated System

While researchers continue to investigate the role of vitamin D in cancer and disease, much is already known about the important role of vitamin D in maintaining organ systems. "It's a very elegant system," said Hector DeLuca, PhD, Harry Steenbock research professor at the University of Wisconsin-Madison at a 2007 AACC Annual Meeting Symposium, "Vitamin D Reinvented." DeLuca's laboratory has been devoted to the understanding of metabolism and mechanism of action of vitamins A and D since 1960.

About 80 percent of vitamin D used in the body is produced photochemically when ultraviolet radiation from sunlight reacts with 7-dehydrocholesterol, a precursor sterol in the skin, producing vitamin D₃ or cholecalciferol. This is the opening action of the multi-sequenced vitamin D endocrine system (See Figure). The liver then metabolizes vitamin D₃ into 25-hydroxycholecalciferol (25[OH]D₃) or calcidiol, the main form of vitamin D circulating in the blood. The kidney, functioning as an endocrine gland, converts this precursor into two principal dihydroxylated metabolites or calcitriol, 1 α ,25[OH]₂D₃—the primary active form of vitamin D—and 24R,25(OH)₂D₃. After binding to the vitamin D-binding protein, 1 α ,25[OH]₂D₃ is transported to various target organs, including bone, the intestines, and kidneys, where it binds to receptors and carries out further biochemical actions.

The primary and best understood action of vitamin D is to maintain plasma calcium and phosphorus levels at concentrations that support normal neuromuscular function and skeletal mineralization. Vitamin D deficiency results from inadequate intake coupled with inadequate sunlight exposure. Shortages of the prohormone leads to rickets in children and osteomalacia in adults (See Sidebar). Both develop when, with no other available source, the body robs calcium from the bones. This also leads to secondary hyperparathyroidism from the parathyroid gland's continual attempts to open the calcium floodgates.

In Target Cells

What has researchers excited is the possibility of preventing or at least slowing the progression of many diseases like cancer via vitamin D's actions in target cells. A nuclear receptor protein, termed VDR, with ligand- and DNA-binding domains has been found in more than 30 different cell types. Researchers have discovered at least 50 genes that are regulated by this complex; however, there appears to be a threshold amount needed to initiate activity, according to DeLuca. "A certain level of receptor is required before it functions," he noted. "Why is it expressed in some tissues and not others? I don't know the answer, nor do I know anyone in the field who knows the answer."

In cells where the vitamin-D receptor is active, including lymphocytes, promyelocytes, and keratinocytes, it forms a complex with the retinoid-X receptor, which binds to DNA—often to activate transcription, but in some instances to suppress it. In most if not all the target tissues, vitamin D also induces the enzyme CYP-24 hydroxylase that starts the pathway to break down vitamin D itself into water-soluble calcitriol, which is then excreted into urine. "By initiating its own destruction, there's a system to prevent overactivity. It's an auto-regulation," explained David Feldman, professor of medicine at Stanford University (Palo Alto, Calif.).

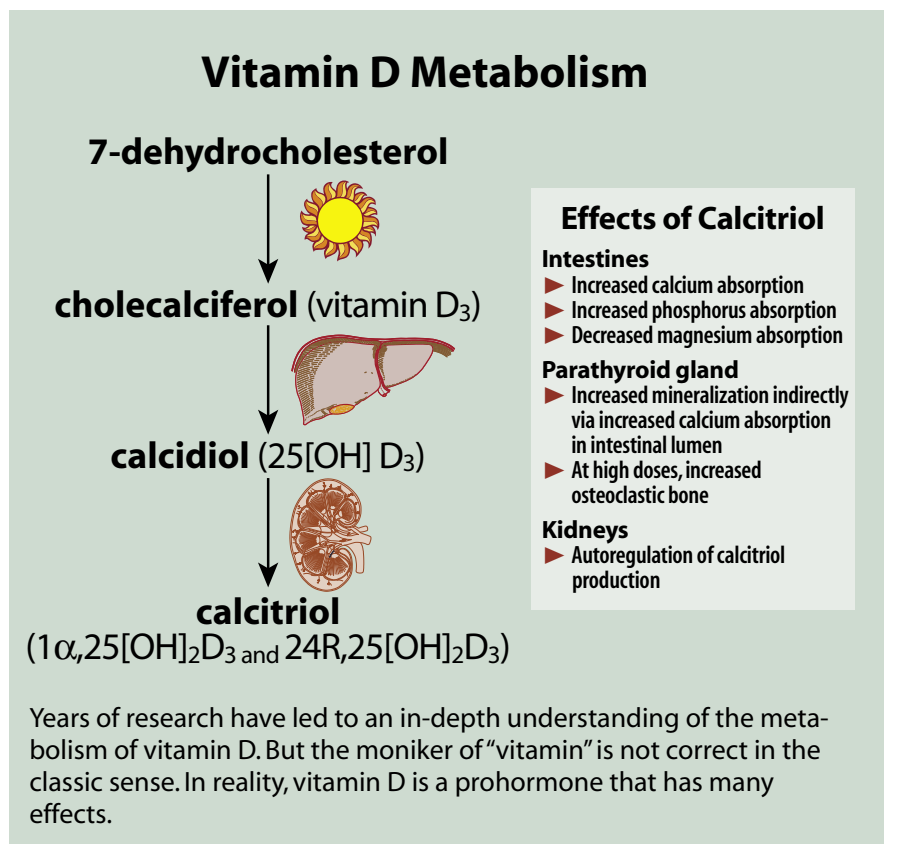
The mechanism of how vitamin D plays a role in the prevention or progression of various diseases has not been established definitively. In the case of cancer, 1 α ,25[OH]₂D₃ apparently inhibits cell proliferation and stimulates cell differentiation. In certain malignancies, like prostate cancer, it also appears to act as an anti-inflammatory agent by downregulating the activity of cyclo-oxygenase II (COX-II), the critical enzyme for synthesis of inflammatory prostaglandins, while at the same time stimulating prostaglandin dehydrogenase, which destroys prostaglandins. An analogous process may be at work in multiple sclerosis.

"Right now we believe that 1 alpha, 25-dihydroxy vitamin D₃ downregulates pro-inflammatory cytokines including interleukin 12, interleukin 2, and interferon gamma, and that it enhances cytokines that inhibit inflammation, including interleukin 4 and interleukin 10," explained Christakos.

Vitamin D also has been proposed as a mediator of immune response to bacterial antigens like tuberculosis bacillus. Research indicates that 1 α ,25[OH]₂D₃ stimulates immune response in antigen-presenting cells like macrophages, while also curtailing excessive response in immune system cells like lymphocytes. Low levels of calcidiol have been found in Alzheimer's patients, and its neuroprotective role may work by modulating calcium homeostasis and the production of neurotrophins. In addition, a role for vitamin D has been suggested in treating osteoporosis, in increasing muscle function and thereby preventing falls in the elderly, in preventing the development of preeclampsia in pregnancy, in decreasing the risk of hypertension, and even in lowering the chance of postmenopausal women developing overactive bladder.

Spotlight on Epidemiology

Aside from biochemical discoveries, a host of epidemiologic studies also have pointed to a connection between sunlight exposure, a surrogate for vitamin D levels, and various diseases, including multiple sclerosis, type 1 diabetes, and various cancers, most notably breast, colon, and prostate. In general, people who live closer to the equator, where UV rays are stronger, have a lower incidence of these conditions. As latitude increases, however, so does the incidence of these diseases. For instance, a child in Finland is 35 times more likely to develop type 1 diabetes than one in South China or other countries near the equator, according to Cedric Garland, DPH, cancer prevention specialist at Moores Cancer Center at the



University of California, San Diego, and a speaker at the AACC symposium on vitamin D.

As epidemiologic evidence has accumulated, researchers have been able to establish a dose-response relationship between sunlight and serum vitamin D levels. "Each degree of latitude corresponds to 1 ng/mL of 25-hydroxy vitamin D₃. If you're at latitude zero degrees at the equator, your 25-hydroxy vitamin D₃ is going to be about 60 ng/mL. If you're at 60 degrees latitude in southern Alaska or Scandinavia, it will be near the range of detection, somewhere between zero and 10 ng/mL in the winter," Garland explained. He estimates that the naturally occurring level of vitamin D in people with adequate sun exposure is about 65 ng/mL, which can be quite beneficial. His research indicates that serum calcidiol levels of 55 ng/mL are optimal for cancer prevention.

Using data from GLOBOCAN, a new World Health Organization database of cancer incidence, mortality, and prevalence in 177 countries, Garland estimates that up to 250,000 cases of colorectal cancer and 350,000 cases of breast cancer could be prevented worldwide by boosting vitamin D intake to 55 ng/mL.

How Much Intake?

As strong as the role of vitamin D in preventing various diseases appears to be, the medical community hasn't made sweeping recommendations that the entire population become either sun worshippers or habitual cod liver oil drinkers, for several reasons. First, the sunlight-vitamin D dose-response relationship is multifactorial. Research indicates that the lightest skinned people who sunburn easily and rarely tan need a scant 2 percent to 10 percent the amount of sun exposure to produce a unit of vitamin D as those with the darkest skins.

Latitude, season, cloud cover, pollution, and shade play roles as well. In North America at latitudes above San Francisco and Philadelphia, ultraviolet rays are not powerful enough to generate substantial amounts of vitamin D during the winter. Complete cloud cover halves the energy of ultraviolet rays; shade cuts it by nearly two-

thirds. Sunscreens with a sun protection factor of 8 or higher also block UV rays, effectively cutting vitamin D production by 95 percent.

Obesity and age play roles as well. Since vitamin D is fat soluble, it is deposited in fatty tissue, and people who are overweight can't access it as readily as others. By age 65, changes in the skin may reduce vitamin D production by as much as 60 percent. People with conditions that involve fat malabsorption like Crohn's disease, pancreatic enzyme deficiency, and celiac disease also absorb less vitamin D.

But experts disagree about the proper levels of vitamin D required to promote health or prevent vitamin D toxicity. When the Food and Nutrition Board of the Institute of Medicine (IOM) set dietary reference intakes for vitamin D in 1997, the body of evidence for optimal or safe levels of vitamin D was not substantial. Lacking sufficient data to set a recommended daily allowance for all healthy individuals, the IOM group instead issued adequate intake levels for various age and sex groups, based on maintaining serum calcidiol levels at 37.5 nmol/L. The adequate intakes are 200 IU (5 mg) per day for all individuals from newborns to age 50, 400 IU (10 mg) for people between the ages of 51 and 70, and 600 IU (15 mg) per day for men and women older than 70.

Recent research suggests that vitamin D insufficiency is widespread, particularly among members of minority groups. For instance, data from a National Health and Nutrition Examination Survey found that 42 percent of African American women had hypovitaminosis, compared with just 4 percent of Caucasians.

But what is the optimal level? "My feeling is that no person should have less than 80 nmol [32 ng/mL]. That's based on cumulative data," said Bruce Hollis, PhD, professor of pediatric biochemistry and molecular biology and director of pediatric nutrition sciences at the Medical University of South Carolina in Charleston. He believes the standard for "normal" was set incorrectly decades ago when the first assays for vitamin D were developed. "They sampled asymptomatic populations and

See **Vitamin D**, continued on page 8

Promoting Vitamin D Testing

Vitamin D, from page 6

measured circulating serum 25-hydroxy vitamin D₃ [calcidiol], but if the community was deficient, it's not a good standard," he explained. His recent research suggests that optimal vitamin D status may be when there are equimolar concentrations of calcidiol and the parent compound, vitamin D₃.

At least one person who sat on the IOM committee that set the original standards believes the recommended intake amounts eventually will be raised to 1,000 IU per day. But, cautions Michael Holick, PhD, MD, professor of medicine, physiology, and biophysics at the Boston University School of Medicine, "It's not going to happen in the near future." Christine Stencel, a spokesperson for the IOM, agrees that updates to vitamin D intakes are probably a ways off. "There's been a lot of discussion on the question of the criteria for when to review the science, and we stand ready to conduct scientific evaluations," she noted. But without a mandate from a federal agency to conduct a review, the IOM cannot conduct an evaluation.

Another issue up for debate is the amount of vitamin D one can take before inducing toxicity in the form of kidney

stones, bone loss, and even calcification of certain organs. As with the intake amounts, the Food and Nutrition Board had little scientific evidence to go by in 1997, so it set what is now considered a very conservative tolerable upper intake level of 2,000 IU (50 mg) per day for children older than age one and all adults.

While studies have found no adverse effects from oral doses as high as 10,000 IU, there is not expert consensus about what the upper limit for nontherapeutic consumption should be. In the opinion of Hollis, the present 2,000 IU upper limit is "a disaster. It's totally wrong. I can't tell you how many studies have had doses of 10,000 IUs and not observed hypercalcemia," he proclaimed.

Others sound a note of caution. "I think it should be individualized to some extent," said Feldman. "That's the problem with a public health message intended for thousands of healthy people. There are individuals in the population who tend towards making kidney stones, or who may develop vascular calcification and cardiovascular disease, and you have to take them into account in deciding on the recommended dose. I think the dose recommendation definitely should be increased and the range considered vitamin D sufficient should be

A Brief History of Vitamin D

First identified in the 1920s, vitamin D is not a vitamin in the classic sense of being a nutrient the body needs in small amounts but can't make on its own. In reality, it is a prohormone that is biologically inactive until it is metabolized into a secosteroid, similar to classic steroid hormones like testosterone, progesterone, and cortisol. Vitamin D is available both from sunlight and dietary sources such as cod liver oil, fatty fish like salmon, and vitamin D-fortified milk. Individuals who receive enough sunlight on a regular basis do not need vitamin D from their diet.

A series of discoveries dating from the 1960s established that vitamin D is involved in a tightly regulated endocrine system involving the liver and kidneys, as well as multiple target organs. However, for public health and nutritional reasons it continues to be classified officially as a vitamin. Vitamin D is an umbrella term for vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol), along with metabolites and analogues of these substances.

raised, but I'm leery of a huge dose for the general population until more data proving the benefits in disease prevention are developed. However, in people who already have cancer or other diseases, or who are at increased risk of developing such diseases, higher doses may already be indicated. For example, in such individuals, the risk-benefit ratio is different and they can be given higher amounts." Many vitamin D-related studies are using high-dose analogues, which have less calcemic activity, often in combination with chemotherapy or anti-inflammatory agents, he noted.

In the absence of official updates, some professional groups are beginning to take the lead in recommending higher intakes. In Canada, for example, Health Canada-endorsed adequate dietary amounts mirror those in the U.S., but the Canadian Paediatric Society issued updated guidelines in September 2007 calling for administration of 2,000 IU per day to pregnant and lactating women. For full-term infants, the guidelines recommend 400 IU per day, with 800 IU per day in winter months at higher latitudes.

Accurate Assays a Must

While scientists and professional groups deliberate on vitamin D intake amounts, laboratorians have a role in the here-and-now of patient care. The quality of commercial tests has improved significantly over the past two decades, according to Graham Carter, MSc, organizer of the Vitamin D External Quality Assessment Scheme (DEQAS), a London-based program to en-

sure the analytical reliability of calcidiol and calcitriol assays, in which more than 70 U.S. labs participate. Several of the most commonly used commercial tests are capable of producing consistently accurate results, but there is still a "considerable degree" of variation between laboratories, he says. There also have been problems with certain tests, particularly in having sufficient reactivity to calcidiol. For that reason, Carter urges laboratorians to participate in quality assessment programs and be familiar with the limitations of the methods they are using. "If your test underestimates 25-hydroxy vitamin D₃ [calcidiol], then clinicians should be told about it," he recommended.

In addition to DEQAS, the College of American Pathologists offers quality assessment for vitamin D assays. The National Institute of Standards and Technology (NIST) expects to make standard reference materials available in early 2008, according to Karen Phinney, PhD, a research chemist at NIST.

Laboratorians also can help spread the word about the necessity of vitamin D testing, says Feldman. "They can help put it on the radar screen. Most physicians don't measure it unless they're treating patients with osteoporosis, for example. But it needs to be part of the world of good medical practice, what we think of in terms of management of good health." CLN

Gina Rollins is a freelance writer based in Silver Spring, Md. Email: rollinswrites@verizon.net.

Recommended Reading

For more on the link between vitamin D and reduction in all-cause mortality, see:

Autier P and Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med* 2007;167:1730-1737.

For more on outcomes from the ASCENT trial, see:

Beer TM, Ryan CW, et al. Double-blinded randomized study of high-dose calcitriol plus docetaxel compared with placebo plus docetaxel in androgen-independent prostate cancer: a report from the ASCENT Investigators. *J Clin Oncol* 2007;25(6):669-74.

For more on vitamin D epidemiology, see:

Garland CF, Grant WB, et al. What is the dose-response relationship between vitamin D and cancer risk? *Nutr Rev* 2007;65(8 Pt 2):S91-5.

For more on vitamin D testing, see:

Hollis BW and Horst RL. The assessment of circulating 25(OH)D and 1,25(OH)₂D: where we are and where we are going. *J Steroid Biochem Mol Biol* 2007; 103(3-5):473-476.



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HDL-C

The Changing Testing Paradigm

Part 2

BY ALAN T. REMALEY, MD, PHD, AND G. RUSSELL WARNICK, MS, MBA

Assessing a patient's risk of coronary heart disease (CHD) routinely involves measuring one of the major classes of lipoprotein particles, high-density lipoprotein (HDL). The concentration of cholesterol carried on HDL particles, HDL-C, has been the main metric for this assessment, originating from early studies that first showed the link between cholesterol and atherosclerosis. But some patients whose HDL-C levels and other traditional risk factors fall within the recommended levels set by the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III (ATP III) still suffer adverse CHD events.

Research has now conclusively shown that the major lipoprotein classes are not homogeneous entities, but rather heterogeneous and polydisperse populations of particles with varying composition, physical characteristics, and pathophysiologic significance for the development of atherosclerosis. Researchers have discovered that not all HDL-C particles confer the anti-atherogenic properties ascribed to this so-called "good" cholesterol molecule.

In the first part of this review (*CLN*, November 2007, page 10), we described the latest findings on the atherogenic properties of HDL and the status of HDL lab mea-

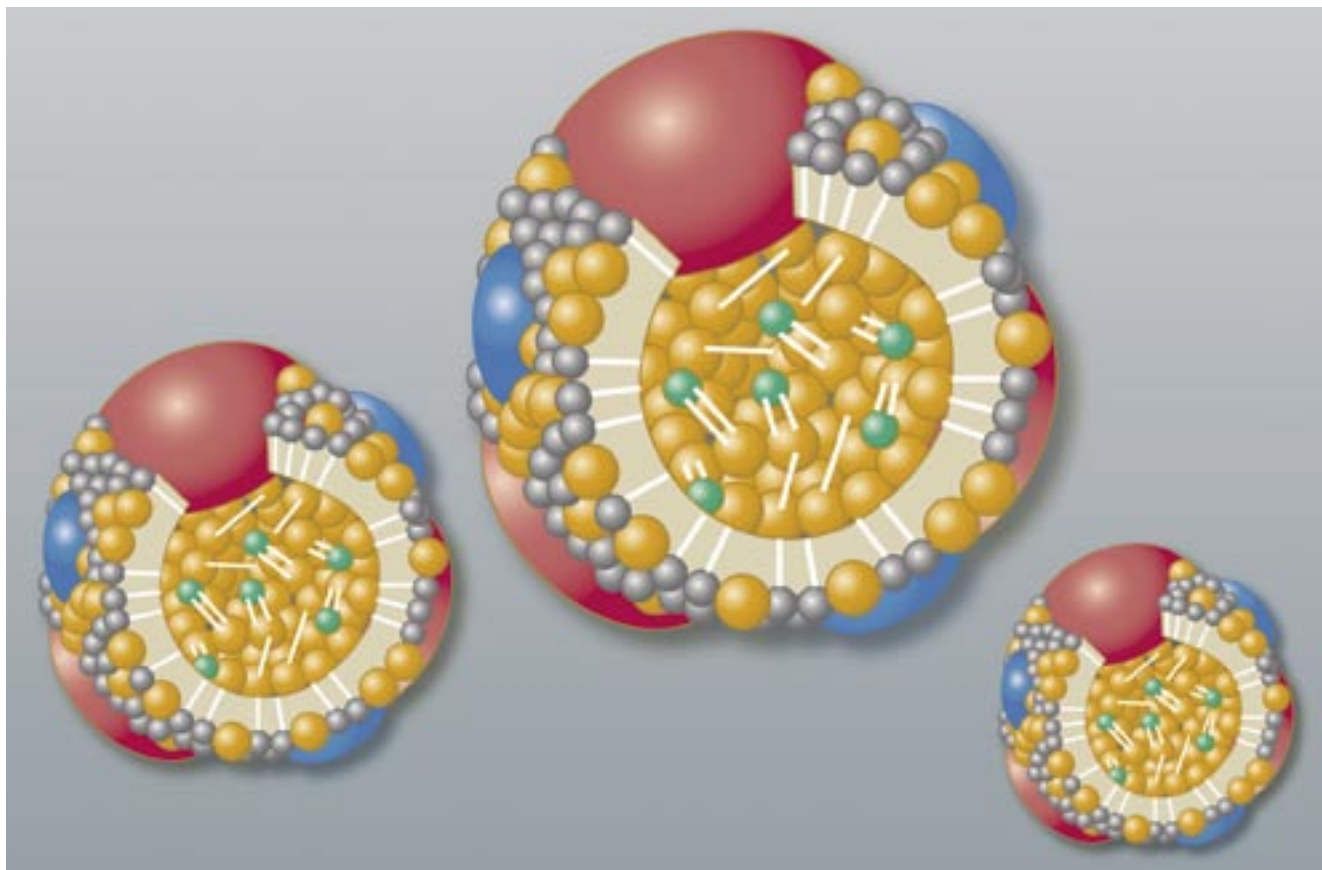
surements. Here we discuss the various lab methods for measuring the subclasses of HDL and the current controversy in the use of these measurements in predicting CHD. method was followed by density gradient ultracentrifugation, which can resolve up to five different subclasses of HDL. While tedious to perform, it is still considered the

tently revealed that the large apoA-I containing lipid-rich, less-dense forms of HDL appear to be atheroprotective. Furthermore, in some studies the smaller, denser forms of HDL positively correlate with CHD risk (1, 2).

The remaining tests in Table 1 are more practical alternatives for HDL subclass identification and are currently offered by reference labs that specialize in advanced lipid and lipoprotein testing. Berkeley HeartLab (Burlingame, Calif.) offers a stepped 4–30%, nondenaturing, gradient polyacrylamide gel separation for HDL subclass separation (2). After the HDL subclasses are separated on the gel and stained, the gel is analyzed by densitometric scanning to quantify the amount of each subclass (Figure 1). Initially validated against analytical ultracentrifugation, this method electrophoretically resolves HDL and an apoE-rich form of HDL that can migrate with LDL into five subclasses similar to that seen in ultracentrifugation. Consistent with other methods, gradient gel electrophoresis reveals that the larger apoA-I containing particles are more atheroprotective (2).

A simpler method for the electrophoretic separation of HDL uses a linear tube gel that separates HDL into three subclasses. Quantimetrix Corporation (Redondo Beach, Calif.) is developing this assay, which is similar to an LDL-subfraction method that is currently offered by the company. This type of assay would fall within the technical capability of most clinical labs.

Another test for resolving HDL subclasses uses nuclear magnetic resonance (NMR). LipoScience's (Raleigh, N.C.) NMR analysis of lipoproteins depends on the proton vibrational signal generated by the terminal methyl groups on the lipids in HDL. The frequency and shape of the NMR signal is affected by the lipoprotein particle size (3). After a mathematical deconvolution of the signal, the method provides the amount of three subclasses



surements. Here we discuss the various lab methods for measuring the subclasses of HDL and the current controversy in the use of these measurements in predicting CHD.

Analytical Methods for Measuring HDL Subclasses

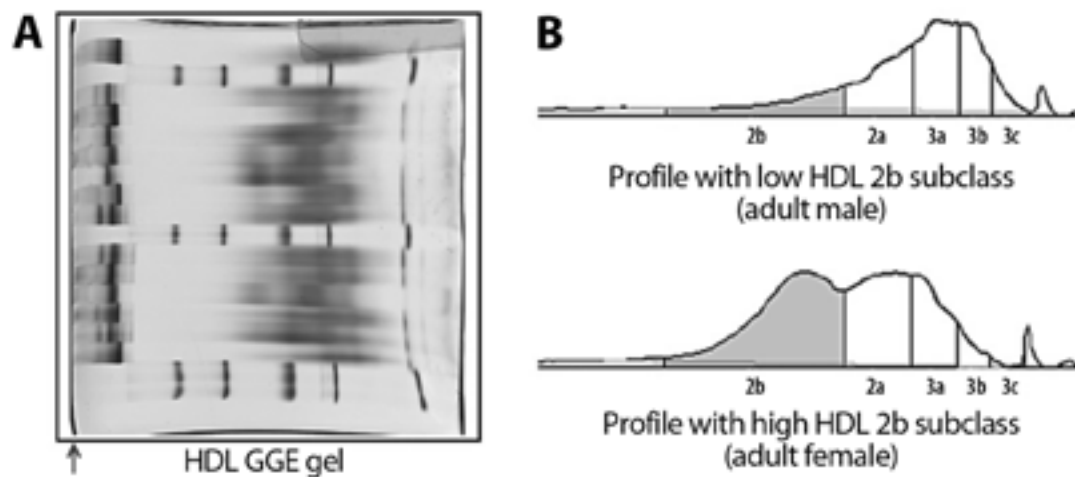
Table 1 lists the various methods for determining HDL subclasses. The assays each resolve and detect HDL subclasses using different physical properties, so the results of different assays are not necessarily interconvertible.

Scientists first used analytical ultracentrifugation to resolve HDL subclasses. This

gold standard for separating HDL lipoprotein subclasses.

A higher resolution method, two-dimensional agarose gel electrophoresis, can resolve up to 14 different subclasses of HDL. In the first dimension, HDL subclasses separate largely based on charge into pre-beta, alpha, and pre-alpha migrating forms. The second dimension separates the particles by size under nondenaturing conditions (1). This method is technically demanding, very tedious, and therefore impractical for clinical laboratories. However, a variety of subclass separation methods, including the two-dimensional separations, have consis-

Figure 1 Analysis of HDL Subclasses By Gradient Gel Electrophoresis



Panel A: Stained polyacrylamide gel showing separation of HDL subclasses. The arrow indicates origin of gel. Size standards are present at ends of the gel and in the middle lane.

Panel B: Densitometric scan of a representative gel. Top right panel is a gel from an individual with a low level of a large HDL subclass (HDL2b), whereas the bottom right panel is from a subject with a high level of HDL2b.

of HDL. LipoScience calibrated the assay using different HDL subclasses isolated by density gradient ultracentrifugation. In addition to HDL subclass information, the method also yields information on HDL particle number, which, like LDL particle number by NMR versus LDL-C, appears to be superior to HDL-C for predicting CHD risk (4).

Another method for separating HDL into two subclasses by density involves a vertical rotor. A continuous-flow cholesterol analyzer, called a vertical auto profiler (VAP), monitors fractions from the centrifuge tube for cholesterol (5). Compared to standard ultracentrifugation procedures, VAP analysis is easier and faster to perform. Atherotech (Birmingham, Ala.) offers this test, along with other lipid and lipoprotein testing.

The final method for separating HDL subclasses is based on a method developed by Okazaki and colleagues (6). The method separates HDL particles into four size forms by high-pressure liquid chromatography (HPLC). Skylight BioTech, Inc. (Akita, Japan) offers this method under the name LipoSEARCH.

HDL Immunoassays

Researchers have also worked to develop immunoassays against specific subfractions of HDL. An ELISA produced by Daiichi Sankyo (Tokyo, Japan) and distributed in the U.S. by Polymedco (Courtland Manor, N.Y.) measures pre-beta HDL, the lipid-poor discoidal-shape HDL that is especially good in mediating cholesterol efflux from cells by the ABCA1 transporter (See Figure 1 in part 1 of this article, *CLN* November, 2007). Interestingly, although pre-beta HDL has anti-atherogenic properties when tested in vitro, its concentration in plasma positively correlates with CHD risk (7). It may be that the accumulation of pre-beta HDL in vivo indicates that there is an aberration in the HDL maturation process,

and when this occurs there is a decrease in the net flux of cholesterol by the reverse cholesterol transport pathway. In fact, genetic defects in HDL metabolism—such as mutations in the ABCA1 transporter in Tangier disease—lead to an overall decrease in HDL but to a relative increase in pre-beta HDL and an increased risk for CHD (8).

HDL Functional Assays

Based on the knowledge of HDLs in vivo function, researchers have also attempted to develop functional assays for measuring it, such as the ability of HDL to remove cholesterol from cells, to inhibit the expres-

sion of the adhesion protein VCAM-I on endothelial cells, and to inhibit the production of pro-inflammatory cytokines from macrophages. At this time, these assays are mostly reserved for research studies, but they may lead to new insights into developing more practical tests for assessing the anti-atherogenic functions in the clinical laboratory.

One approach that shows early promise is a test for the anti-oxidative capacity of HDL (9). The first assays of this function were complicated, involving the use of cells, but cell-free assays have now been developed that could potentially be automated.

The anti-oxidative capacity of HDL appears to be particularly useful in distinguishing fully-functional good HDL from dysfunctional or even bad-good HDL. In small studies, the assay has even been shown to be better than HDL-C in predicting CHD risk (9).

Outlook for HDL Testing

Clearly, there is still much uncertainty about how HDL works to protect against CHD and how its composition impacts its function. In terms of testing in clinical labs, compositional assays are almost always easier to implement than functional assays. But the current analytical procedures used to isolate and measure HDL may alter its composition, particularly for those constituents that are weakly associated.

There are many ongoing studies examining the clinical utility of HDL subclass assays in different populations, and more information on their clinical utility should be available shortly. Importantly, at a December 2006 meeting of the FDA's Clinical Chemistry and Clinical Toxicology Devices Panel, members of the advisory board recommended that HDL and LDL lipoprotein subfraction tests may be useful to help determine the need to treat patients at intermediate risk for CHD as determined by more conventional tests. They further advised that more data are needed to support more widespread use of these assays.

In summary, although HDL-C has been useful for predicting CHD risk and for managing patients with dyslipidemia, its predictive ability is still rather limited as demonstrated by the Torcetrapib (Pfizer, New York, N.Y.) clinical trial discussed in part one of this review. Briefly, the drug raised HDL-C approximately 50% by

Table 1
Methods for Analysis of HDL Subclasses

Test Type	Methods	Lab/Manufacturer	Principle	Measurement	Number of HDL Subclasses
Research Tests	analytical ultracentrifugation		density	reflectance	5
	density gradient ultracentrifugation		density	cholesterol	5
	2D-gel electrophoresis		charge and size	immunoblot of apolipoproteins	14
Reference Tests	gradient gel electrophoresis	Berkeley HeartLab (Burlingame, Calif.)	4-30% stepped gradient gel of d<1.21	protein by Coomassie dye	5 + E-rich
	nuclear magnetic resonance	LipoScience (Raleigh, N.C.)	proton shift 0.8ppm	terminal methyl group protons of phospholipids, triglycerides, free and esterified cholesterol	3
	vertical auto profile	Atherotech (Birmingham, Ala.)	vertical rotor ultracentrifugation	cholesterol	2
Products	gel electrophoresis (Lipoprint)	Quantimetrix (Redondo Beach, Calif.)	linear gel	Sudan black pre-stain	3
	high-pressure liquid chromatography (LipoSEARCH)	Skylight BioTech (Okazaki, Japan)	gel permeation	cholesterol	4

increasing the amount of large-sized HDL; however, the trial was stopped because participants experienced a significant increase in cardiovascular deaths (10).

Future advances in HDL testing will most likely go beyond just measuring cholesterol content. Evidence is accumulating that HDL-C is not synonymous with HDL and that this single parameter of HDL does not embody all its diverse compositional, structural, and functional characteristics. Better indices of HDL function, structure, and quality are clearly needed now for research and drug development, and perhaps ultimately in the establishment of HDL-based treatment goals for patients.

For most clinical labs, the only viable alternative to measuring HDL-C is apoA-I, which is probably underused, given its superior predictive ability over HDL-C in many clinical studies. Consequently, there

is a growing interest and support in the possible inclusion of apolipoprotein tests in national testing guidelines for CHD risk as an alternative to HDL-C and LDL-C tests.

While HDL subfraction tests currently available from specialty reference labs can be useful for deciding how to treat patients at intermediate risk for CVD, such tests will likely have limited use until more convenient formats become available that are suitable for routine clinical lab use. With new insights gained from research on HDL metabolism and better characterization of the proteome and lipidome of HDL, clinical laboratories will likely have tests that better distinguish the good and "bad-good" forms of HDL. Such tests represent the next major advance in lipid and lipoprotein testing for CHD risk assessment. **CLN**

REFERENCES

1. Asztalos BF, Collins D, Cupples LA, et al. Value of high-density lipoprotein (HDL) subpopulations in predicting recurrent cardiovascular events in the Veterans Affairs HDL Intervention Trial. *ATVB*. 2005; 25:2185-91.
2. Warnick GR, McNamara JR, Boggess CN, et al. Polyacrylamide gradient gel electrophoresis of lipoprotein subclasses. In: Smith DA, guest editor. *Clinics Lab Med*. 2006; 26:803-46.
3. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. In: Smith DA, guest editor. *Clinics Lab Med*. 2006; 26:847-70.
4. Otvos JD, Collins D, Freedman DS, Shalurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ. Low-density

lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation* 2006; 113(12):1556-63.

5. Kulkarni KR. Cholesterol profile measurement by vertical auto profile method. In: Smith DA, guest editor. *Clinics Lab Med* 2006; 26:787-802.

6. Okazaki M, Usui S, Fukui A, et al. Component analysis of HPLC profiles of unique lipoprotein subclass cholesterol for detection of coronary artery disease. *Clin Chem* 2006; 52:2049-53

7. Hirayama S, Miida T, Miyazaki O, Aizawa Y. Pre beta1-HDL concentration is a predictor of carotid atherosclerosis in type 2 diabetic patients. *Diabetes Care* 2007;30(5):1289-91.

8. Zannis VI, Chroni A, Krieger M. Role of apoA-I, ABCA1, LCAT and SR-BI in the biogenesis of HDL. *J Mol Med* 2006;84:276-94.

9. McMahon M, Grossman J, FitzGerald J, Dahlin-Lee E, Wallace DJ, Thong BY, Badsha H, Kalunian K, Charles C, Navab M, Fogelman AM, Hahn BH. Pro-inflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum* 2006;54:2541-9.

10. Schaefer EJ, Asztalos BF. Where are we with high-density lipoprotein raising and inhibition of cholesteryl ester transfer for heart disease risk reduction? *Curr Opin Cardiol*. 2007;22:373-8.

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**Clinical
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The Growth of Mass Spectrometry Analysis

New Guidance Addresses Lack of Lab Resources

BY MELISSA D'ARCHANGELO

Mass spectrometry (MS) has been widely used in science and industry for decades, but its considerable analytical advantages have only become available to clinical laboratories in recent years. The technology is now taking on an increasingly important role for analysis of biological samples due to the development of more user-friendly, affordable, and versatile instruments, as well as greater availability of internal standards. Known for its ability to produce high-quality results with superior selectivity, MS is particularly useful for analyzing multiple analytes, such as in expanded newborn screening programs.

Although mass spectrometers have decreased in price and complexity, clinical laboratories interested in employing this technology still must overcome some significant challenges. Developing assays, acquiring high-quality standard materials, training and maintaining highly qualified staff, adopting methods for quality control, and reporting results remain difficult for many laboratories.

"Many clinical chemists are intimidated by a mass spectrometer because it is not a tool that most are trained on or historically have even had an opportunity to use," said Donald H. Chace, PhD, of Pediatrix Analytical in Bridgeville, Pa., who is chairholder of the Clinical and Laboratory Standards Institute (CLSI) (Wayne, Pa.) subcommittee that developed a recently released guideline for the use of MS in the clinical laboratory. The support networks are not nearly as large as those for commercial auto-analyzers in hospitals, and mass spectrometrists skilled in its operation are often difficult to find."

Alan L. Rockwood, PhD, of the University of Utah Department of Pathology and ARUP Laboratories in Salt Lake City, Utah, and a member of the CLSI committee, notes that more clinical labs are adopting the technology, but that until now, few applicable resources have been available to them. "Mass spectrometry is in an exponential growth phase, but it is necessary to support that growth with reliable, useful resources to enable the clinical laboratory to address the challenges of using this complex instrument," he advised. The recently released guideline from CLSI, *Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline (C50-A)*, offers a general understanding of MS and the principles that dictate its application in the clinical laboratory (See Sidebar).

Mass Spectrometry: Then and Now

Because of the cost of equipment, and complexity of sample preparation and interpretation of results, laboratories using MS were often specialized reference or university-based medical centers. However, MS has been a mainstay of metabolic profiling. "The roots of MS in clinical applications are in metabolic profiles, more specifically organic acid analysis in urine extracts,"

explained Chace. "A compound could be identified by its retention time and mass-to-charge value. Due to this power and the advent of improved chromatography such as capillary gas chromatography (GC), urine organic acid analysis by GC/MS became the gold standard. Other assays were developed on this platform, such as steroids, amino acids, and carbohydrates, but generally only in highly specialized metabolic laboratories or centers of excellence."

The development of more user-friendly, affordable, and versatile mass spectrometers has since allowed a large increase in the use of MS for clinical applications. In addition, over the last two decades, advances in ion source design have made it possible to analyze more water-soluble, polar compounds, including peptides, proteins, oligonucleotides, DNA, and trace elements.

One advance that greatly enhanced the application of MS in clinical labs is a process called ionization. "New ionization techniques that allow analysis of polar compounds without the need for extensive derivatization enabled applications in clinical analysis in the late 1980s and early 1990s. For example, some of the metabolites of special interest were organic acids in blood that mark metabolic disease. But, the real developments at this time were not so much in the area of mass analysis, but in ionization—the process of making ions from molecules in solution."

Thanks to those developments, newborn screening by MS became feasible and continues to be an area where MS is used extensively. "Expanded newborn screening measures many analytes simultaneously from two or more classes of molecules, such as amino acids and fatty acid conjugates known as fatty acylcarnitines," noted Rockwood. "A mass spectrometer, in particular a tandem mass spectrometer, has good specificity and the ability to measure many analytes simultaneously using a relatively small sample and relatively simple sample preparation." Mass spectrometers also have high throughputs, Rockwood pointed out. "These qualities combine to make expanded newborn screening practical, whereas it may not be practical when using most other technologies, such as immunoassays."

Challenges of Using MS

Despite the growth of testing by MS and its many advantages, there remain several challenges to its use in the clinical laboratory. "Few graduates enter the workforce with practical, hands-on experience with mass spectrometers, and without a good understanding of this technology it is possible to generate meaningless or misleading results," noted Mark W. Duncan, PhD, of University of Colorado Health Science Center Fitzsimons (Aurora, Colo.), and a member of the CLSI committee.

Although the basic MS technology has been around for decades, a mystique still surrounds its use. "MS has been a rather specialized field, and it is a technology that is somewhat unfamiliar to most people working in clinical laboratories," Rock-

How to Obtain the Mass Spec Guidance Document

CLSI welcomes comments and questions about its guidance documents. This feedback serves as the basis for updating published documents. All comments and responses are formally addressed and published in the next edition of the document. *Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline, Document C50-A*. Visit www.clsi.org.

wood observed. Along with training and understanding of the equipment, there are other problems with the widespread adoption of MS. "Acquiring high-quality standard materials, such as calibrators and controls, is sometimes a challenge. Maintaining and operating instrumentation to a high standard can also be difficult because mass spectrometers are complex instruments that have not yet been optimized for use in clinical laboratories." And, as Rockwood points out, "Proficiency testing is still being developed for some analytes for which an MS peer group has not yet been established."

A Reference and Resource

To help laboratorians offer MS-based testing, the new CLSI MS guideline provides accurate and state-of-the-art information. It is divided into six areas: advantages and disadvantages of the methodology; precautions for the use of MS; quality control considerations; assay verification/validation; approaches to reporting results; and communication of the data. To illustrate these concepts, the document uses concrete examples of how MS is currently being used.

Members of the committee are enthusiastic about what the new guideline offers to both novice and expert laboratorians. "The guideline has been carefully crafted using CLSI's consensus process with input from a group with diverse experience," noted Duncan. "If you have little or no experience with mass spectrometry, this is a good place to start. For the expert, the document highlights the areas of importance and draws the critical information together in a single document."

Rockwood echoes that enthusiasm. "In many cases, the CLSI MS guideline will be where a laboratorian will find answers to questions such as how to set up a selection matrix for matching application classes to instrument types, or what factors to consider when setting up quality control programs.

Committee members also believe that the new document will provide a reference for a large audience serving as an educational resource for both the practitioners of MS and the medical professionals who use the results produced by the instruments for the diagnosis, characterization, or monitoring of disease. In particular, the committee aimed to help medical professionals better understand why MS may be preferred for a clinical application, and allow them to be more informed consumers when selecting a diagnostic laboratory to provide MS services, noted Chace. "A physician may refer to the CLSI MS guideline to determine


what advantage a mass spectrometer offers in the analysis of testosterone. A physician may ask, 'Are MS results more reliable, or simply better for my patients because of a more accurate diagnosis?' The guideline would be a place to find the answer," he noted.

What's Next?

The use of MS in clinical labs will no doubt continue to expand, with many exciting possibilities on the horizon. "Soon, nearly every infant in the United States will be screened using tandem MS," predicted Chace. "Numerous other countries around the world are also embracing this technology. Applications in other areas, including steroid analysis, demonstrate how a mass spectrometer can be used to improve both precision and accuracy when quantifying structurally similar compounds that are present at widely different concentrations. This is an area where immunoassays are often prone to failure or are notoriously unreliable," he said.

"Although newborn screening is a major clinical application, it primarily resides in specialized laboratories, including public health laboratories. As the larger commercial and private laboratories recognize the advantages of MS, its application will expand to other areas where it can serve to complement other analytical approaches," he added.

The field of proteomics will also bring more MS applications to labs. As scientists conduct studies aimed at identifying protein biomarkers, MS will be at the heart of most of these biomarker discovery studies. "So far in the clinical laboratory, MS has mainly been applied to the quantitative analysis of small molecules. In the future, we are likely to see this expand into other areas, such as protein and peptide analysis, genetic analysis, and infectious disease testing," added Rockwood.

Duncan emphasizes the analytical capabilities of MS as the reason that the technology will expand. "The mass spectrometer offers exceptional selectivity as a chromatographic detector. This leads to an increase in both precision and accuracy. Decreasing system costs and complexity now make these benefits accessible in many more situations," he said. "We will increasingly see mass spectrometers take the place of other systems in the routine testing laboratory." 

Melissa J. D'Archangelo is a marketing and communications consultant based in Chester Springs, Pa. Email: mdarchangelo@comcast.net

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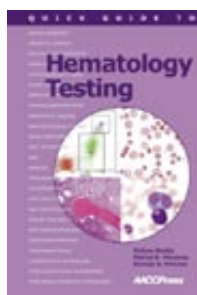
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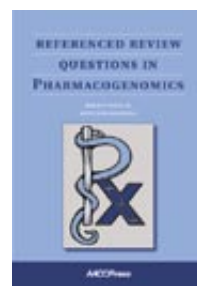
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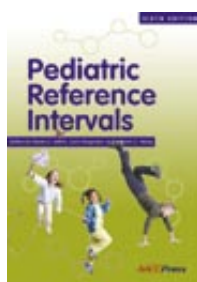
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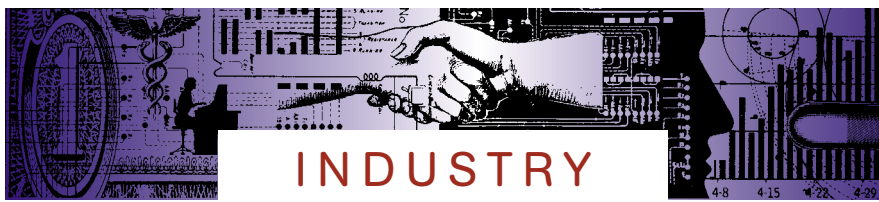
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Beckman Coulter Licenses Johns Hopkins Cancer Data

Beginning with 200 genes linked to breast and colon cancer, two licensing option agreements with Johns Hopkins University (Baltimore, Md.) give Beckman Coulter (Fullerton, Calif.) first access to Hopkins' cancer genomics studies, including sequencing services and options

to license intellectual property from ongoing studies on six other cancers. "These unprecedented agreements put Beckman Coulter in a unique, leading-edge position in the molecular diagnostics field," said Bruce Wallace, VP of Beckman Coulter's Molecular Diagnostics Business Center. "Beckman Coulter will have the exclusive option to license any of the genetic mutations discovered in these studies that have

diagnostic potential." Financial terms were not disclosed.

Quest Licenses HIV Co-Receptor Tropism Test

Quest (Madison, N.J.) announced a deal with Pathway Diagnostics (Malibu, Calif.) to offer a molecular HIV co-receptor tropism assay based on Pathway's SensiTrop test technology. The non-exclusive license agreement covers the heteroduplex tracking technology that the SensiTrop test is based on. According to Quest, the assay will help physicians identify patients with certain strains of HIV that make them unresponsive to entry inhibitor anti-retroviral

drugs, such as Pfizer's (New York, N.Y.) Selzentry. Selzentry is the first FDA-cleared therapy in this new class of entry inhibitor drugs that work by blocking the CCR5 co-receptor, hindering the virus's ability to bind to and infect cells. Terms of the agreement were not disclosed.

LabCorp and Medco Ink PGx Research Deal

LabCorp (Burlington, N.C.) and pharmacy benefit manager Medco Health Solutions Inc. (Franklin Lakes, N.J.) announced a research agreement focused on developing a genetic test for patient response to the breast cancer drug tamoxifen. The project will use Roche's (Pleasanton, Calif.) AmpliChip CYP450 test. Approximately 10% of women using tamoxifen do not fully benefit from the drug because of variations in genes coding drug metabolizing enzymes, according to LabCorp.

New Warfarin-Response Test in the Works

Osmetech (Pasadena, Calif.) announced plans for a genetic test to help determine the initial warfarin dose a patient should receive. Osmetech licensed research on a new biomarker from Marshfield Clinic Research Foundation (Marshfield, Wis.) that it plans to incorporate into the test, which CEO James White said the company expects to market in the first half of 2008. "The marker provides additional information to improve the dosage accuracy over any other product on the market and will be run on our recently introduced XT-8 instrument. The addition of unique content to our compelling XT-8 instrument will further enhance our ability to become a significant player in the molecular diagnostics market," White said. Financial terms of the licensing agreement were not disclosed.

PAML and MountainStar Sign Deal for Lab Outreach Services

Pathology Associates Medical Laboratories (Spokane, Wash.) announced an agreement with MountainStar (Cottonwood Heights, Utah), a healthcare system that operates eight hospitals in Idaho and Utah, to enhance outreach laboratory services in Utah. The joint enterprise will be known as MountainStar Clinical Laboratories and will bundle services from the labs at two of MountainStar's hospitals with the esoteric and outreach services of PAML. "The partnership with MountainStar is an important milestone for PAML in the continued expansion of our laboratory joint ventures with leading hospital systems," said PAML CEO Tom Tiffany. "We are confident that our partner health systems will realize the benefits of working in a collaborative fashion to expand outreach lab businesses. Our goal is to provide a turn-key program that will enable a competitive offering to physicians in their local communities." PAML said it will employ its recent investment in a suite of laboratory IT products and services to connect the two hospitals and PAML for the joint venture. Financial details were not disclosed.

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San Diego Area First Location for CMS Competitive Bidding Demonstration; Fires Force Conference Postponement

In mid-October, CMS announced it had selected the San Diego–Carlsbad–San Marcos, Calif. metropolitan area as the first of two locations for a competitive bidding demonstration for clinical laboratory services. The 3-year demonstration, required by the Medicare Modernization Act of 2003, will help determine if competitive bidding can be used to provide laboratory services under Medicare Part B at fees below current Medicare payment rates, while maintaining quality and access to care.

A Bidders' Conference, scheduled for October 31, was canceled due to the fires in the area. At press time, CMS had not yet announced a new date for the event, which will help laboratories in the region understand the purpose of the demonstration project and how it will be implemented, as well as provide a forum to address questions. More information about the Bidders' Conference can be found at www.cms.hhs.gov/center/clinical.asp, click on "Demonstration."

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CDC Awards \$35 Million to Increase HIV Testing Among African Americans

As part of a \$45 million program to expand access to HIV testing, the CDC announced in September it has earmarked \$35 million of these funds for state and local health departments to increase HIV testing opportunities among African Americans. The program is being targeted to 23 states and major metropolitan areas in which African Americans have been most severely affected by HIV/AIDS.

The states receiving funding are: California, Connecticut, Florida, Georgia, Louisiana, Maryland, Massachusetts, Michigan, Missouri, New Jersey, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, and Virginia. Washington, D.C. also will receive support. Other cities receiving funding are: Chicago, Houston, Los Angeles, Philadelphia, and New York City. Awards will range from \$690,000 to \$5.4 million and are based on the percentage of AIDS cases among African Americans in each locale. Disbursement of funds began on September 30.

A focus of the program will be to integrate HIV testing with screening and prevention activities for other infections, such as hepatitis, other sexually transmitted diseases, and tuberculosis. Additional information on HIV prevention is available on the CDC Web site at www.cdc.gov/hiv.

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New Healthcare Codes for Reporting Alcohol, Substance Abuse Screening

New Current Procedural Terminology codes—99408 and 99409—issued in October by the American Medical Association

and scheduled to become effective as of January 1, 2008 will allow U.S. physicians to screen their patients for substance abuse and provide an appropriate intervention. The new AMA Level 1 CPT Codes for medical services are expected to streamline reporting and the reimbursement procedure for physicians who perform structured screening for alcohol and/or substance (other than tobacco) abuse and brief intervention. Current codes do not identify performance of evidence-based screening and brief intervention (SBI), which primarily is a general medical service provided by physicians who are not psychiatrists or behavioral health specialists.

New SBI codes specify that discrete, protocol-driven procedures are appropriately coded by general medical practitioners. According to the Office of National Drug Control Policy, using these new codes will be cost-effective because screening and intervention generally provide for shorter in-patient visits, fewer emergency room visits, improvements in overall health, and reduced substance use. Additional information can be viewed at www.whitehouse-drugpolicy.gov.

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Joint Commission Begins Study Of Rapid Tests for Influenza

A 3-year project begun by The Joint Commission's Division of Quality Research and Measurement in October and funded by the CDC will study how rapid tests for influenza are implemented in outpatient medical settings, including solo and group practice physician offices, community health centers, and acute care hospital emergency departments. The project will examine the types of rapid tests in use and how they are selected; the training and competency of individuals performing testing; the extent to which good laboratory practices and testing guidelines are being followed; the impact of rapid test use on antiviral and antibiotic prescribing practices; and the perceived advantages and disadvantages of using rapid influenza testing.

The first phase of the project involves surveying 5,000 outpatient medical settings to determine how rapid tests for influenza are implemented. In a future phase, 300 of these participants will be interviewed to identify factors that influence adoption of these tests, barriers to implementation, and strategies to overcome these barriers.

Recent accreditation surveys by The Joint Commission found: test controls are not always used as directed by the manufacturer; test kits may be improperly stored and used past their expiration dates; individuals conducting the tests are not always trained to use the test; people conducting or interpreting the tests are not always evaluated with regard to their competence in these areas; staff are confused about the use of confirmatory tests and whether the test is used as a screening or diagnostic tool;

and insufficient policies and procedures in place to support conducting the tests. Additional information can be found at www.jointcommission.org/NewsRoom/News-Releases/nr_102907.htm.

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HHS Announces Health IT, Electronic Records Storage Projects

The move toward using methods to store and exchange medical information electronically took two steps forward in October when Department of Health and Human Services Secretary Michael Leavitt announced two new initiatives. In one, HHS awarded contracts totaling \$22.5 million to nine organizations representing state and regional health information exchanges to begin trials of the Nationwide Health Information Network (NHIN). In the other, Leavitt announced a 5-year demonstration project that will offer extra compensation from Medicare to 1,200 physician practices for using electronic health records in lieu of paperwork.

In a statement Secretary Leavitt said, "These trial implementations are taking place in communities across America that are leading the way to healthcare transfor-

mation using secure, interoperable health information technology. Trial implementations of the NHIN will bring us steps closer to a health IT system that will improve quality of care, increase efficiencies in healthcare, and improve disease prevention." More details on these contracts are available at www.hhs.gov/healthit.

The second initiative, conducted by CMS, will begin in 2008 and will provide financial incentives to physician groups using certified electronic health records (EHRs) to complete certain tasks online, such as recording laboratory test results. Bonuses also will be available. "We want to revolutionize the way vital health data is managed and maintained, so we are taking steps to change from a paper-based medical record to an electronic health record," said CMS Acting Administrator Kerry Weems. "This project will appropriately align incentives to reward doctors in small physician practices who use certified EHRs as tools to deliver higher quality care." CMS also is encouraging private insurers to offer similar incentives for adopting EHRs. Additional information can be viewed at www.hhs.gov/news/press/2007pres/10/pr20071030a.html.



Study Emphasizes Extra Troponin Testing in Non-ST ACS

Because even mild but persistent minor cTnI elevation can predict mortality during long-term follow-up after a non-ST ACS (NSTACS) episode, further troponin testing is warranted after hospital discharge, suggests a new study (*Circulation* 2007; 116:1907–1914).

To assess the prevalence and prognostic importance of persistent cTnI elevation in stable patients who have had an NSTACS event, Swedish researchers measured cTnI in 1,092 stabilized patients at 6 weeks, 3 months, and 6 months after enrollment in the FRagmin and Fast Revascularization During InStability in Coronary Artery Disease (FRISC-II) trial. The researchers measured cTnI with the Access AccuTnI assay (Beckman Coulter, Fullerton, Calif.) using

different prognostic cutoffs, and assessed outcomes through 5 years.

At 6 weeks, 48% of patients had elevated cTnI levels >0.01 µg/L, while 36% had similar levels at 6 months, and 26% had such levels at all 3 points. cTnI elevation was associated with increased age and other cardiovascular high-risk features. The lowest tested cTnI cutoff (0.01 µg/L) was most useful for prognosis and was independently predictive of mortality (HR 2.1, 95% CI, 1.3–3.3) in multivariable analysis adjusted for cardiovascular risk factors and randomization to an invasive versus non-invasive treatment strategy. But the lowest cutoff was related to myocardial infarction only in univariate analysis. “From a prognostic perspective, cTnI 0.01 µg/L as cutoff performed at least as well as the previously described 99th percentile. In view of these results, the question of how to define ‘tro-

ponin elevation’ needs to be addressed,” the researchers wrote. They suggested that when identifying high-risk patients, troponin elevation should be defined by the lowest reliably measurable concentration rather than the 99th percentile.

Early CRP Measurement Advised in ACS

Because increased baseline concentration of CRP is strongly associated with mortality and heart failure across the ACS spectrum, CRP measurement with index diagnosis-specific cutpoints should occur soon after presentation, according to recent research (*Clinical Chemistry* 2007; 53: 1800–1807). Researchers from Brigham and Women’s Hospital in Boston, Mass. measured CRP upon admission in 3,225 patients who participated in the Orbofiban in Patients with Unstable Coronary Syndromes (OPUS)—Thrombolysis in Myocardial Infarction (TMI) 16 trial of patients with unstable angina, non-elevation myocardial infarction (NSTEMI), and STEMI. The researchers compared CRP concentrations in patients who suffered an adverse cardiac outcome within 10 months of study entry and in patients who had no adverse events. Samples were collected within 48 hours of symptom onset. Patients in the highest quartile of CRP, compared to those in the lowest quartile, were at increased risk of death at 30 days (adjusted HR 4.6) and at 10 months (adjusted HR 3.9). In patients with unstable angina (NSTEMI), CRP >3.0 mg/L was associated with 10-month mortality (adjusted HR 2.3), whereas in STEMI, a relationship with mortality was seen at CRP > 10 mg/L (adjusted HR 3.0). Increased concentrations of CRP were strongly associated with the development of heart failure at 30 days (adjusted HR 8.2) and at 10 months (adjusted HR 2.6).

Low LDL-C Doesn’t Mitigate Risk from High HDL-C Levels

HDL-C levels can predict major cardiovascular events (CVEs) in patients on statins, even among those with LDL-C levels below 1.8 mmol/L, according to a recent paper (*The New England Journal of Medicine* 2007; 357: 1301–1310). While researchers have long known that a low HDL-C level is a powerful predictor of increased cardiovascular risk, it’s been unclear whether a low HDL-C level would remain a significant risk actor in people whose LDL-C has been reduced to very low levels.

In a posthoc analysis of the Treating to New Targets (TNT) study, researchers from seven sites in the U.S., the Netherlands, and France assessed the predictive relationship between HDL-C and clinically evident CHD using time to a first major cardiovascular death as an outcome measure in 9,770 patients age 35 to 75 with clinically evident CHD. In both univariate and multivariate analyses, the research team determined the predictive relationship between both HDL-C levels at the third month of statin treatment and first major cardiovascular event. The team also assessed specific LDL-C strata, including subjects with LDL-C <1.8 mmol/L. The HDL-C levels in patients who

received atorvastatin were predictive of major cardiovascular events across the TNT study cohort, both when HDL-C was considered as a continuous variable and when subjects were stratified according to quintiles of HDL-C level. When the analysis was stratified according to LDL-C level, the relationship between HDL-C level and major cardiovascular events was of borderline significance. Even among study subjects with LDL-C levels <1.8 mmol/L, those in the highest quintile of HDL-C were at less risk for major cardiovascular events than those in the lowest quintile.

White Blood Cell Count Linked to Cancers

For the first time, researchers have demonstrated that a high WBC is associated with incident invasive breast, colorectal, endometrial, and lung cancer among postmenopausal women, providing a boost to a hypothesis of a causal link between inflammation and the initiation, promotion, and progression of these tumors (*Archives of Internal Medicine* 2007; 167: 1837–1844). In a prospective cohort study performed at 40 U.S. clinical centers, researchers examined data from 143,748 postmenopausal women who were free of cancer at baseline, age 50 to 79 and participants in the Women’s Health Initiative. The main outcome measures were incident invasive breast, colorectal, endometrial, and lung cancers.

In multivariate models, researchers observed a graded association of WBC count with incidence of all four types of cancer. Compared with the lowest quartile of WBC count (2.50–4.79 X 10⁹ cells/L), women with WBC counts in the upper quartile (6.80–15.00 X 10⁹ cells/L) had a statistically significantly higher risk of invasive breast cancer (HR 1.15, 95% CI, 1.04–1.26), colorectal cancer (HR 1.19, 95% CI, 1.00–1.41), endometrial cancer (HR 1.42, 95% CI, 1.12–1.79), and lung cancer (HR 1.63, 95% CI, 1.35–1.97). The findings were similar when cancer that occurred during the first 2 years of follow-up was excluded. Statistically significant associations remained for invasive breast cancer and endometrial cancer when the analyses were limited to nonsmokers. The WBC count also was statistically significantly associated with breast cancer, lung cancer, and overall mortality. “Before these finding can be applied clinically, they should be replicated in other populations, preferably with two measures of WBC count, and expanded to include other biomarkers of inflammation that may be more specifically linked to underlying causal mechanisms of neoplasia,” the researchers wrote.

CORRECTION: A diagnostic profile entitled “New High Sensitivity TnT Assay Is Useful in Heart Failure” that appeared on page 18 of the November 2007 issue incorrectly cited research about a new high sensitivity cardiac troponin T assay’s usefulness in chronic heart failure and incorrectly reported the name of the relevant study. The study’s name is the Valsatran Heart Failure Trial and the correct citation is *Circulation* 2007; 116: 1242–1249. CLN regrets these errors.

NEWS FROM THE FDA

Second Test Cleared for Nanosphere's Verigene System

Nanosphere (Northbrook, Ill.) announced the FDA clearance of its Verigene F5/F2/MTHFR nucleic acid test that detects gene mutations associated with blood coagulation disorders and problems metabolizing folate. The assay identifies mutations in three genes—F5, F2, and MTHFR—that together can increase a person's risk for developing blood clots. Both the F5 and F2 genes encode proteins involved in coagulation, and the F2 protein is an active precursor of thrombin. The MTHFR gene encodes an enzyme important to synthesis of the primary circulating form of folate.

CDRH Posts List of Guidance Docs for 2008

The FDA's CDRH released a list of the guidance documents it wants to tackle next year, encouraging stakeholders to submit comments, draft language, as well as suggestions for new or different guidance documents they'd like to see the agency consider. CDRH emphasized that it probably will not get to all the items on the list, as its staff are frequently called off to review premarket submissions or to work on unplanned guidance documents for de novo devices. Highlights of the list include Assay Migration Studies for IVDs, Clinical Trials Using De-identified Leftover Samples, IVDs for Detection and Differentiation of Influenza Viruses, HCG Tests, IVDMIAs, and CLIA Categorization Procedures and CLIA Waiver Applications. The full list, including instructions on how to submit comments, is available online at www.fda.gov/mdufma/guidance/agenda/fy08.html.

Rapid HIV Test Gets CLIA Waiver

Chembio Diagnostics (Medford, N.Y.) announced that the FDA has granted a CLIA waiver for its rapid HIV test kit, which is marketed by Inverness Medical In-

novations (Waltham, Mass.) as the Clearview COMPLETE HIV 1/2 test. The test is a single-use, self-contained, closed system for collecting, processing, and analyzing whole blood, serum, or plasma for the detection of HIV 1 and HIV 2 antibodies.

Two Quest POC Tests Cleared

Quest Diagnostics (Madison, N.J.) announced that its HemoCue (Lake Forest, Calif.) and Focus Diagnostics (Cypress, Calif.) subsidiaries received FDA clearance for two new POC tests. The HemoCue WBC analyzer uses a whole-blood test performed on finger-stick samples to help physicians diagnose infection, inflammation, and other conditions. The Herpe-Select Express HSV-2 test, from Focus Diagnostics, aids in the diagnosis of herpes simplex-2 virus.

TB Test Approved

Celtestis (Valencia, Calif.) announced FDA approval of its QuantiFERON-TB Gold In-Tube assay. The blood test detects cell immune responses to proteins associated with TB infection, replacing the original QuantiFERON-TB Gold test. According to Celtestis, the new assay offers the same specificity and accuracy, but adds the in-tube format to simplify testing and better fit with existing lab equipment.

Sepsis Risk Assessment Assay Cleared

bioMérieux (Marcy l'Étoile, France) announced FDA clearance to market the VIDAS BRAHMS PCT test. The first automated test for measuring procalcitonin in the U.S., the assay is intended for use with critically ill patients on their first day of admission to the ICU as an aid to determine their risk for progression to sepsis and septic shock. bioMérieux licensed the use of procalcitonin from BRAHMS AG (Hennigsdorf, Germany) in 2005 for use on its VIDAS immunoassay instrument.

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March 25–26 in San Diego, Calif.

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