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In this issue’s “Special Feature” article, the success of any biobank biobanking management. The participation and support of the public is important for their samples stored. In this way, community engagement is a central component of incidence and endurance of biobanks depends on people’s willingness to donate and encourage them to donate samples.

Still, the high cost of the enterprise is an obstacle; potential investors are being asked to make long-term commitments in a new business whose model is still a hazy one. According to Rodrigo Gutierrez Gamboa, Managing Analyst for GBI Research, “Most biobanks are reportedly employing relatively vague cost models, suggesting a lack of financial strategy. Failure to accurately capture costs may lead to the early termination of projects, and may prove to be the downfall of various biobanks.”

The other challenge, public skepticism, may be even more difficult to overcome. Gutierrez Gamboa explains: “The public’s attitude towards biobanking is mixed, with some people having concerns over disclosing personal and medical information. Public support also depends to a large extent on what the samples are used for, as the treatment of disease is generally valued highly, while other interventions [e.g., the development of cosmetics] are seen as less acceptable.” To overcome these concerns, he says, biobanks must reassure the public that information is safely stored and encourage them to donate samples.

GBI Research concludes that despite the useful applications of biobanking, the creation and endurance of biobanks depends on people’s willingness to donate and have their samples stored. In this way, community engagement is a central component of biobanking management. The participation and support of the public is important for the success of any biobank.

The full GBI Research report is called “Biobanking: Developing Smart, Sustainable and Ethically Compliant Biorepositories for the Future.”

In this issue’s “Special Feature” article, “A2O modulation: a potential biological threat that can be mitigated by immunohistochemistry” (pp.16-17), Maj. Michael A. Washington, PhD, (M(ASCP), Chief of Microbiology Research in the Department of Clinical Investigation at Tripler Army Medical Center in Honolulu, HI, makes a chilling observation; he suggests that we are now in “an environment in which it is possible to engineer a new biological threat agent in a matter of days, while the characterization of the threat and the development of countermeasures can take months to years.” The nightmare scenario of international terrorism unleashing a bio-threat that will be difficult to counter promptly is no longer just a staple of “techno-thriller” paperbacks; it is real. Maj. Washington goes on to describe efforts by researchers to combat that threat, particularly through the techniques of immunohistochemistry.

The author concludes that “the clinical laboratory staff is on the frontlines of bio-defense and will undoubtedly play an important role in the detection and response to future biological threats, whether natural or manmade. In order to be prepared for novel threats, it is essential that laboratory staff have a thorough understanding of what is possible and are provided with the tools to respond to unusual and novel situations.

No lab director would prefer to use the specter of a cataclysmic bioterror event as a way to induce hospital management to loosen the budgetary purse strings. But legitimate threats are legitimate threats, and decision-makers should be aware of them and of the role labs can play in averting or responding to them. Can institutions afford to invest appropriately in their labs? - Alan Lenhoff
1 test. 14 pathogens. All in about an hour.

A fast diagnosis of meningitis is essential, but difficult due to overlapping symptoms. The new FDA-cleared Meningitis/Encephalitis Panel helps with this medical emergency by detecting bacterial, viral or fungal infectious agents in about one hour. This can influence better patient management, leading to reduced healthcare costs and improved outcomes.

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Pathogens

Bacteria
Escherichia coli K1
Haemophilus influenzae
Listeria monocytogenes
Neisseria meningitides
Streptococcus agalactiae
Streptococcus pneumoniae

Fungi
Cryptococcus neoformans/gattii

Viruses
Cytomegalovirus (CMV)
Enterovirus
Herpes simplex virus 1 (HSV-1)
Herpes simplex virus 2 (HSV-2)
Human herpesvirus 6 (HHV-6)
Human parechovirus
Varicella zoster virus (VZV)

Correction
Due to a printing error, several lines were left out of the Continuing Education article in the January 2016 print issue of MLO. The last paragraph on page 10 should read as follows: Clearly, there is a pressing medical need for highly accurate detection of cervical cancer and high grade abnormal lesions, especially in developing countries where the use of standardized Pap tests is limited. This test must involve a low-cost, quick, disposable, cervical cancer screening system that is sufficiently inexpensive to be employed as a primary screen globally. Limited laboratory infrastructure and instrumentation should be required to quantitatively screen the cervical samples and provide analysis quickly without the need for expensive, trained personnel.

Ebola
Study notes high frequency of spontaneous mutation in Ebola virus. In late December, nearly two years after the epidemic began, the World Health Organization declared the African country of Guinea to be free of Ebola virus infections. But the race to find a cure and therapies to combat the disease is forging ahead as officials warn that inattention could lead to another epidemic.

Texas Biomedical Research Institute scientists had been working on therapies, diagnostics, and vaccines for years before the 2014 epidemic, and a recent study by Dr. Anthony Griffiths published in the Journal of Virology shows a promising mechanism for attacking the virus. Essentially, Ebola virus has the potential to evolve rapidly, but the genetic changes result in viruses that are weakened or not viable. Due to the unprecedented numbers of individuals infected in the latest outbreak, researchers have learned that Ebola virus does evolve in humans. Therefore, a better understanding of the capacity of the virus to evolve could lead to better diagnostics and potential therapies.

To determine whether Ebola virus was sensitive to increasing mutation rate, Griffiths’ group tested a drug called ribavirin. Preliminary experiments with mice suggested ribavirin could be a potential therapy and did cause the desired effect of increasing the mutation frequency enough to make the virus non-viable. Further testing in monkeys showed ribavirin reduced production of infectious Ebola virus, but results were not strong enough to recommend ribavirin as a treatment protocol.

Cancer
Cancer cells poised for growth when opportunity knocks. Researchers have identified a mechanism that allows cancer cells to respond and grow rapidly when levels of sugar in the blood rise. This may help to explain why people who develop conditions in which they have chronically high sugar levels in their blood, such as obesity, also have an increased risk of developing certain types of cancer. The findings were published in the journal eLife by Susumu Hirabayashi, who leads the Metabolism and Cell Growth group at the MRC Clinical Sciences Centre based at Imperial College London, and Ross Cagan of the Icahn School of Medicine at Mount Sinai, in New York.

People with obesity often have persistently high levels of glucose and insulin in the blood. Over time this fades to background noise and the body tunes out, or becomes “insulin resistant.” With the gate closed, glucose can’t be absorbed efficiently so it builds up in the blood, and this accumulation can ultimately lead to type 2 diabetes. But not all cells tune out. In fact, Hirabayashi and colleagues have previously shown that tumor cells in the fruit fly Drosophila melanogaster actively tune in. Hirabayashi found that in flies fed a high-sugar diet, the “normal” cells became insulin-resistant, but the tumor cells didn’t. The tumor cells actually became more sensitive to insulin because they turned on a metabolic switch that triggered them to produce extra receptors for insulin. With insulin binding to many more receptors than usual, more glucose channels opened up and the tumor cells became a “sink” for the glucose that had nowhere else to go in the insulin-resistant body of the fly.

Pregnancy/prenatal
Infertility treatments do not appear to contribute to developmental delays in children. Children conceived via infertility treatments are no more likely to have a developmental delay than children conceived without such treatments, according to a study by researchers at the National Institutes of Health, the New York State Department of Health, and other institutions. The findings, published online in JAMA Pediatrics, may help to allay longstanding concerns that conception after infertility treatment could affect the embryo at a sensitive stage and result in lifelong disability.

Study authors found no differences in developmental assessment scores of more than 1,800 children born to women who became pregnant after receiving infertility treatment and those of more than 4,000 children born to women who did not undergo such treatment.

When the researchers considered only children conceived through ART (assisted reproductive technology), they found that they were at increased risk for failing any one of five domains, with the greatest likelihood of failing the personal/social and problem-solving domains. However, twins were more likely to fail a domain than were singletons (single-born). So, when the researchers compensated for the greater percentage of twins in the ART group than in the non-treatment group (34 percent vs. 19 percent), they found no significant difference between the ART group and the non-treatment group in failing any of the domains.

Similarly, the researchers found no significant differences in the percentage of singleton children in the two groups who were referred for evaluation by developmental specialists (21.2 percent vs. 20.7 percent). Of the children diagnosed with a disability at three-to-four years old, no significant difference was found between the treatment and non-treatment groups: 13 percent, compared to 18 percent.

Innovations in gestational diabetes testing may better assess risk. Susan Hammond, who serves as Global Reagents Manager for Randox Laboratories, UK, writes in with news of a development in gestational diabetes testing: “More and more women in the United States are waiting until they’re older to start having children. The number of births to women between the ages of 45 and 49 rose 14 percent in 2012, according to the Centers for Disease Control and Prevention’s National Vital Statistics Report. With this comes a responsibility for clinicians and laboratories to better assess those at risk of gestational diabetes and to aid better control of the condition for those who already have it. Quick and precise detection of risk of gestational diabetes and associated complications by clinical labs will provide women with the autonomy to take control of their maternal health.”
"Innovations in maternal health testing have meant that analysis such as adiponectin and enzymatic fructosamine are now available in automated biochemistry formats and with more accurate methodologies; allowing laboratories to assess gestational diabetes risk and evaluate control of the condition with ease, speed, and accuracy. Such analyses have historically been non-routine and not easily accessible for clinical laboratories, but now, with little adjustment within the laboratory, these can be added to the test menu, allowing for detailed patient testing profiles."

"Current innovations in the area of gestational diabetes testing will ultimately secure the health, both during and post-pregnancy, of both mother and baby."

**Autoimmune disease**

Researchers find links between processed foods and autoimmune diseases.

The convenience of processed foods may come with an even bigger price tag than previously known, says an international team of researchers. In findings published in *Autoimmunity Reviews*, researchers from Israel and Germany present evidence that processed foods weaken the intestine’s resistance to bacteria, toxins, and other hostile nutritional and non-nutritional elements, which in turn increases the likelihood of developing autoimmune diseases.

The research team examined the effects of processed food on the intestines, and on the development of autoimmune diseases—conditions in which the body attacks and damages its own tissues. More than 100 such diseases have been identified, including type 1 diabetes, celiac disease, lupus, multiple sclerosis, autoimmune hepatitis, and Crohn’s disease.

The researchers focused on the increase in the use of industrial food additives aimed at improving qualities such as taste, smell, texture, and shelf life, and found "a significant circumstantial connection between the increased use of processed foods and the increase in the incidence of autoimmune diseases."

Many autoimmune diseases stem from damage to the functioning of the tight-junctions that protect the intestinal mucosa. When functioning normally, tight-junctions serve as a barrier against bacteria, toxins, allergens, and carcinogens, protecting the immune system from them. Damage to the tight-junctions (also known as "leaky gut") leads to the development of autoimmune diseases.

The researchers found that at least seven common food additives weaken the tight-junctions: glucose (sugars), sodium (salt), fat solvents (emulsifiers), organic acids, gluten, microbial transglutaminase (a special enzyme that serves as food protein "glue") and nanometric particles.

**Infectious disease**

Antibiotics pave way for *C. difficile* infections by killing beneficial bile acid-altering bacteria.

New research finds that bile acids which are altered by bacteria normally living in the large intestine inhibit the growth of *Clostridium difficile*. The work sheds light on the ways in which some commonly used antibiotics can promote *C. diff* infections by killing off the bile acid-altering microbes.

*C. diff* exists in the environment as a dormant spore. To colonize the gut, *C. diff* spores need to germinate and become growing bacteria that produce toxins and damage the large intestine. Researchers know that the use of certain antibiotics lead to a higher risk of *C. diff* infections, particularly among hospital patients. Casey Theriot, PhD, of North Carolina State University, wanted to know exactly how *C. diff* spores were interacting with the microbiota, or natural bacterial environment, within the gut.

“We know that within a healthy gut environment, the growth of *C. diff* is inhibited,” Theriot says. “We wanted to learn more about the mechanisms behind that inhibitory effect.”

Bile acids are made from cholesterol and aid in the digestion and absorption of fats. They also control lipoprotein, glucose, drug, and energy metabolism. Primary bile acids are made in the liver and travel through the intestinal tract. In the large intestine, bacteria convert these to secondary bile acids. Theriot found many bile acids have an inhibitory effect on *C. diff* growth.

Researchers looked at the intestinal contents of mice before and after treatment with many different antibiotics. They identified 26 different primary and secondary bile acids, and defined the concentrations of those acids before and after treatment. Then they added *C. diff* spores to the contents in order to find out how the bacteria may germinate and grow in an actual gut environment.

Interestingly, the primary bile acids in the small intestine allowed spores to germinate, or begin to grow, regardless of the antibiotic treatment. But when the spores reached the large intestine, where normal gut bacteria generate secondary bile acids, those secondary bile acids stopped the *C. diff* from growing. When those bacteria—and the secondary bile acids—were not present following antibiotic treatment, the *C. diff* was able to quickly grow.

**Hematology**

A microfluidic biochip for blood cell counts at the point of care.

The blood cell count is among the most ubiquitous diagnostic tests utilized in primary healthcare. The “gold standard” that is routinely used in hospitals and testing laboratories is a hematology analyzer, which is largely manual and expensive and requires trained technicians and physical sample transportation. It slows turnaround time, limits throughput in hospitals, and limits accessibility in resource-limited settings.

Now, researchers from the University of Illinois at Urbana-Champaign led by Rashid Bashir, PhD, have demonstrated a biosensor capable of counting blood cells electrically using only a drop of blood. Bashir’s team has developed a biosensor to count red blood cell, platelet, and white blood cell counts, and its three-part differential at the point of care, while using only 11 microl of blood.

The microfluidic device can electrically count the different types of blood cells based on their size and membrane properties. To count leukocytes and its differentials, red blood cells are selectively lysed and the remaining white blood cells are individually counted. Specific cells, like neutrophils, are counted using multi-frequency analysis, which probes the membrane properties of the cells. For red blood cells and platelets, one microl of whole blood is diluted with peripheral blood smear on-chip and the cells are counted electrically. The total time for measurement is under 20 minutes.

“Our biosensor exhibits the potential to improve patient care in a spectrum of settings, including resource-limited settings where laboratory tests are often inaccessible due to cost, poor prevalence of laboratory facilities, and the difficulty of follow-up upon receiving results that take days to process,” says Bashir.

“There exists a huge potential to translate our biosensor commercially for blood cell counts applications,” says lead study author Umer Hassan, PhD. “The translation of our technology will result in minimal to no experience being required for operation of the device. In addition, patients can perform the test at home and share the results with their primary care physicians via electronic means.”

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Understanding the CDC’s updated HIV test protocol

By Robert Kapler

A
fter the human immunodeficiency virus type 1 (HIV-1) was identified as the cause of acquired immunodeficiency syndrome (AIDS) in the early 1980s, publicly and privately funded scientists worked quickly to develop tests that could detect the antibody to the retrovirus. Although imperfect, these tests have been used for the clinical diagnosis of HIV infection in both symptomatic and asymptomatic patients and for blood-donor screening for three decades.1

In 1985, the Food and Drug Administration (FDA) approved for marketing the first antibody-based human immunodeficiency virus (HIV) screening test, an HIV-1 enzyme immunoassay (EIA). In the same year, the Centers for Disease Control and Prevention (CDC) released an interim HIV test algorithm and guidelines, and four years later published its first formal guidelines. The algorithm is a recommended step-by-step testing process that is designed to overcome the limitations of any one test. It is also a way of combining the best attributes of several tests to get more accurate results than any single test can deliver. Under the algorithm, which was updated slightly in 1992, if an initial antibody assay was repeatedly reactive it was to be followed by a supplemental test. For 23 years, the protocol required labs to perform either a confirmatory Western blot (WB) or immunofluorescent antibody (IFA) assay on repeatedly reactive specimens.2 Market dynamics made WB the favorite.

But in June 2014, the CDC changed its algorithm to one that no longer recommended the use of the WB to confirm the presence of HIV-1 antibodies (though the test is still used for a few other applications). Technological advancements had led to the approval of an HIV-1/HIV-2 antibody differentiation test that was more sensitive than the WB, especially early in an infection. Moreover, it was a rapid test, which shortened the turnaround time for confirmation of HIV infection. Thus, technological advancements in testing championed by the private sector made the CDC’s original blood-specific HIV algorithm obsolete.3,4

Generations of HIV tests

To detect HIV, a lab either must detect markers of the human immune cellular response to the infection, usually antibodies, or the genetic material of the virus itself, using a nucleic acid amplification test (NAT), or a derivation such as a polymerase chain reaction test (PCR). From 1985 to 1999, the year the first molecular tests obtained FDA approval, antibody tests and some antigen tests basically comprised the HIV test arsenal.

EIAs became the most widely used antibody tests in the United States due to their high sensitivity and standard methodology, making them suitable for high-volume testing. EIAs are designed to detect antibodies (immune cells) or antigens (proteins on the virus that stimulate an immune response) that indicate the presence of HIV infectivity by producing a color change caused by a reaction to an enzyme. The FDA has licensed approximately 10 EIAs.

Since 1985, when commercial immunoassays for HIV-1 detection first became available, there have been five new generations of tests for screening and diagnosis.1 (Figure 1, page 12). (The CDC has published a guide that describes the qualities and differences among the first four generations of tests.) Each new generation of HIV assays further reduced the detection window period—-the time between potential exposure and an accurate test result—and, therefore, the time to the diagnosis and treatment of early infections.

• First-generation EIAs used an antigen consisting of viral lysates to detect immunoglobulin G (IgG) antibodies. The window period of infectivity detection was 56 days.

• Second-generation tests relied on recombinant HIV proteins or synthetic peptides to detect HIV-1/2 IgG antibodies. The window period was reduced to 42 days.

• Third-generation tests are basically combination or “combi” tests that can detect HIV-1 Group M (for “major,” the common U.S. AIDS-causing strain) and O (for “outlier,” the rare African strain), as well as HIV-2. They also use recombinant/synthetic peptides to detect IgG antibodies, as well as immunoglobulin M (IgM) antibodies produced by B cells. The window period was reduced to 22 days.

• Fourth-generation assays, introduced in 2000, could detect HIV-1, Group M, and HIV-2 IgG and IgM antibodies, as well as the HIV-1 p24 antigen. This advancement enabled labs to detect infection in 15 to 17 days.

• The first fifth-generation assay is a multiplexed screening test that detects and differentiates all three HIV analyte markers: HIV-1 antibodies, HIV-2 antibodies, and the HIV-1 p24 antigen.

While the Western blot was for two decades the confirmatory test of choice in the majority of U.S. laboratories, that same time period saw HIV test manufacturers pouring millions of dollars into research to improve first-line tests. They found new methods to make antibody-based EIAs more sensitive and specific and began to develop different technologies to detect both antibodies and antigens, to differentiate infection by type, and to shorten the time to results.

WB: popular but problematic

The first WB kit for HIV-1 was licensed in the U.S. in 1991, and as a result of its apparent reliability and adequate specificity, WB assays proliferated and outpaced IFAs in popularity. The test, made from the inactivated virus itself, was named, in a sort of play on words, after the Southern blot, a technique used for DNA detection developed by Edwin Southern. In the WB

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Describe the history of HIV testing and the purpose for developing an HIV algorithm.

2. Describe the limitations of the first testing methods that were produced for the detection of HIV.

3. Discuss the need for an updated algorithm.

4. Identify the new objectives in the current algorithm and describe the test methods involved.
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- IgA
- IgG
- IgM

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Allergy
- Total IgE

Diabetes
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- Fructosamine
- Hemoglobin A1c
- Insulin
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- α-1 Anti-Trypsin
- α-1 Microglobulin
- Haptoglobin

Allergy
- Total IgE

Diabetes
- Cystatin C
- Fructosamine
- Hemoglobin A1c
- Insulin
- Microalbumin

Coagulation
- D-Dimer
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Continued from page 8: The test is a multiplex flow immunoassay intended for the simultaneous qualitative detection and differentiation of the individual analytes HIV-1 p24 antigen, HIV-1 (groups M and O) antibodies, and HIV-2 antibodies in human serum or plasma (fresh or frozen K2 EDTA, K3 EDTA, lithium heparin, sodium heparin; fresh citrate). This assay is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2, including acute (primary) HIV-1 infection. The assay may also be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in pediatric subjects as young as two years of age, and pregnant women.

The BioPlex® 2200 HIV Ag-Ab assay is not intended for use in screening blood donors, as the effectiveness of this test for use in the screening of these donors has not been established. However, in urgent situations where traditional licensed blood donor screening tests are unavailable or their use is impractical, this assay can be used as a blood donor screening assay.

WARNING: FDA has approved this test for use with serum and plasma specimens only. Use of this test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results. This test is not intended for use in children younger than 2 years of age.

CAUTION: United States federal law restricts this device to sale by or on the order of a physician, or to a clinical laboratory.

Revisiting the algorithm: the momentum gathers

The problem of the WB missing early acute-stage HIV infections, as well as missing HIV-2 cases—and hospitals losing touch with patients—would eventually lead the CDC in 2008 to launch a six-year project to change its recommended HIV test algorithm. Suggestions that the CDC consider changing the protocol actually came three years earlier, in 2005, when the CDC and the Association of Public Health Laboratories (APHL) co-hosted the first HIV Diagnostics Conference. Some 200 researchers, laboratorians, and industry representatives attended the meeting, which was billed as a forum for sharing “the latest information on testing technologies and alternative methods to increase the uptake of testing and diagnosis of persons with HIV infection,” according to a summary of the 2005 conference.

Several presentations dealt with the vulnerabilities of the WB compared with other assays, and others suggested different tests or alternative algorithms. S. Michele Owen, PhD, head of the Lab Branch of the Division of HIV/AIDS Prevention of the CDC, reported the results of a study of 713 specimens that were tested by an initial EIA, retested on an alternate EIA if the original test was non-reactive, and then tested again using WB if the second test was reactive. Results showed that 675 specimens were initially reactive and deemed HIV-1-positive. Of the 38 non-reactive specimens, 26 were reactive on the alternate EIA, but of that number, 17 were reactive on the WB, zero were non-reactive, and nine, or nearly one-third, of the repeatedly reactive specimens were indeterminate.

An official with the American Red Cross (ARC) reported that of 12.4 million blood donations from 1989 through 1999, 11,080 were EIA repeatedly reactive, and of these, 7.1 percent were WB positive, 46.6 percent were WB indeterminate, and 46.3 percent were WB negative. She explained that the rate of indeterminate and negative WB results could be partly attributed to an FDA requirement that any background discoloration or band must be reported in addition to clearly viral bands.

Several presentations explored other algorithm options that would reduce or eliminate the use of WB. These included using NAT or more advanced-generation EIA for supplemental confirmatory testing; or using combinations of rapid tests; or using a particular rapid test in combination with HIV-1 and HIV-2 assays and returning the initial visit, or the hospital lost touch with them entirely.

One large study of 3.6 million tests comparing the WB with IFA found that the use of the IFA had 13 times fewer indeterminate samples than the WB. And when pregnant women’s HIV tests were repeatedly reactive on an EIA, they were more likely to be negative or indeterminate on the WB. What’s more, the WB sometimes confused the relatively rare HIV-2 type for the common HIV-1 strain. The CDC reported in 2011 that 60 percent of a study group of 163 HIV-2 cases were HIV-1 reactive on a WB.

As new generations of first-line assays came on the market, laboratory supervisors, including those at public health laboratories (PHLs) in states with known hot spots for HIV incidence, began to notice a disturbing trend. More specimens were producing repeatedly reactive results on initial screening but negative or indeterminate results on the WB. A significant number of those specimens came from people known to be at high risk.

PHLs experiencing unconfirmed HIV diagnostic results with the old algorithm often became aware of clinical manifestations in the person being tested that caused them to suspect that the patient could be in an acute, or early stage of HIV infection. If a laboratory issued a negative or indeterminate report, it would be up to the public health provider to advise the person that he should return to provide another blood sample in two or three weeks to capture seroconversion. (The variability of individual immune responses also played a part, considering that the body starts producing antibodies between two and 12 weeks of infection). But many people who might have been infected with HIV did not return after the initial visit, or the hospital lost touch with them entirely.

The BioPlex® 2200 HIV Ag-Ab assay is also intended for use in testing plasma specimens to screen organ donors when specimens are obtained while the donor’s heart is still beating.

The BioPlex® 2200 HIV Ag-Ab assay is not intended for use in screening blood donors, as the effectiveness of this test for use in the screening of these donors has not been established. However, in urgent situations where traditional licensed blood donor screening tests are unavailable or their use is impractical, this assay can be used as a blood donor screening assay.

Continued on page 12
Early detection saves lives

The 5th generation BioPlex® 2200 HIV Ag-Ab assay sniffs out acute infection early in the window period

BioPlex® 2200 HIV Ag-Ab assay uses its multiplex technology to simultaneously detect and differentiate:

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- HIV-2 Ab

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HIV

A higher concentration of the virus in their bodies and thus were more likely to infect others. There was also mounting evidence that the sooner an individual started therapy, the less damage was wrought on his or her immune system, and thus, the greater likelihood of an increased life span.

The objective to more accurately diagnose HIV-2 was inspired by the fact that if a patient is misdiagnosed as being infected with HIV-1, he or she might be treated with ineffective drug therapy. Some of the reverse transcriptase inhibitors used to treat HIV-1 are effective in fighting HIV-2. However, other classes of drugs, such as the protease inhibitors, are not.

And getting results back on the same day instead of a week later is important because a significant number of people who come in for screening leave their specimen but fail to return to obtain results. At the 2010 conference, Dr. Branson said that at the time about 20 percent of those tested in the U.S. never obtained WB confirmatory results.

Revised recommendations

After years of development, the CDC in June 2014 published a new HIV test algorithm and recommendations. The new protocol is intended to help laboratories detect chronic, or established, infections, as well as acute, or new, infections up to a month sooner than the previous testing protocol. Thus, public health officials and clinicians can concentrate on people who are in the early stage of HIV infection.

The revised algorithm enables the detection of the p24 antigen as the viral load ramps up. By exposing infectivity earlier, the revised algorithm helps clinicians get HIV patients into treatment faster and supports public health efforts to restrict the spread of the disease by making HIV patients’ sexual partners aware that they may be at risk and should be tested. The protocol also differentiates HIV-1 from HIV-2 and eliminates most indeterminate results because of the greater sensitivity of today’s supplemental test technology.

The first level of testing is with an HIV-1/2 “combo” immunoassay, which can be either a fourth- or fifth-generation test. If the serum specimen is positive in the first-line test—meaning the qualitative detection of HIV-1 p24 antigen or HIV-1/2 antibodies—it is subject to the HIV-1/2 antibody differentiating assay. A positive result of the differentiating assay for either HIV-1 or HIV-2 will be interpreted and diagnosed. A negative or indeterminate test will require a NAT test, which will confirm the accurate detection of an early infection or indicate a false positive by the fourth-generation test. A positive test will trigger a diagnosis of acute HIV-1 infection. A negative result will indicate the individual is HIV-negative.

The FDA has approved three non-differentiating, fourth-generation “combo” assays: (1) the Bio-Rad HIV-1/2 Ag/Ab EIA; (2) the Abbott Architect HIV Ag/Ab chemiluminescent assay; and (3) the ADVIA Centaur Ag/Ab CIA.

Five Generations Of HIV Tests

![Five Generations Of HIV Tests](image)

**First generation**
- **EIA:** Used an antigen consisting of viral lysates to detect IgG antibodies. Mean window period: 56 days.

**Second generation**
- **Recombinant HIV proteins or synthetic peptides detect HIV-1/2 IgG antibodies. Window period: 42 days.**

**Third generation**
- **Combination or “combo” tests can detect HIV-1, group M and O, as well as HIV-2. Use recombinant/synthetic peptides to detect IgG and IgM antibodies produced by B cells. Window period: 22 days.**

**Fourth generation**
- **HIV-1 Group M and HIV-2 IgG and IgM antibodies as well as the HIV-1 p24 antigen. Window period: 15-17 days.**

**Fifth generation**
- **A multiplexed screening test that detects and differentiates all three HIV analytic markers: HIV-1 antibodies, HIV-2 antibodies, and the HIV-1 p24 antigen.**

Figure 1. Five generations of HIV tests
The FDA has approved one combo differentiating fifth-generation assay, the BioPlex 2200 HIV Ag-Ab. This assay detects and differentiates antibodies to HIV-1 and HIV-2, as well as the HIV-1 p24 antigen. Bio-Rad’s Multispot HIV-1/2 assay is scheduled to be withdrawn from the market as of December 2016.

The FDA has approved a number of “rapid tests,” including (a) the Bio-Rad Geenius HIV-1/2 Supplemental Assay; (b) the Alere Determine HIV-1/2 Ag/Ab Combo assay; (c) the INSTI HIV-1/2; (d) the DPP HIV-1/2; and the (e) OraQuick Advance HIV-1/2.

Labs may continue to use a third-generation “combi” HIV-1/2 antibody-only assay, but they run the risk of missing cases in which the patient is HIV antibody-negative but HIV-1 antigen positive.

It is clear that the pace of technological advancements in HIV testing is continuing to accelerate. To keep abreast of the changes, the CDC is expected to recommend more improvements to the HIV test algorithm in coming years.

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3. Some of the information used in this article was derived from a previous article by the author published in this journal. Kapler R. HIV test algorithm matches protocol with latest technology. MLO. 2015;47(4):38-40.
4. The original algorithm is still used for testing other bodily fluids.
11. The three stages of HIV infection are acute (or early) HIV infection, chronic (or established) HIV infection, and acquired immunodeficiency syndrome (AIDS).
13. Much of this information was provided by Barry Bennett, MPH, head of the Retrovirology Unit of the Florida Bureau of Public Health Laboratories, during his lecture on 8/16/15 at the Bio-Rad CE Event, East Elmhurst, NY.
TEST QUESTIONS

1. What was the first type of HIV screening test approved by the FDA in 1985?
   a. HIV-1/HIV-2 enzyme immunoassay (EIA)
   b. Western blot (WB)
   c. NAT testing
   d. HIV-1 EIA

2. What was the purpose for the CDC to develop an HIV algorithm?
   a. so that limitations of the HIV test can be overcome
   b. to produce more work for the lab technicians
   c. both a and b
   d. neither a nor b

3. In the first algorithm produced, what test(s) was/were considered the most popular and most recommended confirmatory test(s) to HIV-1 EIA?
   a. NAT testing
   b. WB test
   c. immunoassay antibody assay (IFA)
   d. both b and c

4. After more than two decades of the first published HIV algorithm, what was the biggest change in the updated algorithm?
   a. the elimination of recommending the WB test as a confirmatory test
   b. the addition of more testing methods, along with the WB test
   c. the elimination of an algorithm
   d. none of the above

5. EIAs are the most popular and widely used test methodology for detection of HIV antibodies because they are highly sensitive and have a standardized methodology.
   a. True
   b. False

6. How many generations of HIV screening tests have been developed since 1985?
   a. 10
   b. 1
   c. 7
   d. 5

7. What factor was improved upon with the development of each new generation of HIV screening tests?
   a. decreased turnaround time of results to the physician
   b. reduction of the detection window period
   c. decreased amount of blood specimen required
   d. none of the above

8. The improvement of first-line screening tests involved the methodology to be more sensitive and more specific, as well as discovering technologies that could detect antibodies, along with antigens; differentiating the infection by type 1 or 2; and reducing the turnaround time of retrieving results.
   a. True
   b. False

9. What test methodology is used in the Western blot (WB) test?
   a. electrophoresis
   b. nephelometry
   c. agglutination
   d. EIA

10. The Western blot (WB) test was the most widely used confirmatory test because it was easy to manufacture, easy to use, and provided a fast turnaround time.
    a. True
    b. False

11. What problem was identified with the Western blot (WB) test, as newer generations of screening tests were being used?
    a. reactive screening tests, with reactive on the WB
    b. nonreactive screening tests, with reactive on the WB
    c. reactive screening tests, with negative or indeterminate on the WB
    d. none of the above

12. What main limitations led the CDC to revisit the current HIV algorithm and make changes to the protocol in 2008?
    a. screening tests leading to many false positive results
    b. screening tests leading to many false negative results
    c. WB test results missing early infection and HIV-2 infection
    d. limitations were not identified and the protocol was not changed.

13. Recommendations for a new algorithm included the diagnosis of people early in infection, distinguishing HIV-1 from HIV-2 more accurately, and faster turnaround time of results.
    a. True
    b. False

14. What is the importance of distinguishing HIV-1 from HIV-2?
    a. It detects chronic/established and acute/new infections.
    b. It differentiates HIV-1 from HIV-2.
    c. It detects infection up to a month sooner than the previous protocol.
    d. all of the above

15. What is the importance of a timely turnaround time of HIV results?
    a. less confirmatory testing performed
    b. for follow-up appointments for results
    c. for proper drug treatment protocol
    d. all of the above

16. What is the importance of obtaining results early in the infection period of HIV?
    a. to reduce the infectivity of the virus to others
    b. for proper drug treatment protocol
    c. for follow-up appointments for results
    d. for less confirmatory testing

17. In what year was the most recent HIV testing algorithm published?
    a. 2010
    b. 2011
    c. 2013
    d. 2014

18. What enhancements have been made to the new HIV algorithms?
    a. It detects chronic/established and acute/new infections.
    b. It differentiates HIV-1 from HIV-2.
    c. It detects infection up to a month sooner than the previous protocol.
    d. all of the above

19. What combination of tests is used in the new algorithm?
    a. HIV-1 qualitative immunoassay, NAT test, WB test
    b. HIV-1/2 qualitative immunoassay, WB test, HIV-1/2 differentiating assay
    c. HIV-1 qualitative immunoassay, HIV-1/2 differentiating assay, NAT test
    d. none of the above
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A20 modulation: a potential biological threat that can be mitigated by immunohistochemistry

By Maj. Michael A. Washington, PhD, M(ASCP)

During the past two decades we have witnessed tremendous strides in the advancement of the biological sciences. In particular, molecular cloning has become a common activity, and the ability to develop genetically modified organisms has migrated from the confines of secure biological containment suites onto the open benches of undergraduate teaching labs.

Nowhere has this progress been more dramatic than in the fields of immunology and microbiology. Within a short period of time, our understanding of the immune system has been transformed from that of an intractable “black box” to an intricate series of networks in which a specific and defined set of signal transducing proteins control the initial activation and modulation of an expanding set of effector molecules, leading to outputs that are both predictable and tractable.1

Such progress has yielded an understanding of the mechanisms of disease at the molecular level and the ability to diagnose and treat both infectious and non-infectious ailments by rational means. However, detailed knowledge of the mechanisms of immunity can also be utilized to design specific and effective biological weapons, or to augment the capabilities of traditional biological threat agents.

Intentional biological attacks on the immune system

The vertebrate immune system has been under continuous selective pressure for approximately 500 million years.2 The architecture of this system can be divided into two distinct but interdependent branches: the innate and adaptive immune systems.

- The innate immune system uses germline-encoded receptors to detect pathogens via the recognition of a group of conserved constituents on the surface of invading microorganisms.
- The adaptive immune system is a complementary defense mechanism that utilizes gene rearrangement to form antigen receptors of nearly unlimited diversity, enabling the precise discrimination of self from non-self, the recognition of the majority of pathogens encountered throughout the life of the organism, and the constitution of an immunological memory.3

The age and complexity of the immune system, coupled with the high degree of diversity among the organisms with which it must contend, leads to the expectation that numerous genetic redundancies have accumulated over evolutionary time. However, research suggests that the necessary and sufficient functions of the immune system can be performed with a relatively small number of genes.2 The products of these genes are often targeted by pathogens, as a means of evading the host immune response, and it has been demonstrated that the modulation of these genes directly results in compromised immunity.2 These genes therefore represent critical vulnerabilities of the immune system, vulnerabilities that could potentially be exploited by adversaries through engineered biological weapons.

Currently, there are very few methods available to detect genetically engineered organisms or to rapidly respond to such a threat. It can be argued that the ability to manipulate biological systems has increased exponentially in this era, whereas the ability to detect and develop effective countermeasures has increased linearly. This discordance has led to an environment in which it is possible to engineer a new biological threat agent in a matter of days, while the characterization of the threat and the development of countermeasures can take months to years.

A20 as a critical vulnerability of the immune system

Tumor necrosis factor alpha induced protein 3 or Tnfaip3 is an example of a critical vulnerability of the immune system. Tnfaip3 encodes a 790-amino acid protein known as A20.2 A20 was initially identified in the early 1990s by a group working at the University of Michigan. This group was interested in the effects of a cellular mediator known as tumor necrosis factor alpha (TNF-alpha) on endothelial tissue.2 Their approach was to expose human endothelial cells to TNF-alpha, followed by molecular cloning of any gene products induced by this treatment. Several new proteins were identified, including A20. A20 expression was detected in nearly all tissues and all cell types, with high levels present in lymphoid tissue.

Further investigation revealed that the molecular activity of A20 involves the “editing” of ubiquitin chains.3 Ubiquitin is a small peptide which is covalently attached to substrate proteins as a monomer or multimer in one of several spatial conformations. Depending on the particular form of ubiquitin substitution, substrate proteins are targeted for either degradation or functional modification.3 Through these ubiquitin editing activities, A20 has the potential to modulate the activity of a broad range of intracellular signal transducers.

Recently, a group working at the University of California in San Francisco showed that A20 plays a crucial role in the immune system. Their studies employed a “knock-out” mouse model in which the gene encoding A20 was inactivated in the mouse genome.3 The resulting mouse strain was evaluated for defects resulting from this deletion. The results of these studies showed that A20 knockout mice exhibit systemic multi-organ inflammation, in response to commensal flora, leading to early death. Interestingly, if these mice were given large doses of antibiotics, systemic inflammation was reduced and the mice survived.3 This result suggested that A20 holds immune responses in check, as the absence of A20 leads to an exaggerated and ultimately fatal immune response to non-pathogenic commensal bacteria.

Later studies revealed that A20 plays a key role in limiting the extent of both innate and adaptive immune responses. It has been shown that A20 is directly involved in regulating both the morbidity and mortality resulting from acute viral infection and intracellular parasitism.4 In 2012, a Belgian group reported that the deletion of A20 in myeloid cells protects mice from lung damage during mild influenza A virus infection.

The views expressed in this publication/presentation are those of the author(s) and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the U.S. Government.
Myeloid-specific A20 knock-out mice are also protected from the mortality resulting from a lethal dose of influenza A virus. This study demonstrates that A20 is indeed a central gatekeeper of the immune response, since the deletion of this gene alone is able to alter the outcome of a viral infection.

The central role of A20 has been exploited by various groups of pathogen to evade the host immune response. For example, the measles F protein has been shown to up-regulate the expression of A20 as a means of dampening the innate immune response, and a group working in India reported that the protozoan parasite Leishmania donovani has evolved a mechanism for modulating A20 activity as a means of facilitating intracellular survival. A20 is also a target for biological toxins. There is data to suggest that A20 is involved in maintenance of epithelial barriers and in the destruction of allergens and toxins via an intracellular degradation pathway involving vesicles called endosomes and lysosomes. It has been shown that cholera toxin forms complexes with A20 in the cytoplasm. Cholera toxin sequesters A20, preventing interaction with substrate molecules. The end result is that cholera toxin-mediated inhibition of A20 leads to the breakdown of epithelial barriers, with the concomitant release of allergens and toxins into the cytosol.

Modulation of A20 as a biological threat

The fact that A20 plays a critical and non-redundant role in the immune response to bacterial, viral, and protozoan pathogens makes this molecule an attractive target for biological weapons development. There are several means by which the activity of A20 could be modulated by a biological weapons designer. The most likely method would be to genetically modify a traditional biological agent or an emerging pathogen to interfere with the activity of A20 in such a way as to enhance pathogen virulence. For example, Bacillus anthracis could be engineered to deliver an A20-activating enzyme, together with chromosome-encoded protective antigen and edema factors. Since A20 down-regulates both the innate and adaptive immune systems, the activation of A20 in the context of an anthrax infection could enable the pathogen to escape the immune response, thereby increasing virulence. In addition, this strategy might allow a smaller number of spores to initiate infection, increasing the efficiency of either line-source or point-source dissemination.

Another possible scenario is that anthrax and cholera could be delivered simultaneously as a binary weapon. Complex formation between cholera toxin and A20 would lead to a breakdown in epithelial barriers, causing increased spread of anthrax and anthrax-associated toxins resulting in increased virulence. Similarly, a viral pathogen such as influenza could be engineered to express an A20-degrading enzyme. This modification may have the effect of exacerbating the immune response to the virus in the upper respiratory tract, leading to increased pathology. The technology to modify Bacillus anthracis and the influenza virus not only exists, but is widespread. A20 is being actively researched on at least three continents. Thus, there is potential for the diffusion of technical knowledge regarding A20 and its properties to potential adversaries.

A20 as a biomarker in detecting malevolent biological engineering

Although A20 analysis is not currently performed in the clinical setting, it is an emerging biomarker with the potential to augment the analysis of novel (engineered) host-pathogen interaction. Since the clinical laboratory is essential to the detection of unusual pathogens in the patient population, this may be the ideal environment for the detection of overt A20 modulation. While there are several methods which can be used to monitor A20 activity, immunohistochemistry is the most attractive. This is due to the fact that immunohistochemistry allows direct visualization of the protein and requires no prior knowledge of how A20 modulation may have been achieved. Normally A20 tends to localize into discrete punctate structures in the cytoplasm. These structures have been visualized with the aid of fluorescently-tagged antibodies. Such antibodies can be used to determine whether A20 is present in clinical samples, whether it is under-expressed or over-expressed, and possibly to determine whether it is properly localized. In addition, antibodies can be developed to target individual A20 domains. This will make it possible to determine whether an organism is expressing a specific domain, if a specific domain from endogenous A20 has been re-targeted, or whether A20 has been cleaved by an anti-A20 enzyme resulting in a separation of the domains.

Conclusion

As technology advances and our knowledge regarding the molecular mechanisms underlying the delicate interaction between bacterial and viral pathogens and the host immune response becomes more sophisticated, the possibility of the malevolent application of this knowledge will also increase. The clinical laboratory staff is on the frontlines of biodefense and will undoubtedly play an important role in the detection and response to future biological threats, whether natural or manmade. In order to be prepared for novel threats, it is essential that laboratory staff be familiar with A20 and that the specific reagents be prepared to monitor its activity.

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Biomarkers and personalized cancer medicine

By Nancy I. Alers, MS, MT(ASCP)CM

The American Cancer Society (ACS) estimated that 1.6 million people would be diagnosed with cancer and more than 500,000 would die from the disease in 2015.1 ACS also projects that almost 40 percent of men and women will be diagnosed with cancer at some point in their lifetime. With cancer having such an impact on society, it makes sense that cancer research has focused on developing more effective treatment options. Resistance to anticancer drugs, however, is a leading contributor to death in cancer patients.2 Here is a brief review of how biomarker research is addressing that problem and revolutionizing cancer management.

Drug resistance: one size does not fit all
The standard of care for cancer patients has been a combination of systemic drugs, surgery, and/or radiation. Yet treatment may or may not work, based on a number of factors. For this reason, scientists have been trying to decode and understand cancer in hopes of developing more advanced diagnostic techniques and being able to offer more effective treatment options. Until relatively recently the knowledge base and technology needed to understand the molecular processes behind cellular resistance to treatment was simply not available.

The emergence of genome-wide fields of study such as proteomics (the study of proteins encoded in genes) and pharmacogenomics (the study of individual genetic variations in drug response) are bridging that gap. Using new advances in technology and information learned from human genome mapping,2 researchers are beginning to have a better understanding of cancer cell biology, as well as some intrinsic resistance mechanisms.

In addition to increased understanding of the molecular pathogenesis of various cancer types, information on how a person’s genes affect his or her response to drugs has been instrumental in the development of targeted treatment strategies. Targeted therapies increase the efficacy of medication by targeting specific biomarkers while reducing the toxicity associated with systemic treatments.

Predicting treatment resistance
Biomarker identification is an essential element of personalized cancer medicine. Identification is achieved by testing the patient’s tumor tissue, blood, or other body fluids for the presence or expression of certain molecules. Once a specific biomarker or genetic mutation is detected in the patient’s tumor, the information obtained is used to predict treatment effectiveness.3

Biomarker research has shed light on the genetic variations and resistance mechanisms of cancerous cells: what triggers their uncontrolled proliferation, what fuels their self-sustaining capabilities, and what makes them immune to apoptosis (programmed cell death). This information has been used for the design of “smart drugs,” often used in addition to chemotherapy, which target specific cell growth factors—proteins involved in signaling pathways—and inhibit factors that promote angiogenesis (formation of blood vessels to support tumors). Targeted drugs work by suppressing key activities necessary for cellular division and differentiation.4,5

For colorectal cancer patients, for example, tumor testing for KRAS mutation, a gene that stimulates cell growth as a downstream effector of the activated EGFR (epidermal growth factor receptor) signal, has become a standard prior to using EGFR antibody therapy.4 Up to 40 percent of colorectal cancer tumors have a detected KRAS gene mutation. Patients with wild type (i.e., normal) KRAS genes may or may not respond to such therapies, depending on alternative resistance mechanisms.6,7 Therefore, it is imperative to determine the patient’s KRAS mutation status prior to selecting EGFR antibody therapy as a choice.

In the case of melanoma, about 50 percent of cases have mutations in the BRAF gene. BRAF, a protein kinase that helps activate the RAS/MAPK pathway, is important for cell division, growth, and differentiation.8 A mutated BRAF gene in cells can potentially make normal cells turn cancerous by signaling the cells to grow and divide at abnormal rates. Treating melanoma BRAF mutation-positive patients with a targeted therapy agent, such as Vemurafenib, helps inhibit the mutated BRAF gene function. In turn, this will increase treatment effectiveness.

Biomarkers and the lab
Understanding cancer’s pathogenicity and multiple pathways for cell growth, division, and treatment resistance at a molecular level remains a complex goal for researchers. However, the potential benefits to patients more than justify the effort, one which will only increase as the theories and practices of personalized medicine move increasingly into the clinical realm.

Laboratory testing will play a vital role in aiding physicians to select the most appropriate treatment for patients. Expect more biomarker testing to enter the clinical laboratory as more biomarkers are discovered and translated to clinical practice.

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Management of diabetes: the future is now

By Ross Molinaro, PhD, MLS(ASCP)℠, DABCC, FACB, and Carole Dauscher

Diabetes is a worldwide epidemic. Its prevalence continues to rise globally at an average rate of 8.7 percent, and it currently affects 382 million of the world’s population. Significant increases in populations diagnosed with diabetes have been reported by many nations as their lifestyle and dietary norms evolve with globalization. National healthcare budgets bear the financial burden of treating diabetes and its complications, exceeding $548 billion dollars globally.

Through the power of diagnostic testing to help screen, diagnose, and monitor, a patient’s chronic condition can be kept in balance and not allowed to escalate to a critical state that lessens quality of life and may require hospitalization and more expensive intervention.

The impact of diabetes

Diabetes is defined as a chronic disease that occurs when the pancreas is no longer able to make insulin or when the body cannot make good use of the insulin it produces. Not being able to produce insulin or use it effectively leads to raised glucose levels in the blood (known as hyperglycemia). Over the long-term, high glucose levels are a threat to well-being, and are associated with damage to the body and failure of various organs and tissues.

Alarmingly, the growing number of people with diabetes worldwide will place even more individuals at risk for developing the comorbidities associated with diabetes. Left untreated, complications may affect the proper functioning of all organ systems, including increases in the likelihood of cardio-renal syndrome exhibited by progressive chronic kidney disease (CKD) and cardiovascular disease (CVD) that result in premature mortality.

Diabetes is the leading cause of CKD, which is more prevalent in diabetics than non-diabetics. Uncontrolled high blood glucose and high blood pressure cause damage to small blood vessels, leading to decreased kidney function.

Kidney failure is the most costly chronic disease, accounting for five percent of annual healthcare budgets (over $30 billion in the U.S. alone). People with early CKD are generally asymptomatic; hence, early detection and treatment are crucial for preventing or slowing progression to end stage renal disease (ESRD), complications, and premature death.

The power of HbA1c and ACR

Hemoglobin A1c (HbA1c) is recognized as a reliable and convenient biomarker for the screening, diagnosis, and management of long-term diabetes. It is recommended as a marker for the management of diabetes in CKD patients; the prevalence of diabetes-related complications rises at higher HbA1c (> ~six percent to seven percent). Glycemic control, as reflected by normoglycemic HbA1c concentrations, leads to reduction in diabetic complications, including nephropathy.

Higher levels of urine albumin (albuminuria) are determined by a measurement of urine albumin to creatinine ratio (ACR). They are possibly the earliest indication of diabetic and other glomerular kidney diseases and are associated with all-cause and CV mortality, adverse outcomes (ESRD, acute kidney injury, and progression of CKD), and mortality in CKD patients.

CKD is defined as abnormalities in kidney structure or function of greater than three months. The Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend classifying CKD as to (1) Cause, (2) GFR category, and (3) Albuminuria category (CGA). A CKD diagnosis is made when one or both of the following are present for greater than three months:

• A decline in kidney function as defined by eGFR < 60 mL/min/1.73m². (Normal ≥ 90 mL/min/1.73m²)
• Kidney damage (albuminuria: ≥30 mg urine albumin per gram of urine creatinine (urine albumin to creatinine ratio, ACR); imaging abnormalities, genetic, or renal transplant history).

Additionally, guidelines recommend that all CKD patients, including children, should be considered at increased risk for CVD because lower eGFR and abnormally high levels of albumin in the urine associated with CKD are also associated with cardiovascular mortality. The most common cause of death in the dialysis population is CVD; CVD mortality is twice as high in dialysis patients as in the general population. Increased risk for CVD is observed in the early stages of CKD.

Monitoring for comorbidities

Diabetes is a multi-faceted chronic illness requiring continuous monitoring with multi-faceted risk reduction strategies. Today, physicians require immediate access to the diagnostic tools that provide comprehensive data to diagnose and evaluate the progression of diabetes, CKD, and CVD.

Physicians require the sensitivity and specificity of specialty assays performed in the clinical laboratory. Tests such as HbA1c, c-peptide, insulin, glyceded albumin, and glucose each have their role as laboratory tests, including diagnosis and monitoring for diabetes. Creatinine, eGFR, cystatin c, and urine albumin are key assays to assess the progression of CKD within the KDIGO guidelines.

Markers to aid in the assessment of cardiovascular events and disease include BNP (B-type natriuretic peptide), NT-proBNP (N-terminal pro-brain natriuretic peptide), and cardiac troponin (cTn).

continued on page 22
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POC enhances management

Physicians’ ability to perform key assays such as HbA1c and albumin/creatinine ratio in-office is important to establish an effective consultative relationship with patients and to be able to consider timely therapy modifications to enhance well-being and quality of life.

As healthcare becomes more connected, so has the in vitro diagnostic industry. The harmonization and standardization of cost-effective end-to-end solutions across reference labs, hospitals, clinics, and physician offices ensure consistent, accurate, and reliable results regardless of where testing takes place.

Point-of-care (POC) informatics can maintain control and visibility from a central location to facilitate high-quality results and enhanced patient outcomes. Although there remains a resource disparity in many regions of the world that can affect the level of care, the availability of innovative technologies surrounding informatics is empowering physicians and patients with ready delivery of customizable reports, remote 24/7 monitoring, and control support services.

The future is here. Diagnosis, screening, and monitoring the progression and treatment of diabetes has been shaped and transformed by the delivery of clinical and workflow excellence through end-to-end solutions including informatics, automation, and pre-post analytics. These innovations help enable patient treatment that can advance human health today.

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Paving the way for prediabetes diagnostics: biomarkers that reflect impaired glucose tolerance

By Doug Toal, PhD

In 2014, the global prevalence of diabetes was estimated to be at nine percent among adults age 18 and over, with approximately 1.5 million deaths directly attributed to the disease. Furthermore, the World Health Organization (WHO) estimates that by 2030, diabetes will be the seventh-leading cause of death worldwide.1,2 Type 2 diabetes (T2D) has become a worldwide pandemic that continues unabated, and there remains a great public health need for biomarkers that can detect early signs of the disease (prediabetes) so that those at greatest risk can implement lifestyle changes that delay or prevent the disease.

Prediabetes is currently defined using one or more glycemic-based criteria, including fasting plasma glucose (FPG, 100-125 mg/dL), hemoglobin A1c (A1c, 5.7 percent to 6.4 percent) and two-hour plasma glucose (2hPG, 140-199 mg/dL).3 Unfortunately, these criteria have been shown to identify only partial overlapping groups of subjects and likely reflect different pathophysiologic states along the metabolic continuum leading to T2D. In order to complement the diagnosis of prediabetic states such as impaired glucose tolerance, it is necessary to identify and quantify disease-specific biomarkers that provide more information than the currently used glycemic measures.

Impaired glucose tolerance (IGT) is a prediabetic state of hyperglycemia that occurs when blood glucose levels remain high for an extended period after oral ingestion of glucose but not high enough to be diagnosed as T2D. It is associated with insulin resistance, obesity, dyslipidemia (high triglycerides and/or low HDL cholesterol), and hypertension.4 Persons with IGT have an increased risk of developing T2D and cardiovascular disease.5 It is estimated that 10 percent to 15 percent of adults in the United States have IGT.6

Historically, IGT has been diagnosed via the oral glucose tolerance test (OGTT), with two-hour plasma glucose (2hPG) values of 140-199 mg/dL. The use of the OGTT has diminished considerably in recent years, as it is time-consuming, expensive, and unpopular with both patients and physicians. Due to the greater convenience of measuring FPG and A1c, patients at risk for diabetes are more likely to have these two parameters measured in routine examinations rather than to undergo an OGTT. As a consequence, IGT subjects with normal FPG and A1c may not be identified.

Since glycemic measures alone are insufficient in identifying IGT and the costly OGTT is inconvenient and unpopular, researchers have recently turned to metabolomics to identify IGT-specific biomarkers. For instance, Cobb et al7 applied global metabolomic profiling to identify 23 candidate biomarkers from fasting plasma samples taken just prior to an OGTT from a cohort of 1,623 nondiabetic subjects. This work confirmed the idea that multiple metabolic pathways, not directly involved in glucose metabolism, are perturbed in IGT and further demonstrated that metabolites from these perturbed pathways can be used in models predicting IGT.

Two metabolites, α-hydroxybutyric acid (α-HB) and linoleoyllycero-phosphocholine (LGPC), performed equally as well as FPG in predicting IGT. Moreover, there were a number of metabolites—including two small organic acids (α-HB and 4-methyl-2-oxopentanoic acid [4MOP]), two lipids (oleic acid and LGPC), a ketone body (β-hydroxybutyric acid [BHBA]), an amino acid (serine), and a vitamin (pantothenic acid [vitamin B5])—that are complementary with and additive to FPG when utilized in multivariate models for the prediction of IGT. Using a multivariate logistic regression algorithm, a panel of the above metabolites and glucose were measured from 955 fasting plasma samples to predict IGT with a sensitivity of 78 percent and specificity of 72 percent.

The metabolites described in that paper appear to represent a broad sampling of the body’s ability to dispose of a glucose load like that seen in an OGTT from multiple perspectives beyond that of purely glycemic parameters. Global metabolic approaches to biomarker discovery allow translational research scientists an opportunity to improve upon the status quo, and they are leading to the development of new diagnostics that can significantly improve our ability to identify prediabetic conditions such as insulin resistance and impaired glucose tolerance. Such work will provide clinicians better tools to help identify at-risk patients and ultimately may help individuals to avoid the devastating effects of diabetes by placing more focus on preventive measures.

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Doug Toal, PhD, serves as Vice President of CLIA Laboratory Operations for North Carolina-based Metabolon, Inc., where he leads the development of metabolomics-based assays for use in the clinical laboratory. Metabolon has launched Quantose IR and Quantose IGT as LDTs for use in patients at risk for development of prediabetes.
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Train the trainer: taking control of your lab’s software education

By Craig Madison

Your lab has installed new software, which you hope will improve the quality and efficiency of its work. You want to get your staff up to speed as soon as possible so everyone can effectively use and benefit from the software without delay. But you find that your staff’s learning curve is considerable. How can training be made painless, quick, and successful?

Training staff to use new software has inherent challenges. If the software is meant to be used by the majority of the lab’s staff, then a mid-to-large size laboratory could have dozens or even hundreds of trainees. In such cases, it may not be possible for the software vendor to train everyone. Clearly, not everyone can get off the bench at the same time, nor does everyone work the same shift. Also, many laboratory organizations consist of physically separated facilities, which can also be challenging.

These challenges can be met, however—with “train-the-trainer” sessions. In this approach, the software vendor trains a select group of “super-users,” first. Those users, in turn, train the remaining staff. Some advantages of this approach include:

• **Cost control:** When vendors tell you the training is included in the price of the software system, this means the cost of training has been added to the price of the system. If more training is needed six months or a year later, that will likely be an add-on.

• **Schedule simplification:** Since you’re training your own lab staff with in-house trainers, there’s less potential for scheduling conflicts as opposed to using the vendor’s trainers, who must coordinate sessions with multiple lab clients.

• **Solid knowledge base:** While the initial group of core users provides training to others, they are themselves becoming increasingly proficient with the software. They become a core team who know the product and can always help users, without having to go to IT or vendor support for front-line questions.

• **Ongoing training:** With the knowledge brought in-house, “refresher” training can be provided as needed. To accommodate new staff, you can schedule new user training as appropriate.

Like most good ideas, it only works, however, when it is well-executed. Here are some tips for making the most of in-house training.

**Planning in advance**

Trainers need to do a fair amount of planning in advance, and the vendor can help with that. The vendor can provide a list of training activities, starting with the easiest activities and getting progressively more sophisticated. Trainers need to determine how many sessions will be needed; there may be just one, but two or more may be needed for complicated systems. They must define the specific objectives for each session—that is, the software functions that staff should be able to complete by the time each session is complete. Then, for each session, the trainers need to create step-by-step “lesson plans.” One way to do this is to start with the desired outcome, and work backwards to discover the best way to bring their students to it. Trainers can revisit their own learning; how did they absorb and integrate this material—that is, in what order and at what pace? With the material divided into sessions and each session organized around objectives, the training will go more smoothly.

**Grouping staff members**

For most software systems there will be different types, or sub-groups, of lab users. User types are determined based on what those particular staff members are allowed to do with the software—such as, read-only users, editors, administrators, or system administrators. Each sub-group will have its own training session(s). Rule of thumb: the more the sub-group has to learn, the smaller the number of trainees it should have.

**Timing “buy-in”**

A common pitfall of software training sessions is training providers providing too much background information: what the software is, what it does, and why the lab has it. This strategy is meant to get the staff’s buy-in that the software is a positive change. This is, in fact, a critical part of the education of the lab’s users; however, it should take place weeks before the first training session. If staff are first hearing that they’ll need to learn to do things differently for the first time at the training session, then most of their questions and concerns will be around “why” the lab is doing it this way, instead of “how” to do it this way. Buy-in should be accomplished prior to training, or it will dominate the session, and training will suffer.

**Providing hands-on interaction**

Users that will be directly interacting with the software need to have hands-on training, including sitting at a computer workstation and actually using it. If users need to learn how to navigate through screens and retrieve or manipulate information, their training must include practice on those software functions as well. Trainers should walk users through each step on their computers, while providing constant support and instruction. The end goal: users can do it on their own.

**Reinforcing through quizzes**

Whether via pop quizzes along the way, or more formal tests at the end of the training session, evaluation is an effective tool for reinforcing what your users have learned; it’s also helpful for identifying areas that may need more emphasis and review. And yes, we’re all still the middle-schoolers we once were at heart: when it is most important for staff to remember certain software functions or how to use specific commands or screens, successful trainers tell them, “This will be on the test.” Even the people who aren’t in the front row will take notes on these points.

Train-the-trainer is a viable approach to learning new software, and not just from a cost standpoint. It also allows easy access to in-house experts, along with the ability to set up quick training sessions on an as-needed basis. The best way to take advantage of the power of the train-the-trainer approach is to follow a template that includes these five essential steps:

1. **Creating a training plan with measurable objectives**
2. **Differentiating user groups and tailoring the training**
3. **Making sure that buy-in takes place before the training**
4. **Structuring the training for hands-on experience**
5. **Quizzing staff to reinforce what they’ve learned.**

With this process-based approach, lab leaders can get the most out of staff software training—and that’s definitely the key to getting the most out of a new software system.

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ne branch of laboratory medicine that has remained little changed for nearly a century is the identification of bacterial species from clinical specimens. The traditional methods are based on dilution, plating on semi-solid media (broadly supportive or selective), and incubation in the appropriate environment—such as aerobic or anaerobic—leading to the growth of distinct individual colonies representative of the bacterial species present in the original sample. Identification then proceeds on these clonal microbial colonies by a combination of morphological classification following microscopic examination (by properties such as shape, motility, and cell wall structure as indirectly determined through Gram staining) and biochemical characterization (by properties such as ability to metabolize various carbon sources).

During the early days of microbiology, when many bacterial species were first identified, these methods were not only cutting-edge; they were the only methods available. Consequently, many microbes are defined to this day by these simple techniques, and thus these techniques remain in important use as the “gold standard” method(s) for classification of many bacteria. While not very fast, these methods are relatively cheap, don’t require complex laboratory infrastructure, and even come pre-packaged in simple manual or automated biochemical test panel formats readily useable even by the non-specialist (as the author can attest from personal experience).

Despite their past primacy and assured future relevance, these methods are not, however, perfect, or ideal in all cases. Morphologic and biochemical variability can mean in some instances that bacterial species which are nearly indistinguishable by these traditional methods can have very different clinical implications when detected in patient specimens. It has been in this context that molecular diagnostic (MDx) methods first started entering clinical use more than a decade ago, and they continue to do so today.

Cell biology: a quick mini-review

To understand this application, a refresher on some basic biology is required. Recall that the critical “machinery” of the cell for the assembly of polypeptides (proteins) from single amino acids, as directed by messenger RNA (mRNA), which in turn is derived from the organism genome (DNA), is the ribosome. This large complex consists of an organized arrangement of a number of protein and structural (that is, non-coding and biologically active through their physical shape) RNA molecules. These specific RNA molecules are called ribosomal RNAs (rRNAs) and are highly conserved across species. The individual rRNA types are historically classified on their hydrodynamic separation properties in Svedberg units “S,” with prokaryotic ribosomes having 23S, 16S, and 5S rRNA components and eukaryotic ribosomes having analogous 28S, 18S, 5.8S, and 5S rRNAs.

As a cell requires many individual ribosomes to function, large numbers of these rRNA molecules are needed, and to provide this most organisms contain multiple copies of the DNA sequences coding for them (known as rDNA) within their genomes; for example, the E. coli genome contains seven copies of its rDNA genes. While the hallmark of MDx methods is sensitivity, having a multicopy genetic target like rDNA sequences just improves lower bound sensitivity, and the polymerase chain reaction (PCR)-based amplification of rDNA is particularly easy—perhaps even too easy in some cases, as we’ll see below.

In the bacterial (prokaryotic) side, sequence analysis across many species revealed that the 16S rRNA is both very highly conserved overall (that is, the 1542 nucleotide sequence is identical regardless of source in almost all nucleotide positions) and contains particular regions which, while well conserved within a single bacterial species, have a number of small but consistent changes between species. This combination is a molecular diagnostician’s dream come true, for it provides the opportunity to design almost universally conserved PCR primers flanking a relatively short (few hundred base pair) region which contains a few consistent variations among different bacterial species. The size of the amplicon is readily amenable to Sanger sequencing methods.

Enter molecular diagnostics

As this became automated through the development of capillary sequencing instruments, a powerful MDx method for the identification of isolated bacterial samples (colonies or broth monocultures) came into being. The method consists of extracting DNA (even by very crude, rapid methods as we’re looking for a high copy number target); PCR amplification with “universally conserved” primers against a part of the 16S rDNA sequence; sequencing of the amplicon; and comparison of this sequence for an identical (or closest) match against libraries of 16S rDNA sequences from known bacterial organisms. While proprietary, curated libraries of 16S rDNA sequences were (and are) probably the most accurate libraries to compare against, publicly accessible libraries such as GenBank queried by open access tools (usually a variant of the old standby “Basic Local Alignment Search Tool” [BLAST]) have been demonstrated to be clinically viable in this approach. Lest we fear eukaryotes are left out of this approach, it should be noted that both this method and the more recent advances described below in 16S prokaryotic
context can be applied to eukaryotic 18S rDNA sequences for the identification of fungal species.

As described, this approach suffers from a significant failing which it shares in common with the traditional morphological/biochemical methods. That is the requirement for isolated single colonies of bacterial species to start from. Mixed samples are not amenable to analysis prior to separation through dilution and one or more rounds of plating. Bacterial species which do not grow under the conditions used (and even with a wide range of media and growth conditions, many bacteria remain challenging or impossible to grow in the customary semi-solid plate agar format) are therefore not amenable to identification either through traditional methods or this MDx technique.

A second and partially overlapping problem sometimes encountered with this method derives from the fact that NGS employs by nature DNA polymerases; these are generally derived by purification from bacterial expression systems; DNA polymerases exhibit sequence non-specific DNA binding affinity; rDNA sequences are often multiply repeated in bacterial genomes, including those bacteria used for DNA polymerase production; and PCR is highly sensitive. Add those up, and there’s a constant risk that the method may amplify contaminating trace rDNA from the polymerase production strain. While this was a distinct nuisance in early iterations of the method, an understanding of the source of these spurious sequences led to the availability of specialized “low DNA content” polymerases, and to better bioinformatics in the detecting and rejection of results which most likely arise from contamination. As is always the case with lab testing, understanding and rational consideration of the test results in clinical context is required!

Resolving the problems
Both of these problems—a need for isolated pure cultures, and a need for the capacity to discriminate results arising from bacteria associated with case-associated pathology as opposed to clinically irrelevant co-detected bacteria—are resolved by more modern iterations of this bacterial identification strategy. Specifically, the application of next-generation sequencing (NGS) methods, with the capacity to sequence very many product amplicons simultaneously in parallel, provides solutions to both while allowing for the extraction of even more useful data.

In its NGS guise, this method can work starting with a direct patient sample containing very small numbers of a significant pathogen even in the presence of other non-significant bacteria. The approach proceeds similarly as above, with bulk amplification of the sample by “universally conserved” 16S primers and generation of a mixed pool of amplicons arising from their respective bacterial sources. These individual amplicons are then separated and independently sequenced in parallel by the NGS process. Regardless of the exact NGS technology used, a “behind the scenes” tiling, sequence assembly, and bioinformatics strategy takes place to eventually output both full representative sequences of each bacterial 16S type present in the sample, and the count or relative frequency of each sequence compared to the total number of sequences determined.

The identities of each bacterial species contributing a 16S type to the milieu are identified by the same library comparison approaches as used for the single sequence, Sanger approach. The amplicon frequency values, while not linearly representative of the true frequency of the occurrence of each underlying bacterial species type in the sample (due to issues such as different numbers of rDNA copies per genome of different bacterial species, or sequence-based differences in PCR amplification kinetics), can be corrected back by bioinformatic processes to yield meaningful numbers on the relative frequencies of each bacterial species identified in the sample. This in turn can help to differentiate low-level contaminant signals (such as rDNA from polymerase) from meaningful signals, and allows for the detection of known pathogenic species even when mixed with apathogenic or commensal organisms. Complex, multipathogen communities and their evolution over time in contexts such as cystic fibrosis sputum samples can be directly observed by this technique with a level of detail not previously available in traditional culture and enumeration approaches.

In the clinical lab
Either of these approaches may thus be of use to today’s clinical laboratory scientist. Neither, however, fully replaces traditional agar plate methods for a full sample workup, primarily because they currently lack an effective way to evaluate absence or presence (and magnitude) of specific antibiotic-resistance profiles with the bacterial species detected as present. Antibiotic resistance is defined in a phenotypic method, such that detection even of a known antibiotic resistance determinant gene in the context of an isolated pure organism is not absolutely definitive of clinical antibiotic resistance (note, however, it can be very strongly suggestive, and would in most cases be a powerful assistant in establishing initial empiric therapy choices).

In a multiorganism NGS-based context, however, the detection of antibiotic resistance markers is much less meaningful, as they generally cannot be unequivocally assigned as having come from a particular bacterial species in the mix, and thus have little capacity to inform therapy choices. Approaches to address this shortcoming through various bioinformatic techniques are under active development; however, until they are validated and reach mainstream use, and the cost and time to result of NGS methods both decrease, we won’t see these as a substitute for the agar plates we know so well. For now, bacterial 16S identification methods remain useful adjuncts to classical microbiology rather than full replacements.

John Brunstein, PhD, is a member of the MLO Editorial Advisory Board. He serves as President and Chief Science Officer for British Columbia-based PathoID, Inc., which provides consulting for development and validation of molecular assays.
The laboratory’s contribution to advanced medical analytics

By Kim Futrell, BS, MT(ASCP)

The laboratory represents only about three percent of healthcare costs, but when it is used properly, it can impact downstream costs far beyond the cost of performing lab tests. As healthcare moves into the future, proactive, preventive, and patient-focused care will require new innovations and a fresh perspective. Laboratorians are encouraged to see the value of the information they are processing and to become active participants in analytics that advance the value of lab information.

If you shut down labs, you shut down healthcare

In response to the continual threat of reduced lab reimbursements, one of the keynote speakers at the 2015 Lab Quality Confab, John Waugh, MS, MT(ASCP), of Henry Ford Health Systems, presented the question, “What if we shut down ALL laboratories?” He displayed an excerpt from “The Waugh Street Journal” (Figure 1).

His humorous parody of a Wall Street Journal article makes two excellent points that laboratorians should take heed of: 1) If labs shut down, healthcare shuts down; and, 2) Of the $3.2 trillion healthcare spending bill, the lab accounts for a very miniscule portion. However, while the lab is not the problem in rising healthcare costs, it can be a huge part of the solution. Providers cannot take care of patients without the lab, so the lab will not go away as a tool in healthcare. In fact, it is just the opposite; the lab actually becomes more valuable. Because lab testing is healthcare’s highest-volume activity, providers rely heavily on laboratory data.

Labs will remain, but where they remain will be a question of cost and value. Labs remain essential no matter what happens to reimbursements. Yet what will change is which labs are successful and which facilities decide to outsource lab testing. This will come down to the level of value provided by the specific laboratory; and analytics can play a substantial role in demonstrating a lab’s value. Laboratory-driven analytics projects not only demonstrate lab value, but can actually drive down costs and support care improvements.

The lab is the hub of diagnostics

The changes taking place in healthcare offer great opportunity for the lab because the lab sits on the hub of clinical data that feeds diagnostic decision-making—information that will change the way we deliver and pay for care. The transition to value-based reimbursements is requiring providers to use quantitative metrics to justify quality and associated reimbursements.

For example, healthcare organizations within an Accountable Care Organization (ACO) must track a multitude of measures to establish quality performance standards in order to achieve shared savings. There are interactions between ACO quality reporting and other CMS initiatives, such as the Physician Quality Reporting System (PQRS) and the Meaningful Use EHR Incentive Program. Patient-centered Medical Homes use the Healthcare Effectiveness Data and Information Set (HEDIS) to track performance.

In addition to required performance measures, healthcare data is being used to glean actionable knowledge to drive decisions regarding patient care and find ways to reduce healthcare spending. The shifts we are seeing in healthcare reimbursement and the new methodologies for tracking quality will only substantiate the laboratory as an even more valuable part of the patient care plan. How can the lab, which holds the bulk of the clinical information, be more involved in gaining value from their data?

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The lab’s analytic opportunities

- Redefine your strategy and mission
- Implement a test utilization plan
- Reduce unnecessary testing, promote preventive testing, etc.
- Implement a test formulary
- Address Population Health Management goals
- How can the lab contribute?
- Be innovative
- Offer Direct Access Testing
- Get out of the lab and collaborate

Figure 4. The lab’s analytic opportunities

Internal views: a fresh perspective on data analytics

Laboratories are already known for their efficiency and focus on reporting quality results, and are accustomed to being on a tight budget. Now it is time to branch out beyond those mastered areas and make a bigger impact. To take this to the next level, labs can use internal analytics to support the ordering provider by offering expertise in test order optimization and interpretation. This can involve one of many interventions, from offering duplicate test alerts or testing cascades at the time of Computerized Physician Order Entry (CPOE), to using variation analysis studies, test formularies, or comprehensive test utilization plans to reduce waste and promote best orders. Additionally, analytics can be used to pursue a Lean culture by tracking staffing to workload, inventory control, and monitoring turnaround times (TATs) (Figure 2, page 30).

External impact: increasing the lab’s effectiveness

Lab value comes from results that drive diagnosis and treatment protocols, but results of lab testing can have a far-reaching influence because many other downstream decisions are based on lab results. Reflect on this concept and think carefully about where, within your facility, lab results can influence downstream decisions that have the potential to improve patient outcomes or reduce spending.

One of every laboratory’s goals is to make it easier for clinicians to provide the best patient care with cost-efficiency in mind. Laboratories can provide user-friendly test menus and test-order cascades choices. For example, at Tri-Core Reference Laboratories in Albuquerque, NM, providers can order a pregnancy testing care package. If results are negative, no further testing is performed; if results are positive, the lab takes the initiative to notify the provider when the 27-week Glucose Tolerance Test (GTT) and the 36-week Strep B, and any other necessary testing, are due. The Tri-Core lab is involved in risk stratification to identify which patients need more care, and it offers order choices that make it easy for the providers to do what is best for the patients. Another area where the laboratory can have a positive impact and use analytics to support this is in point-of-care testing (POCT). For example, in certain care facilities with urgent care services, adding a POCT for D-dimer to screen for deep vein thrombosis or pulmonary embolism has the potential to reduce unnecessary admissions or imaging (which are much more costly than the POCT).

Review your lab menu with a fresh perspective and think about specific tests that, with a faster TAT, can have a far-reaching impact in costs and care. For example, implementation of MALDI TOF technology can increase diagnostic efficiency via faster identification of bacteria which can potentially reduce length of stay (LOS). Dr. Brad Brimhall and his colleagues at the University of Mississippi Medical Center used analytics to justify the value of this methodology. They reviewed codes for sepsis and found 710 patients with a LOS greater than two days. Their cost for an overnight hospital stay for a sepsis patient is $1,471.79. With this data, the team determined that the MALDI TOF analyzer would be paid for in only 12.6 weeks—an 80.79 percent rate of return on this project.

The lesson here is to think outside of the cost-center box. A certain methodology may appear expensive on the typical lab cost spreadsheet, but the downstream savings can be more than enough to cover the testing costs and provide a better test methodology that creates revenue for the organization.

Getting results faster, particularly in certain scenarios, can not only improve patient satisfaction but can potentially eliminate an unnecessary hospital admission or an unnecessary CT scan, or reduce hospital LOS. The average cost of a night in the hospital is approximately $2,000, so if a lab can report a result faster, and this allows a patient to be discharged a day earlier, across multiple patients, that can save a significant amount of money. Potential savings generated by these interventions will more than pay for the costs of the lab tests. Think through the entire patient episode of care and determine whether a faster lab TAT can decrease hospital admissions, or decrease LOS. And, on the flip side, consider areas where preventive testing can potentially pinpoint a condition before it becomes a more costly chronic disease.

Combining lab data for greater influence

Take this concept of using data for actionable improvements and think about the potential of not only looking at lab data, but combining lab data with other data sets. This business intelligence

continued from page 30
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antibiotic stewardship. Initially it was estimated that as much as $12 million to $15 million per year was being spent on unnecessary pharmaceuticals. The lab and pharmacy performed a study that analyzed the usage of the most expensive antibiotics. Looking at only two antibiotics, they uncovered 2,157 unnecessary doses which equated to a total of $705,877 (variable costs) spent on unnecessary antibiotics. Had those patients been switched to vancomycin this would have cost $56,211, but even subtracting the cost of the vancomycin, this project still resulted in a $649,665 savings (Figure 3, page 32).4

Renew your lab strategy

The lab can offer sophisticated diagnostic tools through algorithms, advanced molecular testing, guidance on test utilization, and contributions to analytics that can guide the provider in daily patient interactions and make a substantial contribution to overall organization-wide savings and patient care coordination (Figure 4, page 32). By leveraging lab data and combining that data with data from other IT systems, valuable analytic insights can be uncovered. Analytics will drive the future of patient-centered healthcare, and laboratorians are the stewards of a vast amount of the clinical data used to drive treatment plans. Make sure you are making the most of your analytic opportunities.

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Improving the molecular diagnosis and treatment of epilepsy with complex genetic testing

By Aaron Elliott, PhD, and Amanda Bergner, MS

Epilepsy is a highly complex disease and is one of the most common neurological conditions worldwide. Some 2,500 years after Hippocrates observed a hereditary tendency for epilepsy, researchers discovered the first causative gene, neuronal nicotinic acetylcholine receptor alpha 4 subunit (CHRNA4), in 1995.\(^1\) With the development and implementation of new genomic profiling technologies, the discovery of epilepsy-associated genes has increased rapidly in the past five years. It is currently estimated that 70 percent to 80 percent of epilepsy cases have a genetic component.\(^2\) Incorporating comprehensive genetic testing for epilepsy into clinical practice has enabled physicians to provide a proper diagnosis for many patients, resulting in a better understanding of prognosis, family counseling, and targeted treatment options.

Due to both the diverse spectrum of causative mutations, and the large number of genes and genomic regions associated with epilepsy, several molecular technologies are required to perform the most comprehensive genetic testing and produce the highest diagnostic yield. Genome-wide chromosomal microarray analysis (CMA) to detect copy number variants (CNVs) is generally the first line of testing for patients with unexplained epilepsy with co-morbid neurodevelopmental features.\(^4\) Current high-density oligonucleotide microarrays enable the detection of both large scale CNVs and small exon-level deletions and duplications in disease-associated genes. The diagnostic yield with CMA for individuals with epilepsy in association with intellectual disability or an autism spectrum disorder is estimated to be ~15 percent to 20 percent.\(^5\) CMA is also an appropriate first line test for individuals presenting with infantile spasms, with a diagnostic yield estimated at ~11 percent.\(^6\) Target enrichment and next-generation sequencing (NGS) technologies, such as pre-specified candidate gene panels and whole exome sequencing, have revolutionized the field of epilepsy genetics during the last five years. These tests are used routinely in the clinic today and offer clinicians a variety of options depending on the patient’s phenotype.

The cost effectiveness and availability of target enrichment and NGS technologies have resulted in a multitude of commercial laboratories offering a wide range of epilepsy testing options. It is important for clinicians to realize that all tests are not created equal and that the detection rate will vary depending on not only the gene content of the panels ordered, but also the sensitivity and specificity of the technology and bioinformatics used for testing. These factors all impact the quality of the test.

For example, labs using just NGS for testing will miss large repeat expansions, like the dodecamer repeat expansion within the 5′-untranslated region of the cystatin B (CSTB) gene, which is responsible for the vast majority of the Unverricht-Lundborg type of progressive myoclonus epilepsy cases.\(^7\) In addition, labs detecting mutations and CNVs utilizing NGS should use a secondary technology such as Sanger sequencing and microarray analysis or qPCR, respectively, in order to eliminate the potential of reporting false positives. Tests that are marketed as having a zero false positive rate using NGS data alone generally do not utilize a very sensitive bioinformatics pipeline, resulting in a higher rate of false negatives for mosaic mutations, indels, and mutations in highly complex genomic regions.

Although NGS provides a cost-effective method to sequence a multitude of genes concurrently, clinicians may want to consider ordering smaller phenotype-associated panels initially, with the option to reflex to a more comprehensive panel (Figure 1, page 39). This method provides clinicians the ability to target the genes most likely to be causing their patient’s epilepsy, minimize turnaround time (TAT), and limit reporting to only those genes that are highly characterized in the epilepsy phenotype of interest. With such a large number of genes being reported in association with epilepsy, most with very low rates of mutation detection, it is not surprising that commercial labs vary widely in the number of genes offered for nonspecific epilepsy presentations. For example, a recent publication reviewed epilepsy panels from seven different laboratories, and the number of genes analyzed varied from 70 to 465 genes.\(^8\) Some tests included genes with very little or no supporting evidence associating them with epilepsy.

Importantly, the more genes included on a panel, the more complex the test report and the longer the TAT, as the number of variants of uncertain significance increases. Clinicians should consider ordering exome sequencing once they have exhausted targeted panel options, or if they have a reason to be looking to interrogate more than ~200 genes at once.

Exome sequencing, analyzing the coding regions of ~20,000 genes, has significantly contributed to the rapid discovery of epilepsy-associated genes and has shortened the “diagnostic odyssey” for a large number of previously undiagnosed patients. It is estimated that exome sequencing has a diagnostic yield of ~38 percent for epilepsy.\(^9\) Similar to gene panel testing, exome diagnostic yield is dependent on the test design and the quality of interpretation. For example, not all commercially available exome tests utilize the same gene content or quality coverage metrics such as the percent of targeted bases covered at 10X. Likewise, only one or two labs contain the experience and resources needed to report novel genetic etiologies, which can provide a diagnosis to an additional ~9 percent of epilepsy patients.\(^10\)

The introduction of NGS and enhanced diagnostic testing options has enabled clinicians to better understand the complex genetic contribution to epilepsy and determine a causative diagnosis for...
The Surviving Sepsis Campaign (SSC) has produced new recommendations titled *International Guidelines for Management of Severe Sepsis and Septic Shock: 2012*. These new updated guidelines call for lactate assays to direct therapy for septic shock. For patients with lactate greater than 4 mmol/L, SSC recommends quantitative resuscitation targeting normalization of lactate levels.

StatStrip Lactate provides a 13 second assay on a whole blood sample to allow rapid, early, goal directed therapy in septic patients. Testing is as fast and easy as bedside glucose testing. The single use StatStrip Lactate biosensor is pre-calibrated, fast and uses a very small whole blood sample (0.6 microliters) yet provides lab equivalent accuracy.
Mitochondria, organelles found in cells, play a vital role during embryo development—working as specialized “powerhouses” that supply individual cells with energy and perform other critical cellular functions. Over the past decade, understanding of mitochondrial function has advanced, yet little is known about how variations in mitochondrial DNA (mtDNA), or the genetic information found within each mitochondrion, impacts pregnancy outcomes.

With up to one-third of in vitro fertilization (IVF) transfers of “chromosomally normal” embryos failing, this writer and fellow researchers specializing in preimplantation genetic screening (PGS) and preimplantation genetic diagnosis (PGD) investigated the role of mtDNA in early fetal development. We used a new clinical diagnostic test to establish a critical threshold of mtDNA quantity within embryos. Our study, published in the journal PLOS Genetics and also presented at the American Society for Reproductive Medicine (ASRM2015) annual Scientific Sessions, demonstrates that the level of mtDNA has a strong relationship with the ability of a human embryo to implant in the uterus following IVF.

A novel biomarker
Preimplantation development is an energy-demanding process, as cells rapidly divide in early pregnancy. Because mitochondrial functions are critical during the first few days of embryo development, the study examined the possible correlation between mtDNA content and female age, embryo chromosome status, viability, and implantation potential among 379 embryos. It also analyzed at what stage an embryo initiates replication of its own mtDNA and carried out a detailed assessment of the mitochondrial genome sequence, searching for mutations, deletions, and polymorphisms. A combination of microarray comparative genomic hybridization (aCGH), quantitative PCR, and next-generation sequencing (NGS) were used during the course of this study.

The study showed that embryos produced by reproductively older women contain higher levels of mtDNA (P=0.003) than those from their younger counterparts, implicating mitochondria in reproductive aging. Additionally, mtDNA levels were elevated in embryos with an abnormal number of chromosomes (aneuploid embryos), independent of age (P=0.025). Aneuploidy is responsible for the majority of miscarriages and serious genetic disorders. Healthy embryos that successfully implanted in the uterus and resulted in a live birth were associated with lower levels of mtDNA than those that failed to produce a viable pregnancy (P=0.007).

Importantly, an mtDNA quantity threshold was established, above which implantation was never observed. For embryos with quantities of mtDNA below the threshold, there was a better than average chance of producing a pregnancy. Embryos that had high levels of mtDNA—above the previously established threshold—did not implant successfully, thus leading to a 100 percent negative predictive value for these failed cases. The overall pregnancy rate in the group was 38 percent when mtDNA was not considered prior to transfer.

The results of this study suggest that increased mtDNA may be related to elevated metabolism and embryo viability; mitochondria may have a role in female reproductive aging as well as the genesis of aneuploidy. Of clinical significance, mtDNA represents a novel biomarker with potential value for IVF treatment, revealing chromosomally normal embryos incapable of producing a viable pregnancy.

There is an urgent need for new methods to improve the efficiency and success rates of IVF. These important findings show that mtDNA can help to highlight the embryos most likely to produce a pregnancy, allowing them to be given top priority for transfer to the woman’s uterus. The discovery of a new biomarker of embryo viability, independent of standard assessments such as morphology, is a rare event and of great clinical potential.

Subsequently, the predictive value of this threshold was confirmed in an independent blinded prospective study, indicating that abnormal mtDNA levels are present in 30 percent of non-implanting euploid embryos, but are not seen in embryos forming a viable pregnancy.

A new laboratory tool
A first validated clinical test is now available that can measure the quantity of mtDNA in trophoderm biopsies, and be applied to any biopsy specimens sent for PGS or PGD analysis. It can only be offered for treatment cycles involving vitrification of all embryos after biopsy. The turnaround time for results is two weeks or less. By helping to identify the embryos with the greatest probability of forming a successful pregnancy, this new clinical test is predicted to provide a further improvement in implantation rates, above and beyond what is currently done in the laboratory setting using PGS on its own.

Established to work on the same trophoderm samples used for PGS, this clinical test does not require any additional work in the embryology laboratory and, as a result, embryos do not need to be subjected to any interventions beyond those associated with routine chromosome screening. Since IVF can be a costly endeavor for those undergoing this medical approach to pregnancy, this new clinical test brings a simple and inexpensive approach that could help improve chances of successful fertility outcomes.

The success rate of IVF dramatically decreases with increasing female age. The higher levels of mtDNA observed with advancing age raise the question of whether mitochondria and their genome might play a direct role in the decline of female fertility with age. Increased levels of mtDNA may indicate compromised mitochondria that are unable to generate the expected amount of energy to support embryo development.

My colleagues’ and my study demonstrates a clear association between mtDNA quantity and the ability of a human embryo to implant in the uterus. A large (100 IVF cycles) blinded non-selection study is also being conducted in collaboration with the IVF clinic at the NYU Langone Medical Center to further assess the rate of outcome improvement if mtDNA quantification is combined with PGS. By Elpida Fragouli, PhD

Elpida Fragouli, PhD, serves as Lab Director at ReproGenetics UK, in addition to holding a research position at the University of Oxford. She played a key role in the development, validation, and clinical application of comparative genomic hybridization (CGH), the first comprehensive chromosome analysis method to be widely applied to the study of human embryos.
patients, leading to an appropriate counseling and treatment plan. This was recently illustrated by research presented at the 2015 American Epilepsy Society Annual Meeting, which indicated that ~16 percent of epilepsy patients who underwent diagnostic sequencing harbored a mutation in a gene which had immediate treatment implications. As the number of clinicians who are comfortable utilizing complex genetic testing increases, the understanding of epilepsy genetics will increase rapidly, precipitating a paradigm shift in the diagnosis, management, and treatment of the disorder.

**REFERENCES**

Soaring demand for genetic testing highlights need for streamlined data interpretation

By Michael Hadjisavas, PhD, and Ramon Felciano, PhD

The recent surge in number of sequencing-based clinical genetic tests has put a spotlight on associated challenges in data interpretation. While advances in genomics allow for the development of new genetic tests at a breathtaking pace and with unprecedented complexity, the interpretation of results has remained a largely manual, time-consuming process that is simply not scalable. In this article, we review the current landscape of variant interpretation and the challenges it presents, as well as new developments in the field that indicate significant improvements may be on the horizon.

The process

Today, variant interpretation is conducted by clinical geneticists who have tremendous skill and expertise in their field. It is a testament to their dedication that current interpretations are as reliable as they are. However, this dependence on human judgment, coupled with a laborious process, introduces room for error.

As lab directors know all too well, most variant analysis follows the same formula: Run the genetic test, annotate results, investigate variants detected, weigh evidence, integrate and interpret data, and report final results. It’s the middle parts of the process—variant investigation and interpretation—that prevent this process from becoming fully automated and scalable.

Clinical geneticists usually begin this variant interpretation journey with an annotated report from the DNA results of the test, whether that’s a gene panel, exome, or even whole genome. This annotation includes a list of identified high-quality variants that must be pursued to determine which, if any, is causative for disease. Variant interpreters often begin with PubMed and Google, scouring the literature to find mentions of these variants. Next, they have to go through each paper to figure out whether its information about the variant is relevant to the test at hand, tracking details about heterozygosity, disease type, number of subjects, and so forth. Once any available information about the variants has been uncovered, the next stop is databases or websites that predict protein changes based on the DNA variant. This part of the process indicates whether the variant might be affecting a patient’s phenotype. With all of this information, the analysis team draws on its deep clinical expertise to make a judgment call about how to report each variant on the list: pathogenic or likely pathogenic, benign or likely benign, or significance unknown.

Experts in this process say that this interpretation process takes about 30 minutes for each novel variant, a couple of hours for variants that have been reported in the literature, and many days for the most complicated cases. As genetic tests become increasingly complex, covering more and more of a patient’s genome, the time spent analyzing a growing list of variants for each test is expanding drastically. Challenges in efficient and effective interpretation of genetic test data will soon gate our ability to bring these benefits to patients, motivating the need for robust clinical decision support solutions directed at these clinical testing labs.

Key challenges

Whether a genetic test is trying to pinpoint the cause of a rare or unknown disease, find evidence of hereditary disease, or suggest an appropriate treatment course, the need for rapid turnaround of results is imperative. Clinical geneticists are well aware of this, but the growing demand for genetic testing and the increasing number of variants turned up by these tests are doubly burdensome for analysis teams. As the range of testing options soars, most clinical labs no longer have a genetictist with expertise in every test indication; under these conditions, variant interpretation may take even longer.

In order to ensure that results are returned to physicians quickly enough to be clinically useful, it is essential to find ways to automate and streamline as much of the process as possible so that clinical geneticists deploy their expertise where it’s needed most. For instance, many large clinical labs maintain their own variant databases, so if a variant has been seen and interpreted before, analysts can avoid the time-consuming process of researching it all over again. The development of proprietary databases by testing labs and healthcare providers that consume the data is a key emerging trend. Value is being derived mining these databases to determine variant frequencies and their associations with clinical profiles, outcomes, and ethnicities to enhance the value of clinical reports.

Another major challenge lies in the murky category of variants of unknown significance. Obviously, clinical utility is greatest when a variant falls firmly into either the “pathogenic” or “benign” category, with utility weakening as the variant moves toward the center of the spectrum. However, emerging needs are suggesting that variants be classified into further subcategories such as “likely pathogenic” or “likely benign.” But in many cases, the downstream effect of variants—even those already reported in the literature—is not clear. Because many of these variants must be interpreted with minimal information, it comes as no surprise that variant interpretations can differ significantly from one lab to another.

In combination, these numerous secular drivers of inefficiency, together with the need for testing labs to expand their test menu, drive operational efficiencies and turnaround time. The community needs better resources that will help definitively classify variants, pulling more of them out of the “unknown” category and increasing the likelihood of having consistent interpretation across labs. In fact, lack of commercial grade information solutions is compounding these inefficiencies and moderating the development of the market.

Signs of improvement

There are a number of reasons to believe that the genomics and clinical communities are well on their way to addressing these challenges. For example, ClinVar is a publicly available database hosted by the National Center for Biotechnology Information. Users submit genetic variations and their associated phenotype, with supporting evidence, to help others in the community increase their confidence in their own variant interpretations. Other
commercial efforts perform large scale curation and data integration from clinical literature and other sources to power clinical test interpretation pipelines.

Meanwhile, the Clinical Sequencing Exploratory Research (CSER) program, funded by the National Cancer Institute and National Human Genome Research Institute, has established a consortium of laboratories conducting studies that are helping to explain variant classification differences among labs. This data will be quite useful for suggesting standardized approaches to improve lab-to-lab reproducibility of results.

Separately, the Allele Frequency Community (APC) was founded by a number of organizations to share aggregated information about how often variants are seen in various populations, allowing analysts to factor in data for groups that may be underrepresented in existing public databases, including the Exome Aggregation Consortium (ExAC). The APC operates on a share-and-share-alike model, so all members increase the value of the repository by contributing their own data about allele frequency.

Clearly, the challenge of variant analysis and interpretation affects the entire clinical community, and it will take a community-wide effort to overcome this obstacle. But we believe that clearing this hurdle is possible, and that eventually the variant interpretation process will be faster, more automated, and more definitive, giving clinical geneticists an even greater role in patient diagnosis and care.

The trend is expected to continue and drive the use of high-complexity genetic tests toward an industrialized scale. Consistent with industrialized technology markets, the demand for software solutions will shift from open source and homebrew solutions to highly scalable commercial-grade informatics solutions that are enabled with rigorously curated knowledge bases.

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Michael Hadjisavas, PhD, recently joined QIAGEN Bioinformatics as the Vice President of the Clinical Genomics Program. He leads the clinical bioinformatics portfolio in strategy to maximize molecular insight from biological samples delivering actionable reports to laboratory healthcare professionals.

Ramon Felciano, PhD, serves as QIAGEN’s Chief Technology Officer and leads strategy for the recently launched QIAGEN Clinical Insight (QCI) solutions, a clinical decision support platform designed to help labs streamline and accelerate the interpretation of genetic test results.

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The October 2015 MLO “Washington Report” touches on a complex subject that is extremely important to many readers. The risky, off-label use of glucose monitors with critically ill patients is under close scrutiny by the Food and Drug Administration (FDA) and Centers for Medicare and Medicaid Services (CMS), with virtually every hospital reevaluating its options for glucose testing.

However, the article overlooks several important facts pertaining to glucose monitor use with critically ill patients. I believe that providing these additional facts will give readers a better understanding of the FDA/CMS enforcement actions, and what alternatives are readily available to hospitals for glucose testing with critically ill patients.

The October article’s conclusion may have been reached because several very important facts and options for hospital point-of-care (POC) glucose use were overlooked. These omissions may lead to a different conclusion. To explain further, I respond to three statements within the article.

Statement 1. “In a painful irony, the Center for Medicare and Medicaid Services’ proposed action, taken to protect patients, would actually threaten the quality of care.”

The reason for the FDA/CMS action is to protect patient care. CMS enforcement decisions came after more than 13 deaths attributed to hospital glucose monitors had been reported to the FDA.1 Two additional deaths were later reported, causing the New York State Health Department in January 2014 to declare the use of hospital monitors for critically ill patients as “off label” in that state.2 Also in January 2014, the FDA issued a draft guidance document that highlighted the agency’s concerns with the use of POC blood glucose monitors for critically ill patients.3 Since that time both the FDA and CMS have taken actions to warn industry that they wanted to reduce the adverse events associated with off-label use of glucose monitors, which were never tested or cleared by the FDA for use with critically ill patients.

As indicated in their product labeling, glucose monitors have interferences from substances or hematocrit abnormalities that are often found in the blood of critically ill patients, and this can result in the overestimation of glucose and overdosing of insulin. According to the FDA, “Errors in BGMS (blood glucose monitoring systems) device accuracy can lead to incorrect insulin dosing, which, when combined with other factors, can lead to increased episodes of hypoglycemia. For hospitalized patients who may be seriously ill, any inaccuracies in the meters would further increase the risk to these patients.”

Statement 2. “That is, the change would suddenly make many hospitals’ use of the glucose monitor an ‘off-label’ use, and thus turn glucose monitoring into a high complexity test that could not be performed by nurses and other bedside caregivers. Faced with this situation hospitals might have little choice but to discontinue the point-of-care testing altogether.”

This statement implies that hospitals have no practical POC glucose alternative other than discontinuing the use of off-label glucose monitors for critically ill populations. This comment overlooks the fact that the FDA has already cleared two CLIA-waived glucose monitors for POC use with critically ill patient populations: “That waived status will allow a broad variety of healthcare professionals, such as nurses and technicians, to perform the test at the point-of-care, such as at a patient’s bedside, instead of requiring that the test be performed in a hospital lab (or other lab) that meets the CLIA [Clinical Laboratory Improvement Amendments of 1988] requirements for high complexity testing.”

The CLIA waiver will also allow hospital labs to safely provide blood glucose monitoring to their critically ill patients without having to meet the significant CLIA requirements for high complexity testing.

Statement 3. “In the comment letter, AACC asserts that a transitional period will allow hospitals to adjust policies, procedures, and workforce to ensure compliance with the new regulatory requirements.”

As stated earlier, the FDA issued its new draft guidance document for hospital POC glucose monitors nearly two years ago, and CMS subsequently communicated its intention to enforce off-label use of glucose monitors. More than half the hospitals in the U.S. have already transitioned to the FDA-cleared POC glucose monitor that is cleared for all patients, including critically ill. The downside of an additional transition period would perpetuate the risk of using untested glucose monitors on critically ill patients when a safe, FDA-cleared solution already exists.

AACC’s rationale that the implementation of the FDA/CMS decision would cause patient harm is contrary to the facts. Off-label use of blood glucose monitors has been shown to cause adverse events including deaths. A delay in the enforcement will unnecessarily perpetuate the risky use of glucose monitors that have not been cleared for use with critically ill patients when immediate, FDA-cleared, CLIA-waived products exist.

In summary, these FDA/CMS actions do not adversely affect the quality of patient care; they improve the quality of patient care. Neither do these actions result in prohibiting POC glucose testing. Hospitals have a choice of either using a POC glucose monitor tested and cleared for critically ill patients or following the regulations for off-label use in this population. Half of U.S. hospitals have already employed the FDA-cleared monitor.

REFERENCES
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IT solutions to meet the needs of clinical labs

If you were explaining CompuGroup Medical to someone who is not familiar with the organization, how would you characterize its primary areas of expertise? What are the major categories of solutions that CGM provides for its customers? CompuGroup Medical US is the U.S. division of CompuGroup Medical AG—one of the leading eHealth companies in the world. Founded in 1987 with our global headquarters in Germany, we have more than 4,500 employees, are in 43 countries, serve more than 400,000 physicians, and work across all healthcare sectors. Our mission is to synchronize the systems of the healthcare industry with the mission of patient care, enabling providers to achieve the best possible outcomes for patients. In addition to our laboratory information system, CGM LABDAQ, we develop healthcare IT solutions to support the clinical and practice management activities that take place in physician practices, community health centers, and hospitals; practice management software; and productivity tools and EDI/reimbursement. We’ve also recently introduced to the market our robust business intelligence tool, CGM ANALYTICS, as well as CGM SAM, our Software Assisted Medicine solution.

What are the core elements of eHealth for clinical labs and the larger institutions they are part of? What is the minimum that any lab should have? It’s all about connectivity and seamless integration. It’s well recognized that laboratory data and test results are the largest factor used when providers make healthcare decisions for patients. As patient care and outcomes become increasingly important and more lab testing is done outside the walls of the hospital, the need for health systems to be aligned and connected is critical.

In order to run an efficient lab and ensure these outcomes for the patient, it’s imperative that results from EMRs are being sent and received in a streamlined and connected way. The lab requires test orders, administration data, and billing information about the patient and interfaces to ordering locations and various resulting locations to be efficient. A connection to an analytics module provides an extra advantage as labs can gain insight into their efficiency with turnaround times, QA failures, etc., to ensure their operation is running smoothly. Also, ACOs rely on this type of analytics data to determine if they’re meeting quality measures and population health goals.

What distinction would you draw between software that is necessary today for any lab, and options that are perhaps more appropriate for larger or specialized labs? How does CGM customize solutions for organizations with different needs? Different laboratories have very specific needs, based on their size and testing types. For example, physician office laboratories typically perform fairly basic testing with straightforward setups. Larger laboratories like reference labs or hospital labs require multiple work stations and analyzers to be set up, necessitating interfaces that can share and receive orders in real time to multiple offices and mobile applications. For specialized labs like toxicology and pain management laboratories, their unique workflows and specific customizable reports need a streamlined process. Often, larger institutions rely on additional software like data mining or analytics tools to understand and optimize their lab’s performance, population management tracking, and control, to meet regulatory needs for reimbursement and much more.

CGM LABDAQ offers different packages based on each lab’s specific size and workflow in order to ensure efficiency and maximize testing reimbursements. In today’s healthcare setting, it’s critical to have an LIS that not only maximizes the lab’s operational efficiency but offers the latest technology and is streamlined and customizable for each laboratory’s unique needs. What our customers value most about CGM LABDAQ, in addition to its ease of use and intuitive technology, is that it’s scalable and customizable to meet their individual needs, as opposed to some of the more standard dated systems with a one-size-fits-all mentality.

We hear increasingly that aggressive outreach is a key difference between more and less successful clinical labs. Why is effective outreach more important than ever? As reimbursements per test decrease, labs are making their money on higher volumes, unless they move into specialized testing like toxicology and pain management. However, those specialty areas require different workflows, patients, and resources. Higher volumes help offset the lower reimbursements to maintain profitability. In addition, as ACOs work to reduce stay times and readmission numbers, less inpatient testing is being performed at hospitals. Subsequently, they are also turning to outreach testing to provide lab testing to non-patients and generate revenue through other sources.

The new ICD-10 codes and other factors are adding challenges to reimbursement. How do CGM solutions address that? CGM LABDAQ is fully ICD-10 compliant, offering dual views for users to see ICD-9 and ICD-10 results, thus allowing for dual analysis and comparison. LABDAQ also offers medical necessity checking; you can set billing rules to help with reimbursements, and can do insurance/test routing, allowing automated rules in house and billing interfaces so billing goes directly from the LIS to the billing software to avoid missed billing. Within the CGM application is also a custom scanning feature that allows users to scan in insurance info so you have positive ID and positive insurance information to eliminate insurance mistakes.
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