SEVERE PRE-ECLAMPSIA ON THE RISE

Rates of pre-eclampsia have steadily climbed in the U.S. from 1980–2010, driven by a very sharp rise of 322% in the rates of severe pre-eclampsia, according to a new study. Characterized by elevated blood pressure and excess protein in the urine of pregnant women, pre-eclampsia causes complications in approximately 3-6% of all pregnancies.

Researchers examined data on 120 million births in the U.S. from national hospital discharge surveys, making it the largest cohort study to analyze changes in rates of pre-eclampsia in this country. During the study period, pre-eclampsia rates rose from 3.4% in 1980 to 3.8% in 2010. This increase was due to the rise in rates of severe pre-eclampsia—from 0.3% in 1980 to 1.4% in 2010. At the same time, rates of mild pre-eclampsia declined, from 3.1% in 1980 to 2.5% in 2010.

In contrast, the rate of severe pre-eclampsia increased from 1.7% in 1980 to 2% in 2010. Among 30–34 year old women, the rate increased somewhat among older women. For example, among 15–19 year old women the rate dropped from 4% in 1980 to 1.4% in 2010. At the same time, rates of mild pre-eclampsia declined, from 3.1% in 1980 to 2.5% in 2010.

The research also sheds light on how maternal age affects pre-eclampsia rates. The rate of mild pre-eclampsia was at increased risk for mild pre-eclampsia, whereas women born in the 1970s were at increased risk for mild pre-eclampsia. Overall, these results suggest that changes in the population proportion smoking during pregnancy impact pre-eclampsia rates across age, whereas changes in obesity by cohort influence severe pre-eclampsia in younger cohorts only, “the authors wrote.

The rate of mild pre-eclampsia decreased over time among women age <30 years. The rate of severe pre-eclampsia decreased somewhat among older women. Among 30–34 year old women, the rate increased from 1.7% in 1980 to 2% in 2010. In contrast, the rate of severe pre-eclampsia showed a consistent increase over time within every maternal age group.

The authors of the analysis believe that increasing rates of obesity along with decreasing rates of smoking over this time period may explain their findings. For example, women born in the mid-1970s were at increased risk for mild pre-eclampsia, whereas women born in the more recent periods showed an increased risk of severe pre-eclampsia. “Overall, these results suggest that changes in the population proportion smoking during pregnancy impact pre-eclampsia rates across age, whereas changes in obesity by cohort influence severe pre-eclampsia in younger cohorts only,” the authors wrote.

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Preimplantation Genetic Diagnosis

How Should Labs Grapple With Ethics?

BY KAREN APPOLD

A key breakthrough in modern laboratory medicine, preimplantation genetic diagnosis (PGD) detects genetic abnormalities that cause birth defects or fatal illnesses, allowing embryos to be chosen before being implanted into a uterus, thereby avoiding selective pregnancy terminations. While this technology provides a lot of answers, its increasing sophistication is raising new questions about how to resolve the ethical controversies it creates.

A special January 2014 issue of Clinical Chemistry focusing on women’s health explores some of these ethical issues, highlighting how the rapid pace of scientific discovery can sometimes outpace society’s old categories for ethics in healthcare (Clin Chem 2014; doi:10.1373/clinchem.2013.202515). Next generation sequencing and other advancements are enabling labs to use PGD in new ways beyond the scope of simply improving chances for a successful pregnancy and avoiding disease.

The power of this technique will make dealing with the ethical implications unavoidable, wrote ethicist Arthur Caplan, PhD, an author of the Clinical Chemistry article. “I believe that the future of PGD is in both looking for traits that parents do not want in their children and in selecting for traits that they do very much want to try to pass on. The morality of eugenics, both negative—

See Ethical Questions, continued on page 6
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Studies Show AKI Sets Up, Worsens CKD

Kidney Biomarkers, continued from page 1

poor outcomes, are lacking in renal care and would improve it, according to nephrology researchers. Investigations are focusing on several novel biomarkers, some of which might one day provide this information. Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury-molecule 1 (KIM-1) are perhaps the most promising candidate biomarkers for AKI, but investigators are actively studying many others, said researchers from the Chronic Kidney Disease Biomarkers Consortium (CKD BioCon), funded by the National Institute of Diabetes and Digestive and Kidney Diseases.

“The hope is that new biomarkers of AKI and CKD can help better identify high-risk patients so they can be targeted for more intensive monitoring, treatment, or enrollment in clinical trials,” said Chi-yuan Hsu, MD, CKD BioCon researcher and professor and division chief of Nephrology at the University of California, San Francisco. He spoke at the recent 30th Annual Beckman Conference, “Novel Biomarkers of Kidney Disease: False Dawn or New Horizon?” which was co-sponsored by AACC and the American Society of Nephrology.

Paradigm Shift

In contrast to earlier assumptions that AKI generally had no long-term consequences, recent research shows that severe AKI can increase the risk of developing CKD, worsen underlying CKD, and sometimes, cause end-stage renal disease (ESRD) directly. Some studies have found that AKI’s severity, duration, and frequency are important predictors of poor patient outcomes, while others indicate that reduction of renal mass and nephron number, vascular insufficiency, cell cycle disruption, and maladaptive repair mechanisms appear to be important modulators of progression in patients with and without coexistent CKD. To better clarify AKI’s role in renal pathology, investigators have called for long-term follow-up after first episodes of AKI (Kidney Int 2012;82:316–24).

Researchers have also driven the realization that CKD can lead to heart disease, underscoring the need to identify AKI early on because of its role in hastening and worsening CKD, emphasized Harold Feldman, MD, CKD BioCon researcher, senior investigator for the National Institutes of Health’s (NIH) Chronic Renal Insufficiency Cohort Study (CRIC), and chair of the department of biostatistics and epidemiology at the University of Pennsylvania Perelman School of Medicine in Philadelphia. "Nephrologists used to think of CKD principally as a risk factor for kidney failure, dialysis, and the need for transplantation. Now, CKD is also recognized as promoting cardiovascular disease. CKD needs active management not just to prevent ESRD, but also heart failure, myocardial infarction, and stroke," he said, adding that as CKD worsens, comorbidities become severe. CKD patients often don’t get cardioprotective treatments because nephrologists don’t know which patients are at high risk, he noted, adding that more reliable biomarkers will help to identify them.

Problems With Old Markers

Researchers pointed to a number of ways in which current kidney injury biomarkers, especially creatinine and protein in urine, are inadequate. Protein in urine is considered a sensitive marker of kidney injury and a means of determining recovery, as well as for CKD and its progression. But it is not very specific. Levels may rise with use of certain nonsteroidal anti-inflammatory medications, cancers, lupus, and rheumatoid arthritis. Creatinine, measured by labs for more than 100 years, is used to estimate glomerular filtration rate. This analyte also helps determine the magnitude of AKI, but it provides little information about the underlying cause of kidney injuries, and is less accurate for patients with low muscle mass and unusual diets. Challenges inherent in using creatinine as an AKI marker include diagnostic delays and potentially, misclassification of actual injury status. Neither of these old markers tells the location of kidney injury. “We need a marker that will say in real time what’s happening in the tubulointerstitium, the kidney’s tubules and interstitial tissue. The idea is to get a real-time assessment of kidney function during therapy,” said Brad Rovin, MD, CKD BioCon researcher, professor of medicine and pathology, vice president at the Peripheral Vascular Disease Foundation.

See Kidney Biomarkers, continued on page 4
Novel Kidney Markers Aid Drug Safety Studies

Novel kidney markers under investigation for improved clinical care may also prove useful in the preclinical evaluation of new drugs’ safety. Nephrotoxicity resulting from drug exposure causes an estimated 10–25% of all cases of acute kidney injury (AKI) in critically ill patients. The Predictive Safety Testing Consortium (PSTC), a collaboration of the U.S. Food and Drug Administration (FDA) and the European Medicines Agency, seeks to identify kidney damage biomarkers that can be used in animals early in drug development, well before clinical studies are underway. PSTC researchers seek markers that identify kidney injury early, detect degree of toxicity, site of kidney injury, and track progression or injury and recovery.

Kidney injury molecule-1 (KIM-1) was found by the PSTC, and subsequently by the European consortium to stand out among biomarkers studied as indicators of drug toxicity, according to Joseph Bonventre, MD, PhD, chief of the renal division at Brigham and Women’s Hospital and professor of medicine at Harvard Medical School in Boston. KIM-1 was the first injury biomarker of kidney toxicity qualified by the FDA for preclinical toxicity testing and drug development and, on a case-by-case, use in humans. That qualification is based on a study by the PSTC comparing KIM-1 to other biomarkers, KIM-1 significantly outperformed serum creatinine and blood urea nitrogen (BUN) at detecting renal tubular injury in rats (Nature Biotechnology 2010;28:436–40).

Now PSTC researchers are gearing up to test KIM-1, NGAL, and other novel nephrotoxicity markers at the Brigham and Women’s Hospital and six other institutions, Bonventre noted. The study, which uses fresh urine, will test blood and urine samples from 150 cancer patients undergoing treatment with either cisplatin or amonoglycosides, two drugs known to cause injuries to the proximal tubule of the kidney. The study is notable because it will not use creatinine levels as an endpoint, but rather whether patients got these drugs, said Bonventre.

Data generated from the project is intended to advance regulatory acceptance of new kidney biomarkers appropriate for monitoring kidney safety in the clinic and improve clinical diagnoses of drug-induced kidney injury during drug development and patient therapy.
A Role for Laboratorians

Laboratorians can play an important role in adapting research assays to useful tests that function well in clinical labs, which are not as tightly controlled as research labs, Rovin noted. In addition, laboratorians can figure out how to consistently extract biomarkers and test them "in a cost-effective fashion in real time," and develop quality control procedures and standards for assay consistency. Rovin also suggested that laboratorians can help determine the most appropriate testing technologies, evaluate instrumental drift, and develop reference ranges.

Bonventre urged laboratorians to carefully evaluate research on AKI biomarkers, noting that doing so can be difficult because of what he sees as a problem of logic inherent in many studies that compare biomarkers to creatinine. "You cannot judge biomarkers as being better than creatinine, based on creatinine," he explained. "It's important to recognize that information in these markers doesn't get conveyed in large studies that use creatinine as a gold standard."

Another point to consider is that many studies use frozen urine—not fresh as clinical labs typically do—and in more tightly controlled conditions than exist in many clinical labs, said Bonventre. He noted a "need to test biomarkers on urine that has been well-stored if the assays are not performed in a timely way." That's because urine is subject to compositional changes that can interfere with assays, he explained, urging clinical chemists to "validate urine assays in real urine from CKD patients, if they wish to gain information about these patients by making urine biomarker measurements."

Despite these challenges, Bonventre is optimistic that better kidney biomarkers will eventually become available in the clinic. Noting that he and his colleagues liken their work to a search for "the troponin of the kidney," he pointed out that acceptance of troponin as a cardiac marker was a long process. "Different [kidney] markers will find different roles. Keep an open mind regarding their utility," he urged.

Deborah Levenson is a freelance writer who lives in College Park, Md. Her email address is dlwrites@verizon.net.

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eliminating unwanted traits—and positive—selecting for desired traits—will surely loom very large as the key moral question facing those offering PGD and those seeking to utilize it,” Caplan is the director of the division of medical ethics at NYU Langone Medical Center in New York City.

An Evolving Technology

An evolving technique, PGD is not only the concern of researchers and academics. It has been used for more than 20 years to prevent the transmission of specific disorders, including autosomal recessive disorders such as cystic fibrosis and autosomal dominant disorders such as myotonic dystrophy. PGD can also be applied to carriers of chromosomal translocations who are at risk of transmitting an unbalanced chromosomal rearrangement that predisposes to miscarriage. The most up-to-date technique involves genetically analyzing five cells that are removed from an embryo biopsy on day 5 or 6 of development.

“Couples are determined to be at risk of having an affected child because they already have an affected child, they themselves are affected with a condition, or they test positive for a mutation on prenatal genetic screening,” explained Eric Forman, MD. “The typical paradigm is for couples to undergo in vitro fertilization (IVF), produce embryos, and have those embryos tested for the presence of a specific genetic disorder.” Forman is an assistant professor at the Rodgers Robert Wood Johnson School of Medicine in New Brunswick, N.J. and practicing physician at Reproductive Medicine Associates of New Jersey in Basking Ridge.

Another major application of PGD is to screen embryos for aneuploidy. “Embryonic aneuploidy is the principle cause of failed implantation and miscarriage. It also contributes to the age-related increased risk of infertility,” Forman noted. “More recently, those who produce genetic screening, embryos are tested to determine whether they have the normal complement of 46 chromosomes. Over the last few years, comprehensive chromosome screening strategies have been developed to test each chromosome and preferentially replace a chromosomally normal, euploid embryo in the uterus.”

PGD has dramatically improved the success rate of IVF, according to Mark Hughes, MD, PhD, CEO of Genesis Genetics in Plymouth, Mich. “Because approximately 40 percent of IVF embryos have the wrong number of chromosomes, and virtually all of those do not produce a pregnancy, the success rate of IVF has recently skyrocketed because preimplantation genetic screening allows for a single embryo to be transferred—avoiding multiple gestation—which increases the overall pregnancy rate and reduces the number of children with Down syndrome,” he said.

A more controversial application of PGD involves selecting an embryo whose human leukocyte antigen (HLA) profile is a match for an existing sibling with a disease. Called a sibling sibling, such an embryo deemed free of disease is implanted with the intent to be born to serve as a stem cell or organ donor to the diseased sibling, explained Susan Wolf, JD, McKnight Presidential Professor of law, medicine and public policy at the University of Minnesota in Minneapolis.

The first successful use of this technology took place in 2000 at the University of Minnesota. Parents had stem cells harvested from a second child’s umbilical cord to aid their daughter, who suffered from the rare blood disorder Fanconi anemia, which leads to bone marrow failure.

On the Horizon

With rapidly advancing genetic technology, it is possible to learn even more about the genetic status of embryos. Next generation sequencing creates the potential to determine an embryo’s genome. “This may allow embryos to be screened for de novo mutations which are not necessarily transmitted from the parents,” Forman said. “Recent studies suggest that even chromosomally normal fetuses in ongoing pregnancies have a substantial risk of possessing clinically relevant insertions or deletions, called indels. It may be possible to screen for these indels prior to embryo transfer.” Furthermore, innovations such as clustered regularly interspaced short palindromic repeat (CRISPR) technology introduce the possibility of correcting regions of the genome with pathologic mutations, potentially transforming this area of medicine from PGD to preimplantation genetic treatment.

Caplan told CLN he believes it soon will be possible to screen for risk factors of a disease. “Instead of looking to see if an embryo will produce a person with hemophilia, you will be able to check if an embryo is at high-risk for getting certain diseases, such as breast cancer or hemophilia,” he said.

In addition, using PGD to select desirable traits based on personal and social values, called “designer babies,” will continue to be explored, according to Ann Gronowski, PhD, a professor of pathology and immunology and obstetrics and gynecology at Washington University School of Medicine in St. Louis, Mo.

In fact, Mountain View, Calif.-based 23andMe, a genetic testing company that sells at-home DNA kits directly to consumers, announced a patent in 2013 that will allow people to shop for gamete donors using a proprietary, so-called genetic calculator. “This is supposed looking at traits in the genome of a gamete donor—say, a male sperm donor—in order to identify the perfect mate,” Hughes said. But, he believes, there will never be enough sperm donors, let alone oocyte donors, available to make any reasonable genetic profile choices.

“How many donors is diminishing due to privacy, legal, and financial issues, and a recipient’s selection choice will be much more focused on broad categories such as ethnicity, intelligence, and body stature,” he said.

A Legal Perspective on PGD

From a legal standpoint, the major issue regarding preimplantation genetic diagnosis (PGD) relates to the accuracy of diagnosis. “It would be tragic to undergo the burden of PGD and then have a misdiagnosis result in an affected child,” said Eric Forman, MD. “There have been legal cases and judgments brought against reference laboratories due to incorrect PGD results.” Forman is an assistant professor at Rutgers Robert Wood Johnson School of Medicine in New Brunswick, N.J.

Patients have also brought legal action against in vitro fertilization centers over claims of lack of informed consent. Patients have alleged that they were not informed of the facility’s lack of experience in performing PGD, the types of errors associated with PGD, or that they were not counseled about the option of performing PGD, Forman said.

The legal community is trying to define the standard of care in PGD for the purposes of liability and malpractice. “When should PGD be offered to someone who has a family history of a genetic disease?” asked Arthur Caplan, PhD. “If it is not offered, is that considered marketplace?” Caplan is the director of the division of medical ethics at NYU Langone Medical Center in New York City.

The current legal cases are focused on determining liability if a physician offers testing and then someone has a child with a defect that might have been prevented.

Many debates—from both an ethical and legal perspective—have revolved around the concept of savior siblings: specially selected embryos that, after gestation and birth, might serve as a donor to an existing, sick sibling. Parents who are desperate to save a child might have a conflict of interest. “It’s not clear whether they are in the best situation to make decisions about a second child. After all, they went through great lengths to create a second child to serve as a donor,” explained Susan Wolf, JD, McKnight Presidential Professor of law, medicine, and public policy at the University of Minnesota in Minneapolis.

Wolf poses these questions: While the child is young and not capable of consenting, what safeguards should be in place regarding harvesting stem cells or organs of that child? What if a second child turns out to object to such procedures? What if a child’s legal rights to refuse? The legal precedent on organ donation for minors suggests that the second child’s own best interest has to be protected, she added.

Ethical Questions

Although rare, there have also been cases of couples with an inherited condition, such as deafness or dwarfism, requesting PGD with the intent of producing, rather than preventing, a child who is affected with their condition. Wolf questions whether parents should be able to affirmatively select transferred embryos that would result in a child with a physical challenge, such as deafness. “Parents will argue that it’s a disorder, it’s a cultural choice, and that there is something important and valuable about deaf culture,” she said.

Finally, the use of PGD for “designer babies,” such as non-medical gender selection, sparked similar controversy. “What if we could test for things such as height, eye color, or IQ?” Gronowski asked. “Do we allow any kind of testing or are there types of testing that should not ever be allowed?”

Some have called PGD a form of eugenics. Where is the line between preventing disease and eugenics? “Who should draw those lines?”

As advances in PGD unfold, Wolf said many issues will be raised because a massive amount of information will be created about all sorts of traits and risks that a child-to-be may face. “I think there is a real ethical concern that parents and clinicians may not have the wisdom to appropriately deal with that much information and make ethically appropriate choices,” she said.
No Easy Answers

While unanimity doesn’t exist, for most conditions ethicists see PGD as a matter of patient autonomy. “Couples should be educated on the available options with current medical technology,” Forman said. “Some may prefer to roll the dice and hope for a spontaneously conceived unaffected child.” In the case of autosomal recessive diseases, this occurs 75% of the time, although two-thirds of children will carry the mutation. Other couples will opt to undergo IVF and preferentially transfer unaffected embryos or carriers if no unaffected embryos are available.

As an ethicist, Wolf wonders how it is possible to balance the rights and choices of parents and people who wish to be parents with the welfare of the children they create. Currently, no federal or state provisions specifically address the application of PGD. “The decision to proceed is left to the province of the treating physician and the prospective parents,” Forman said. PGD is legal in most countries where assisted reproductive technology (ART) is available, although some countries, such as Switzerland and Australia, have outlawed PGD.

Caplan believes politicians don’t get involved in regulating PGD because it is controversial. However, he thinks their intervention could be beneficial. For instance, “there could be oversight in terms of using a child’s bone marrow at a minimum age,” he said. “There could also be laws regarding which disabilities are severe enough to screen for.” But on the subject of designer babies, “the larger issue, according to Hughes, is what is the difference between a disease and a trait? And, who decides that? ‘Few people would argue that spinal muscular atrophy and Fanconi anemia are terrible diseases, while a person’s eye color and ear wax consistency are traits,’ he said. “But there is a huge gray zone in between, and it is changing and evolving with time.”

For instance, when Genesis Genetics first performed PGD for cystic fibrosis, many children didn’t live through their teens. “Today, with modern pulmonary and gastrointestinal medical advances, many individuals with this disease are now in their 30s and having children of their own,” Hughes said. “This is progress—but it is now less ethical to perform PGD for cystic fibrosis than in 1992?”

The Laboratory’s Point of View

Laboratory professionals who work in the ART field are aware of the favorable safety profile of IVF and embryo biopsy. “Having seen the impact a given medical condition can have on an affected child, most laboratory professionals believe the benefit of preventing an affected child outweighs the risks of ART and is preferable to termination of an ongoing pregnancy,” Forman said.

The safety and reliability of PGD has been validated to a sufficient extent by both the American Society for Reproductive Medicine and Society of Assisted Reproductive Technology, as they consider it “standard clinical practice,” rather than an experimental procedure, Forman noted.

According to Hughes, many couples come to Genesis Genetics seeking a savior sibling for quite a number of genetic disorders—mostly hematological ones such as sickle cell or genetic anemias, and for some inborn errors of metabolism as well. “Some couples achieve a healthy, cord-matching pregnancy on the first try,” he said. “Others have tried many times and the statistics don’t work for them. This is why screening embryos for a set of traits is a silly idea.”

Hughes explained that an IVF cycle may produce one or two embryos that are disease-free and a transplant match. “But, these two must be growing well to have a chance for uterine implantation, and they must have the correct number of chromosomes,” he noted. “So, the danger is that the roller coaster of hope and disappointment occurs over and over.”

Hughes believes most lab professionals are opposed to embryo sex selection. “I went into science and medicine to diagnose, treat, and hopefully cure disease,” he said. “The last time I checked, one’s gender was not a disease. There is no pain or suffering and no reason for a doctor or clinical laboratory professional to be involved in it.” Nonetheless, some couples do come to Genesis Genetics with this goal in mind. Most want a girl.

In speaking with several PGD laboratory directors, Gronowski said they agree that thoughtfull, ethical decision-making is necessary. Most laboratories have committees to discuss the ethics of testing requests and they require patients to undergo genetic counseling.

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“Doping” is as old as sporting events themselves and can be defined as the attempted use of a prohibited substance or prohibited method with the intent of improving athletic performance. As early as 800 B.C., ancient Greek athletes and Roman gladiators ingested a combination of herbs, plants, and mushrooms to gain a competitive edge and mask pain (1). With the debut of the modern Olympic Games in 1896, the practice of doping rapidly spread and different classes of compounds were used depending on the sporting event. For instance, athletes took stimulants (cocaine and amphetamines) to improve performance in speed and endurance sports, whereas they used anabolic steroids to promote muscle mass in sports requiring strength and power.

Drug testing was introduced at the Olympic Games in 1968, but had little impact on the practice of doping since the testing methods had poor analytical sensitivity that significantly reduced the window of detection. Another problem was that some compounds—notably anabolic steroids, erythropoietin and growth hormone—were used before methods to test them became available. Doping scandals continued to mount in the 1980s and 1990s and the public became acutely aware of the problem in sports. Consequently, the International Olympic Committee convened the World Conference on Doping in Sports to address this problem, and in 1999, it created the World Anti-Doping Agency (WADA) to combat sports doping and harmonize all anti-doping principles.

The goal of this article is to provide an inside view of how WADA-accredited laboratories identify athletes that use prohibited substances.

### Testing Methods to Detect Doping

BY ANTHONY W. BUTCH, PHD

WADA is independent of governments and sports federations, but is equally represented by all stakeholders. A major element of the WADA Anti-Doping Program is the Code, a universal document dealing with all aspects of sports doping (2). WADA signatories such as national anti-doping organizations and international federations are responsible for developing a sports drug testing program in accordance with the Code and must have drug testing performed by WADA-accredited laboratories. Accredited laboratories must develop methods to detect substances on the prohibited list of substances as mandated by the World Anti-Doping Program’s International Standard for Laboratories (3). At the time of this writing there were 32 accredited laboratories worldwide, with two in the United States (4). A violation of any of the WADA anti-doping rules is considered a doping offense. Besides the presence of a prohibited substance or metabolite/marker in an athlete’s sample, other activities can constitute an anti-doping rule violation. These include attempting to use a prohibited substance or prohibited method, refusing to submit a sample, failing to provide whereabouts information (location and availability for sample collection), failing to provide a sample, tampering or attempting to tamper with the doping control process, and possessing, trafficking, or attempting to traffic prohibited substances or prohibited methods. Although testing laboratories exist primarily to detect prohibited substances in athlete samples, they occasionally are asked to identify the contents in vials, pills, and powders confiscated from athletes suspected of possessing prohibited substances.

The WADA prohibited list of substances is updated annually and new substances are typically added to the list every year (5). Prohibited substances are typically grouped based on the pharmacological effect on the body (Table 1). Each class contains numerous substances; there are currently 46 exogenous steroids and 64 stimulants on the list. In the case of exogenous steroids, substances with a similar chemical structure, such as dimethazine—2 molecules of methasterone linked by an azine group—are also prohibited. Some classes of compounds are prohibited at all times whereas others are prohibited only when the athlete is in competition (Table 1). Alcohol and beta blockers are prohibited in certain sports during competition, but a few sports prohibit beta blocker use all the time. Prohibited methods are banned at all times and include manipulation of blood (transfusions, artificially enhancing oxygen delivery) and gene doping.

### Collection and Processing of Samples

Collectors witness urine collection to help prevent samples from being adulterated or substituted with fake or drug-free urine.
improve chromatographic and mass spectral properties when these samples are analyzed by gas chromatography mass spectrometry (GC-MS). The complete steroid pretreatment procedure takes approximately 6 hours.

Testing Methods

We use GC or liquid chromatography (LC) separation coupled with MS detection to identify the majority of substances on the WADA prohibited list. GC-MS is routinely used to detect anabolic steroids and stimulants. However, WADA’s recent lowering of the detection limit for anabolic steroid from 5 to 2.5 ng/ml (6) has forced accredited laboratories to switch to GC-triple quadrupole (GC-MS/MS) systems to meet the new requirement. We use LC-MS/MS systems to detect some anabolic steroids and most of the other classes of compounds. Exceptions include isoelectric focusing and polyacrylamide gel electrophoresis, which we use to detect recombinant erythropoietin and analogues such as peginesatide, and isotope ratio MS, which we perform to detect exogenous testosterone and testosterone precursors.

Data Analysis

GC-MS detects target compounds by comparing the retention time and relative intensities of ion fragments in unknown samples to those obtained for reference compounds. The collected data are reduced to dedicated windows consisting of selected time slices and mass-to-charge (m/z) ions corresponding to the expected retention times and mass spectral fragments for each target compound. Interfering peaks and background noise can complicate GC-MS data reading because screening methods are designed to detect entire classes of compounds and are not optimized for individual compounds. Furthermore, a single ion fragment may not be unique and might be shared by compounds. These issues limit the use of automated software programs for GC-MS data reading and require that experienced data readers carefully evaluate all data. In contrast, LC-MS/MS and GC-MS/MS measure the relative abundance of precursor/product ion pairs (transitions). Because the likelihood of a target compound and an interfering substance having the same precursor/product ion pairs is relatively small, the data usually are easier to interpret compared to GC-MS data and can be more easily evaluated by computer software.

GC-MS screening data for six stimulants are shown in Figure 2. The retention times of reference compounds are indicated in each window by a vertical line and the relative abundance of each ion is displayed on the y-axis. Although some of the windows have considerable background signals, the urine sample appears negative for all of the stimulants except amphetamine. A peak appears at the appropriate retention time for all three m/z ions in the amphetamine windows, so this sample would be considered screen positive and would undergo confirmation testing.

For confirmation testing an additional aliquot of urine from the ‘A’ bottle would be analyzed separately using a testing method optimized to detect amphetamine. Ion chromatograms and mass spectra of the trifluoroacetyl derivative of amphetamine are shown in Figure 3. The negative urine (panel A) does not contain ions at the retention time of the positive urine (panel B). In contrast, the screen positive sample (panel C) contains a peak for each of the m/z ions at the same retention time as the positive control in panel B. Relative abundance of diagnostic ions in the screen positive sample (m/z 118 and 91 as a percentage of m/z 140) match those obtained for the positive urine sample and all diagnostic ions with a relative abundance of >10% that are present in the positive control are also present in the screen positive sample based on full scan mass spectra data (right panels), confirming the presence of amphetamine. The confirmation data would be reviewed by two certifying scientists before being reported as an adverse analytical finding for amphetamine and must also fulfill all identification criteria in the WADA technical document (7). If the client requests ‘B’ bottle testing for the substance identified in the ‘A’ bottle, the athlete and/or his or her representative has the right to observe the entire testing process.

Documentation and the Legal Process

WADA-accredited labs prepare documentation packages in support of adverse analytical findings that must contain a table of contents, shipping/receipt documents, and chain of custody documents for bottles and aliquoted samples, as described in the technical document (8). Other documents include a list of staff involved in the testing process, a description of the testing procedure(s), negative and positive control data, instrument performance data (chromatographic performance, tune reports, etc.), compound identification data, and an uncertainty estimate for threshold compounds. These documents must be provided for both the initial screen and the confirmation testing.

Both the collector and athlete certify the urine sample after it is placed into two separate containers (‘A’ and ‘B’ bottle). Urine collection kits contain either plastic or glass bottles (Figure 1). Tamper-evident tape is applied to plastic bottles to spot tampering. Glass bottles, permanently sealed after filling, can only be opened by crushing the plastic sleeve surrounding the cap with a specially designed opening device. Both bottle types are placed into a box provided with the kit and are shipped to WADA-accredited laboratories, usually at ambient temperature.

Chain of custody documentation begins when urine samples arrive at the laboratory. This includes examining each ‘A’ and ‘B’ bottle for evidence of tampering, leakage, and color/clarity, along with measuring the pH and specific gravity of the ‘A’ bottle. Our laboratory routinely screens extremely dilute urine samples with a specific gravity <1.006 for diuretics even when testing for diuretics is not requested. Aliquots from the ‘A’ bottle are then prepared for testing depending on the specific test menu. Between 5 and 7 aliquots of urine are routinely prepared for testing, with each aliquot containing from 1 to 20 mL of urine.

Sample Pretreatment

The amount of sample pretreatment needed varies depending on the screening method. Some urine testing methods such as hormone immunoneassays for human chorionic gonadotropin and luteinizing hormone do not require pretreatment prior to testing. Sample pretreatment removes unwanted interfering substances and isolates specific compounds of interest. For instance, anabolic steroids first require an enzymatic hydrolysis step to deconjugate glucuronide moieties from the steroid molecules, followed by solid phase extraction to recover the relatively nonpolar steroids. We then add trimethylsilyl groups to steroid functional groups to improve chromatographic and mass spectral Table 1

<table>
<thead>
<tr>
<th>WADA 2013 List of Prohibited Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUBSTANCES PROHIBITED AT ALL TIMES</td>
</tr>
<tr>
<td><strong>Anabolic Androgenic Steroids</strong></td>
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<tr>
<td>Exogenous</td>
</tr>
<tr>
<td>Endogenous</td>
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<tr>
<td><strong>Other Anabolic Agents</strong></td>
</tr>
<tr>
<td>Peptide Hormones, Growth Factors, and Related Substances</td>
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<tr>
<td>Erythropoiesis-stimulating agents</td>
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<tr>
<td>Chorionic gonadotropin</td>
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<td>Luteinizing hormone</td>
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<tr>
<td>Corticotropins</td>
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<tr>
<td>Growth hormone</td>
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<tr>
<td>Insulin-like growth factor-1</td>
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<tr>
<td>Fibroblast growth factors</td>
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<tr>
<td>Hepatocyte growth factor</td>
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<tr>
<td>Mechano growth factors</td>
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<tr>
<td>Platelet-derived growth factor</td>
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<tr>
<td>Vascular-endothelial growth factor</td>
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<tr>
<td><strong>Beta-2 Agonists</strong></td>
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<tr>
<td>Hormone and Metabolic Modulators</td>
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<tr>
<td>Aromatase inhibitors</td>
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<tr>
<td>Selective estrogen receptor modulators</td>
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<tr>
<td>Other anti-estrogenic substances</td>
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<tr>
<td>Agents modifying myostatin function</td>
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<tr>
<td>Metabolic modulators: insulin, peroxisome proliferator activated receptor B agonists, PPAR-AMP-activated protein kinase axis agonists</td>
</tr>
<tr>
<td><strong>Diuretics and Other Masking Agents</strong></td>
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<tr>
<td>SUBSTANCES PROHIBITED IN-COMPETITION</td>
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<tr>
<td>Stimulants</td>
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<tr>
<td>Narcotics</td>
</tr>
<tr>
<td>Cannabinoids</td>
</tr>
<tr>
<td>Glucocorticosteroids</td>
</tr>
<tr>
<td>Alcohol (some sports)</td>
</tr>
<tr>
<td>Beta Blockers (some sports, also prohibited out-of-competition in archery and shooting)</td>
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</tbody>
</table>

**JANUARY 2014 Clinical Laboratory News**
The presence or use of a prohibited substance(s) is considered an anti-doping rule violation. The burden of proof under the Code is to the comfortable satisfaction of the hearing panel. This is more than the mere balance of probability but less than proof beyond a reasonable doubt. The length of the sanction depends on the sporting organization; WADA signatories must follow Code guidelines which mandate a maximum of 2 years for the first violation. However, non-signatories like professional sports can enforce shorter periods of ineligibility.

Athletes have the right to appeal a sanction, and as part of the appeals process accredited laboratories reporting the adverse analytical finding may be called upon to testify. Depending on the case, the laboratory may be required to provide a wide variety of documents during the discovery process. Expert testimony usually involves explaining and defending data in the documentation package, providing information as to what the drug is and why it is performance-enhancing, describing side effects, as well as when and how much the athlete took. Some of these questions can be difficult if not impossible to answer since pharmacokinetic data for many of the drugs is unknown. Another very difficult question often asked is if the drug would enhance performance or recovery given the amount detected in the urine and whether the substance was unknowingly (via a contaminated supplement) or intentionally ingested.

External Quality Assessment Program

In order to maintain WADA accreditation, each laboratory must comply with the International Standard for Laboratories document (3) and numerous technical documents that address specific issues related to the testing process. Technical documents must be integrated into the policies and procedures of each accredited laboratory and range from compound identification criteria and decision limits for threshold compounds to chain of custody documentation and preparation of documentation packages.

Laboratories must also participate in the WADA external quality assessment scheme (EQAS) and correctly identify prohibited substances in at least 20 unknown samples received throughout the year. Besides blind EQAS samples recognized as the quality assessment samples, laboratories receive double-blind samples indistinguishable from routine samples, which the lab doesn't know are EQAS samples. EQAS samples sometimes contain substances that normally are not identified in routine doping samples. In addition, EQAS samples may contain a low concentration of a prohibited substance as well as more than one prohibited substance. A typical example would be a low concentration of an anabolic steroid together with a diuretic. A false-positive EQAS result earns a laboratory 25 points, whereas they receive 10 points for a false negative EQAS result. For quantitative substances (threshold compounds) a z-score ≥3 results in 10 points and a z-score ≥2 but <3 results in 5 points. If a laboratory receives >24 points—meaning just one false positive result—in a single EQAS round, its WADA accreditation will be suspended. When a laboratory receives >30 points over the last 12-month period its accreditation will be either suspended or revoked. This quality assessment program is clearly designed to promote high

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**Figure 2**

GC-MS screening data for selected stimulants

Each window consists of selected time slices and mass-to-charge (m/z) ions corresponding to each of the target stimulants. The x-axis is retention time and the y-axis is ion abundance. The vertical line in each window represents the software-predicted retention time based on reference standards analyzed in the same batch of unknown urine samples. Note that the urine sample screens positive for amphetamine.

**Figure 3**

GC-MS confirmation data for the trifluoroacetyl derivative of amphetamine in a negative urine (A), a positive urine control (B) and the urine that screened positive in figure 2 (C). Retention times (x-axis) and ion abundance (y-axis) for selected m/z ions are shown in the left panels. Full-scan mass spectra normalized to m/z ion 140 with the relative abundance of m/z 91 and m/z 118 ions are shown in the right panels. Note that all ions with a relative abundance >10% in the control urine are also present in the screen positive urine. This data confirms the presence of amphetamine.
quality laboratory testing procedures and raises the bar to a very high standard for WADA-accredited laboratories.

The Now and Then

So how big a problem is doping in sports? Of the 267,645 samples tested in 2012 by accredited laboratories, only 1.19% (3,190 samples) were positive for a prohibited substance (9). This low percentage of positivity suggests that either doping is not a significant problem or that doping is really a problem but the cheaters are not getting caught. If one believes the media and recent doping scandals, performance-enhancing drugs are epidemic in sports like cycling and professional baseball. It is more likely that the true story lies somewhere in between: doping is a larger problem than the numbers reveal but is not widespread throughout sports.

Although testing and the associated sanctions for doping violations help deter athletes from using prohibited substances, additional steps need to be taken to win the battle against doping in sports. One approach is to expand unannounced drug testing programs, develop better testing strategies (including longitudinal profiling), and improve current testing methods to catch more, if not all, athletes that dope. Another approach is to provide better athlete education at an earlier age to deliver the message more effectively that doping is unethical and should not be considered or tolerated at any level of sports competition. Lastly, a change in public attitude might be needed. Perhaps a message needs to be delivered to athletes that fans will not continue supporting sports in which doping is perceived to be a major concern.

Anti-doping laboratories face an enormous task in developing and validating testing methods to detect emerging compounds that become available to the athletes from using prohibited substances, and understand new testing modalities as they become available. World Anti-Doping Agency (WADA) laboratories face an enormous task in developing and validating testing methods to detect emerging compounds that become available to the athletes from using prohibited substances, and understand new testing modalities as they become available in the fight against doping in sports.

References

PATIENT SAFETY FOCUS
TA KING AIM AT REDUCING LAB ERRORS

ASK THE EXPERT:
Reducing Errors in Manual Processes
When We Can’t Automate, What’s the Best Way to Reduce Errors?

MIKE ASTION, MD, PHD
EDITOR, PATIENT SAFETY FOCUS
MEDICAL DIRECTOR, DEPARTMENT OF LABORATORIES
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The benefits of automation have been realized in clinical laboratories more than anywhere else in healthcare. Nonetheless, it is hard to reliably automate everything in the lab, and the manual work that remains is copious and tends to be error-prone. For example, in a lab that processes millions of test requisitions, manual handling of 1% of the requisitions means there are tens of thousands of manual requisitioning events annually, with each posing an opportunity for mistakes.

To cope with these manual processes, it’s important for labs not to become overwhelmed, as there are several reliable steps lab managers can take to mitigate errors—and stress. Labs should focus not only on standardization and accountability, but also on creating a quiet, calm work environment.

One of the best ways to error-proof manual processes is to isolate the work. This means coaching staff to focus only on the task at hand, and not be sucked into multitasking. For example, if the manual work is pipetting, then the pipetting occurs in an interruption-free zone where no other tasks—such as answering the phone—are permitted.

To achieve error-free manual work, accuracy rather than speed should be rewarded. Reducing or completely eliminating time constraints can be particularly helpful in this regard.

Smoothing workflow goes hand-in-hand with isolation. In a smooth workflow, staff work at a constant pace, rather than alternating between large peaks and valleys of work. This can be done by having a lead technologist pace the work, or by having those who perform the manual work pull it at a relatively constant pace while receiving feedback that the steady pace is being maintained.

Finally, laboratorians should consider redundant data entry. Also called double entry, redundant data entry involves two separate people entering the data, with data only flowing forward if the two independent entries match. It adds time to data entry, but it dramatically reduces data entry errors when computer interfaces are not an option.

A few years ago, a lab that I worked with tried the interventions in Table 1 to reduce errors in entering the data from manual requisitions into the lab information system. These manual requisitions on paper forms involved tests for which there was no computerized order entry, and they represented a small but significant fraction of the work in the lab.

Figure 1 shows the error reduction that we achieved. The lab sustained this improved error rate, which ultimately led to decreased patient harm, improved client satisfaction, and higher staff satisfaction.

Further Reading


Table 1

<table>
<thead>
<tr>
<th>Strategies for Reducing Errors in Manual Work</th>
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<tr>
<td>✔ Standardize the work</td>
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<tr>
<td>✔ Isolate the work to eliminate multi-tasking and interruptions</td>
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<tr>
<td>✔ Specialize the work to a small group of highly trained people whose error rates are tightly monitored and who are given feedback</td>
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<tr>
<td>✔ Remove or reduce time constraints from the group</td>
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<td>✔ Smooth the work flow</td>
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<td>✔ Consider double-checking the work with joint accountability of a primary worker and the double-checker</td>
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<tr>
<td>✔ For data entry, use redundant entry when feasible</td>
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The causes of ordering too many lab tests are diverse and complex. However, the consequences are only too clear: waste of scarce healthcare resources, and potential for real patient harm. On the patient side, the Googlefication of healthcare raises alarm about problems that might be better left unexamined. What was once a runny nose requiring no doctor visit now becomes, with just a few key clicks, an opportunity to worry about—and demand testing for—the allergens, viruses, bacteria, autoimmune diseases, nutritional deficiencies, and toxins that could be causing it. For the worried well, the Internet can turn a bout of fatigue into an opportunity to think about poisonings, malnutrition, and cancer.

Too often, social media exacerbate the problem because they allow the worried well to broadcast their concerns and find an exceptional case that confirms their worst fears—the friend, who has a friend, whose brother thought he had a common cold but turned out to have a rare and fatal disease. Unfortunately, the most common ambulatory complaints, including headache, backache, stomach pain, anxiety, and depressed mood, have very little useful lab testing associated with them. In fact, lab testing in the context of these common ambulatory complaints is as likely to confuse as to inform.

Patients don’t bear all the blame, however. The U.S. healthcare industry promotes overtesting in outpatients because it is primarily based on a fee-for-service system. Even labs play a role in overutilization because, to the extent that they participate in fee-for-service medicine, overutilization can provide significant financial benefits. Physicians have their own reasons for testing too frequently in the outpatient setting, including practicing defensive medicine and the inability to resist patient and marketing pressures.

Combating Overutilization

Test ordering errors increase the likelihood of test interpretation errors. But the consequences of this fact are not always intuitive. Figure 2 illustrates overutilization with a graph of positive predictive value (PPV) versus pretest probability. Pretest probability is the likelihood that a patient has the disease being tested for, and it is based on the patient’s history and physical. For example, a 40-year-old woman with a history of back pain who wakes up with a headache twice per month has a low pretest probability that the headache is being caused by heavy metal poisoning, an autoantibody disease, a nutritional deficiency, or a stealth virus. PPV is the probability that a positive test result indicates that the patient has the disease for which the test is being performed. A positive result refers to a result outside a
reference range or above a cutoff value. In the figure, a test was chosen that had a specificity of 85% and a sensitivity of 95%. This would be the typical performance range for a number of tests including the antinuclear antibody (ANA) test, or individual tests for specific allergies or infectious diseases.

As the graph shows, the pretest probability very strongly influences the PPV. The diagnostic utility of a positive test is particularly poor when the pretest probability is low. In this example, it means that at a pretest probability of 1%, the probability that a positive result indicates the presence of the disease is only 6%. In other words, the odds strongly favor the patient not having the disease despite testing positive for it.

In my experience, both patients and physicians do not understand just how poorly tests perform diagnostically in the low pretest probability range. Thus, in our example, if the woman with a headache demands an ANA test and receives a positive test result, it is likely to be a false positive. That is why it would have been better to not test.

False positives are due to a variety of causes including overlap of the diseased and healthy state, lab errors, and lab interferences. A false positive result is often not believed by the worried well and this disbelief can lead to a medical misadventure of additional tests, misdiagnosis, and mistreatment.

Today, it is the rare person who does not know a friend or relative who has gone on a testing-based misadventure. The relationships between pretest probability and PPV shown in figure 2 explain why testing too frequently in the ambulatory setting is a significant contributor to diagnostic error. Patients and physicians can avoid this false diagnosis problem by only undergoing or ordering testing when two conditions are met: 1) an actual lab test exists for the disease that is suspected, and 2) the pretest probability of disease is sufficient to allow a meaningful interpretation of a positive result. In the majority of medical encounters, this means that the patient will need to have signs, symptoms, family history, or personal history that suggests the disease has a reasonable likelihood of being present.

It is especially important to avoid testing in the ambulatory setting, where the pretest probability is low. Usually, testing will do more harm than good when the pretest probability of the disease being tested is 1% or below, and this is even the case for tests with sensitivity and specificity above 95%.

Further Reading
Quality and the Sendout Testing Department

Get Sendout Processes Under Control to Improve Quality, Efficiency, and Patient Safety

SUE GARR, BS, MT(ASCP) 
VICE PRESIDENT AND DIVISION MANAGER, CENTRAL SUPPORT SERVICES 
ARUP LABORATORIES

Referral testing, commonly called sendouts, is often underappreciated. Sendouts do not involve instrumentation and reagents, so some laboratory managers may think of them as simply a clerical function. Hospital sendout areas are generally small operations staffed by a variety of employees ranging from technicians and phlebotomists to laboratory supervisors. Because most laboratory staff members are concentrating on testing hospital and outpatient patients in a timely manner, labs commonly segregate sendout specimens outside the normal laboratory workflow. However, sendout testing is an extremely complex operation, where quality lapses are common and patient care can be seriously impacted. This requires that labs maintain a robust quality management program to cope with a test menu of thousands of different tests, the unique specimen requirements for each of those tests, and the various shipping and information handling processes expected by reference laboratories.

Arguably the biggest patient safety category in referral testing is specimen handling. Specimen integrity is paramount in providing the clinician with a viable laboratory result. Specimen handling concludes with packing specimens into shipping containers in such a way that prevents leakage and maintains appropriate temperature.

Laboratorians may not think of sendout testing as particularly time-sensitive because sendouts are not available stat, but timing with these tests is critical for at least two reasons. First, many specimens degrade over time. Ambient specimens are most prone to degradation, but refrigerated specimens can also degrade. This is of particular concern in smaller or rural hospital laboratories, where courier pickups may occur only once a day or less. Even when specimen stability is adequate for initial testing, this may not be the case with all-too-common add-on requests. Due to degraded specimens, add-ons often require either redrawing the patient or running the test with a disclaimer, risking inaccurate results as well as delayed reimbursement.

Another example of timing complications is when errors in the referral testing process increase the turnaround time substantially. In contrast to a misdrawn complete blood count, where the local laboratory might be able to redraw the patient and run the test within 1 hour or so, a misdrawn sample that is caught by a referral lab can add days to their daily tasks. These pillars, along with their associated quality metrics, are the basis for handling problems originating in the reference laboratory. Table 1 lists the five pillars or principles that our sendouts department applies to clinical laboratories of all sizes.

As a national reference lab, ARUP Laboratories receives 16,000 sendout specimens each month and orders from more than 500 laboratory clients. This gives us particular insight into the frequency and consequences of specimen handling problems originating in the sendout areas of local laboratories. In 2013, 13% of specimens our laboratory received were held or canceled for a variety of reasons. Most could have been tested within published turnaround times if the specimens and orders had been placed correctly or had been sent with all the required information.

At ARUP, the most common reasons for delaying or canceling a test include: erroneous information on the test requisition, such as incorrect patient demographic or wrong test selected; incorrect specimens, such as incorrect type, temperature, or pH; and missing or confusing orders, such as duplicate orders, extra specimens, or no test selected. Common errors like these potentially can increase turnaround time from 2 to 20 hours depending on client availability, since the reference lab must contact the client lab for clarification. The turnaround time is sometimes affected even more if patients have to be redrawn.

ARUP also maintains a sendouts department for esoteric tests that we do not perform in-house. Table 1 lists the five pillars or principles that our sendouts department applies to their daily tasks. These pillars, along with their associated quality metrics, are the basis of our system to reduce the likelihood of errors in sendouts. They are readily adaptable to clinical laboratories of all sizes.

Table 1

| PILAR 1 | Sending Out: Ensure specimens are sent out on time and at the right temperature. |
| PILAR 2 | Result Entry: Enter results as soon as they arrive and utilize interfaces whenever possible. |
| PILAR 3 | Overdue Pending: Track delayed results and communicate to physicians. |
| PILAR 4 | Billing: Track vendor invoices and monitor pricing and contracts. |
| PILAR 5 | Test Consolidation With Leakage: Determine if a requested referral test is being performed in house. |

For manual results: Implement a two-step process with bins labeled “To Be Performed” (initial entry) and “To Be Verified” (final approval/completion).

Keep bins separated by feasible space, and color coordinate bins to prevent accidental misplacements.

SUE GARR, BS, MT(ASCP) 
VICE PRESIDENT AND DIVISION MANAGER, CENTRAL SUPPORT SERVICES 
ARUP LABORATORIES
Due to a weak economy and cost-cutting provisions of the Affordable Care Act (ACA), labs in 2014 will see a 0.75% cut across the board on tests paid for by the clinical laboratory fee schedule (CLFS). Although not as sharp a drop compared to some years, it comes on top of several years of deeper cuts, including a 2% cut in 2012 due to the Middle Class Tax Relief and Job Creation Act—known as the payroll tax cut—and an additional 2% cut in 2013 from Congress’s debt-ceiling fallout, which led to broad federal cuts called sequestration.

While leaders from both houses of Congress struck a budget deal in December to avoid another government shutdown, at Congress struck a budget deal in December, it comes on top of several years of deeper cuts, including a 2% cut in 2012 due to the Middle Class Tax Relief and Job Creation Act—known as the payroll tax cut—and an additional 2% cut in 2013 from Congress’s debt-ceiling fallout, which led to broad federal cuts called sequestration.

Final Physician and Outpatient Payment Rules Spell Trouble for Labs

Beginning in 2014, Centers for Medicare and Medicaid Services (CMS) announced it will move ahead with two new payment policies that could have a significant impact on labs. First, in the final physician fee schedule, CMS decided to move forward with a plan that will systematically revalue prices on the clinical laboratory fee schedule (CLFS) over 5 years, trimming reimbursement for tests based on technological innovations that introduce greater efficiency or automation. Under this provision, CMS, beginning in 2014, will conduct a data analysis of codes on the CLFS and propose a batch of codes the agency believes requires review each year. Factors such as how long the codes have been on the CLFS and high-volume or high-dollar codes will determine which codes the agency reviews first. CMS plans to unveil its first batch of codes under review later this year as part of the proposed 2015 physician fee schedule. Any changes in reimbursement would begin in 2015.

The second significant policy change for 2014 will be outpatient bundling of lab tests. This plan comes out of the final 2014 rule for hospital outpatient prospective payment and ambulatory surgical center payment. Now, rather than pay for lab tests separately for outpatients, lab services will be bundled into ambulatory patient classification (APC) groups when the test is ordered on the same date as the primary service, and by the same provider. Similar to diagnosis related group (DRG) payments for inpatients, APCs give a hospital a single payment based on services provided to a patient.

The bundling provision will leave labs stripped of direct payment and competing with other providers and departments for their piece of the APC. The change also makes outpatient lab testing part of deductibles and copays under Medicare for the first time. One piece of good news did come out of the final physician fee schedule. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates. This policy would have cut several common anatomic pathology codes to match APC rates. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates. This policy would have cut several common anatomic pathology codes to match APC rates. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates. This policy would have cut several common anatomic pathology codes to match APC rates. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates.

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The bundling provision will leave labs stripped of direct payment and competing with other providers and departments for their piece of the APC. The change also makes outpatient lab testing part of deductibles and copays under Medicare for the first time. One piece of good news did come out of the final physician fee schedule. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates. This policy would have cut several common anatomic pathology codes to match APC rates. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates. This policy would have cut several common anatomic pathology codes to match APC rates. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates. This policy would have cut several common anatomic pathology codes to match APC rates. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates. This policy would have cut several common anatomic pathology codes to match APC rates. CMS scrapped a proposal to cut several key anatomic pathology codes to match APC rates.
Siemens, Pfizer Collaborate on Companion Diagnostics

Siemens Healthcare Diagnostics and Pfizer have entered a partnership in the terms of which Siemens will provide comprehensive automation solutions for molecular biology laboratories. This selection will ensure compatibility with BioMérieux’s existing PCR assays, most of which are validated on Life’s 7500 instruments and aid in the diagnosis of infectious diseases. In addition to Life’s instruments, bioMérieux’s planned automation solution will include the following modules: NucliSens EasyMag, bioMérieux’s sample purification platform, EasyStream, a liquid handling system for the assay PCR set-up, and NucliSental, a middleware to connect all the platforms.

Cerner, Claritas Partner on Personalized Medicine Initiative

Cerner and Claritas Genomics have entered a collaboration to build tools and connectivity aimed at integrating next-generation sequencing (NGS)-based diagnostic testing into healthcare practice. The two companies plan to develop a rapid, scalable laboratory solution for molecular diagnostics that is adapted to the complexity of NGS workflows and the massive amount of data they generate. As part of this project’s initial phase, Claritas will implement Cerner’s Millennium Helix solution, which incorporates molecular diagnostic data into patients’ electronic medical records (EMRs), and will join Cerner’s Reference Lab Network. This will enable seamless ordering and results return for other Reference Lab Network partners, while also giving Claritas access to an existing scalable computing infrastructure that integrates ordering of genomic sequencing tests, laboratory processing, results interpretation, return of results to the clinician, and incorporation of results into EMRs.

“One of Claritas’s goals is to enable providers at any pediatric center or practice to use genomics in routine medical care. Effective use of genomics in medicine requires integrating genetic information into the context of the patient’s unique clinical presentation,” said Patrice Miles, PhD, CEO of Claritas. “As a leading provider of both laboratory management systems and EMR systems in the world, Cerner has decades of experience synthesizing complex medical information across organizations to inform patient care and we are extremely pleased to have them as a strategic partner.”

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is known to increase in blood after severe TBI but has not been evaluated in mTBI, as part of their search for candidate biomarkers that might identify neurodegeneration following mTBI sooner and with more sensitivity than current imaging techniques, which do not detect up to 30% of long-term and clinically significant mTBI-associated brain dysfunction. Other neurodegenerative biomarkers have been evaluated in mTBI, but none has demonstrated a prognostic relationship with structural brain injury or brain injury symptoms.

The study involved 17 participants with sustained mTBI, 13 with an orthopedic injury, and eight unjured controls. The researchers obtained plasma samples within 24 hours of the subjects’ injuries. Participants also had neuropsychological and brain imaging assessments at baseline and 1- and 3-month follow-up visits. The authors used a sandwich immunoassay to detect SNTF in which the enzymatic amplification and detection steps were replaced with next-generation electrochemiluminescence detection chemistry.

The investigators found that increased SNTF on the day of injury correlated significantly with cognitive impairment that persisted for at least 3 months, in both all study participants and in those with mTBI. SNTF levels were at least twice the lower limit of detection in 7 of 17 mTBI cases and in 3 of 13 orthopedic injury cases, but in no uninjured controls. Elevated levels of SNTF also were associated with significant differences in brain structure detected by diffusion tensor imaging.

96-Gene Panel Sensitive, Specific in Identifying Eosinophilic Esophagitis

Researchers at the University of Cincinnati College of Medicine in Ohio reported developing a 96-gene quantitative polymerase chain reaction assay and associated algorithm that has high sensitivity and specificity for detecting eosinophilic esophagitis (EoE) (Gastroenterology 2013;145:1289–99). The assay also identified patients with EoE in remission from controls, as well as those exposed to swallowed glucocorticoids.

The authors suggested that this novel platform could improve diagnosis and treatment for EoE, an immune-mediated upper gastrointestinal (GI) disorder that has been rising in incidence since it was first characterized 2 decades ago. EoE, typically associated with dysphagia and eosinophilia ≥15 eosinophils/high-power field, accounts for 10–30% of chronic esophagitis refractory to proton pump inhibitor therapy and 7% of patients who undergo upper GI endoscopy. Diagnosis is made by histological analysis of esophageal biopsy, with at least five biopsies needed to achieve sufficient sensitivity, and correct the irregularity of EoE pathology.

Recent characterization of the EoE transcriptome consisting of about 500 genes opened up the possibility of diagnosing the disease at the molecular level. The authors developed a 94-gene panel built on a Taqman®-PCR system. They found the panel identified adult and pediatric EoE patients with approximately 96% sensitivity and 98% specificity. The panel also distinguished EoE patients from controls, and from those with reflux esophagitis.

Risk Factors Characterized for Healthcare-Acquired and Community-Associated C. difficile Infection

A retrospective observational cohort study aimed at distinguishing the risk factors, clinical course, and outcomes between healthcare-associated (HA) and community-associated (CA) C. difficile infection (CDI) found that CA-CDI more often is seen in previously healthy children without antibiotic exposure or comorbid conditions (Clin Infect Dis 2013;57:1665–72). The authors also found that in comparison to HA-CDI, children with CA-CDI had a trend toward more episodes of septic shock, toxic megacolon, and recurrences.

The study involved 200 pediatric patients diagnosed with CDI, of whom 38 had CA-CDI, 144 had HA-CDI, and 20 had indeterminate CDI, meaning their disease started in the community within 4–12 weeks after hospital discharge. In contrast, the researchers defined HA-CDI as disease onset ≥48 hours after hospital admission, and CD-CDI as symptom onset ≤48 hours after admission and more than 12 weeks since the patient’s last hospitalization.

The researchers found that the risk profiles of children with indeterminate CDI are more similar to HA-CDI, suggesting that these cases should be allocated to HA-CDI rather than CA-CDI. Based on their findings the authors also suggested that the diagnosis of CDI should be considered in healthy children even without recent exposure to antibiotics. They called for additional research to understand why CA-CDI has been increasing, as this trend may not be explained solely by traditional risk factors. 9

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AACC Calls for Uniformity in Lab Test Results
Harmonization Essential to Improving Patient Care

AACC has released a position statement on harmonization of clinical laboratory test results to help patients receive appropriate diagnoses and medical treatment. In this statement, the association provides guidance on how medical community stakeholders can help the efforts of the International Consortium for Harmonization of Clinical Laboratory Results, an oversight body formed by AACC to manage the worldwide harmonization endeavor.

The few laboratory tests that have been standardized or harmonized to date, such as those for cholesterol, glucose, and hemoglobin A1c, have markedly improved diagnosis and treatment of heart disease and diabetes. Additionally, harmonizing these tests may reduce healthcare spending. As a striking example, the initiative to standardize cholesterol tests only cost $1.7 million per year, while the health benefits it has yielded now save more than $538 million annually.

“Results of patient lab tests should be comparable regardless of the method used; the time they were analyzed, or the laboratory that produced them,” said AACC President Robert H. Christenson, PhD. “Especially in situations where doctors depend on several risk factors in addition to test results to make treatment decisions, there cannot be discrepancies between test results if they are to be useful within the context of a patient’s overall health and medical history.”

AACC’s position statement on harmonizing clinical laboratory test results is the first of a planned compendium of such statements that will detail the association’s stance on important healthcare issues.

The full statement is available on the AACC website. Visit www.aacc.org/gov/gov_ materials/Position_Statement-HarmonizationOfClinicalLaboratoryResults_V1.pdf.
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