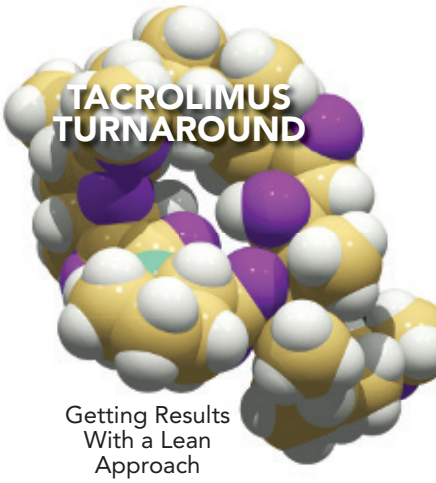
March 2020

CLN Clinical Laboratory News



Getting Results With a Lean Approach

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An AACC Publication | Volume 46, Number 2

THE ROAD TO A PARKINSON'S DISEASE BIOMARKER

NGS Bioinformatics

Budgeting Blind Spots

AACC Better health through laboratory medicine.

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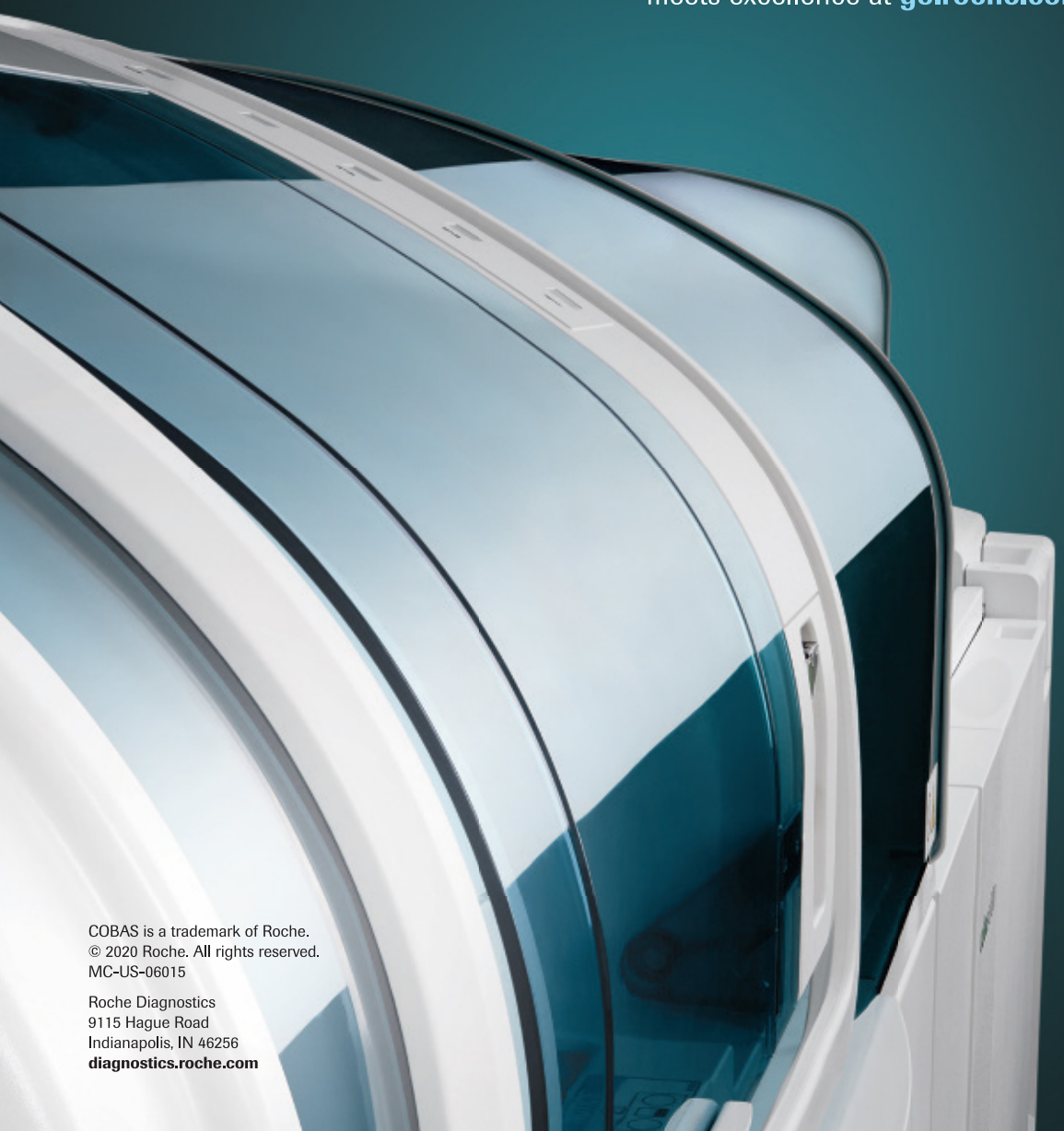

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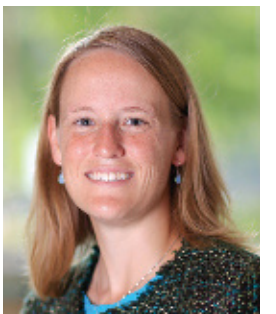

Historically, the diagnostic process for primary immunodeficiency disorders began with a basic immunological evaluation and subsequent functional studies. However, the clinical and genetic heterogeneity of these disorders can make this approach challenging.
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Federal Insider

Lab Act Passes, But Cuts Can Continue

In a victory for AACC and other laboratory advocacy groups that supported the legislation, Congress passed the Laboratory Access for Beneficiaries (LAB) Act, which could fix what advocacy groups say are serious deficiencies in data collection for the Protecting Access to Medicare Act (PAMA). So far PAMA has resulted in significant cuts to federal reimbursement for many clinical laboratory tests. Cuts are based on the Centers for Medicare and Medicaid Services (CMS) assessment of private payer rate data reported by laboratories.

The LAB Act delayed by 1 year the requirement for laboratories to report data on private payment rates to CMS and will commission a study by the Medicare Payment Advisory Commission (MedPAC) on how to improve data collection and rate setting.

However, CMS is still able to move ahead with possible cuts to the clinical laboratory fee schedule in 2021—based on data collected in 2017. This year's cuts are capped at 10%, but CMS can cut up to 15% each year in 2021, 2022, and 2023.

Prior to passage of the bill, AACC had warned Congress that failing to suspend data reporting would "significantly jeopardize seniors' access to vital laboratory benefits in 2020 and beyond." The association also noted that due to the flawed implementation of PAMA, "CMS continues to rely on data from less than 1% of the nation's laboratories, an error that has already led to severe cuts to the most common routine lab tests—some of which will exceed 30% when fully implemented."

PAMA already is having serious consequences for access to clinical laboratory testing. Notably, the Infectious Diseases Society of America found in a survey that more than 79% of respondents would be unable to provide the full range of testing needed to rapidly diagnose infectious diseases following the PAMA cuts of 2018 and 2019.

CMS BROADENS COVERAGE OF NGS TESTING

The Centers for Medicare and Medicaid Services (CMS) expanded its coverage of next-generation sequencing (NGS) testing for patients with germline ovarian or breast cancer after advocacy and medical groups, including AACC, urged the agency to make changes to an earlier, more restrictive draft policy.

AACC had criticized the first draft of the policy for only covering tests approved or cleared by the Food and Drug Administration (FDA). "We are perplexed by this recommendation given that there is no FDA cleared or approved NGS test for hereditary risk assessment of either condition on the market," the association wrote in a letter to CMS.

The final policy does automatically cover appropriately ordered NGS testing only if FDA cleared or approved; however, it allows Medicare's regional contractors to develop their own policies that cover laboratory-developed tests and accommodate other scenarios not spelled out in the national coverage decision memo.

FEDERAL HEALTH IT PLAN FOCUSES ON PATIENT ACCESS

The Department of Health and Human Services (HHS) draft 2020-2025 health information technology plan outlines the administration's goal to put the full weight of the government's policymaking behind promoting patient access to, and use of, electronic health information. Embedded in the plan is also the administration's goal to increase transparency around pricing—even negotiated private payer rates—a subject that's controversial among hospital and healthcare groups, but which the administration believes can help lower costs.

According to HHS, the goal of the plan is to "ensure that individuals have access to their electronic health information to help enable them to manage their health and shop for care."

The plan emphasizes the goal to accelerate the use of secure mobile apps, patient portals, and other tools. To do this, HHS is calling for public and private collaboration that will: improve portability of health information through application programming interfaces; improve access to smartphones and other technologies, especially for at-risk, minority, rural, disabled, and tribal populations; and build the evidence base around the use of health information technology, including on the types of information that benefit patients most and the best ways to present information to patients and caregivers.

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Bench Matters

A Lean Approach to Shortening Turnaround Time for Assessing Whole Blood Tacrolimus Levels

Shohei Ikoma, MD

Kathleen A. Kelly, PhD

The immunosuppressant tacrolimus is a mainstay of post-transplant maintenance therapy, so precisely measuring blood tacrolimus levels remains critical for preventing organ rejection and optimizing transplant recipients' care. Despite the integral role of tacrolimus in transplant outcomes, its unique biological profile poses clinical challenges. In particular, blood tacrolimus levels can be quite variable due to this medication's narrow therapeutic index and susceptibility to drug-drug interactions.

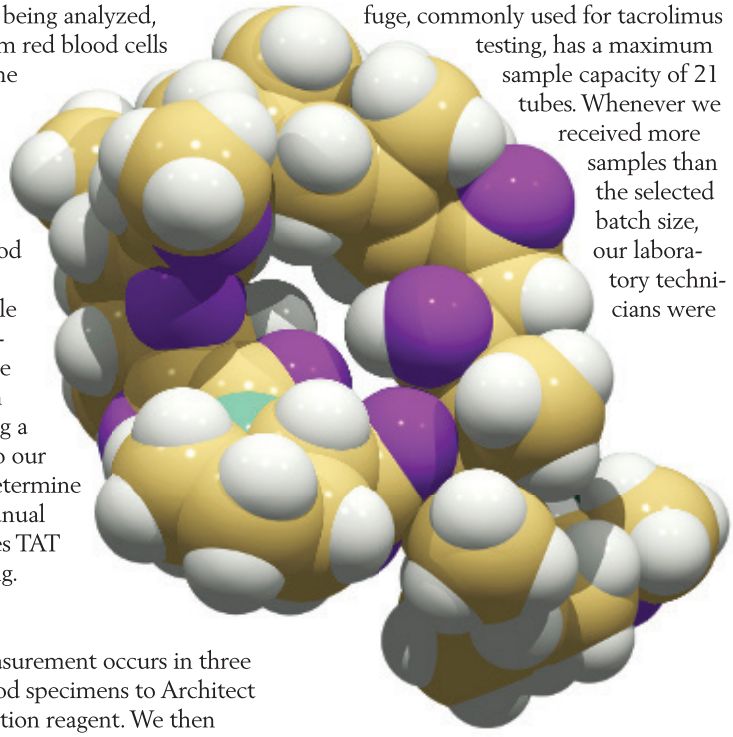
As the largest transplant center in the state of California, the University of California, Los Angeles (UCLA) Health System serves patients from throughout Southern California. To meet the needs of this large population, we have implemented a unique system that expedites transplant patients' follow-up visits. Our patients have routine laboratory tests and see their providers on the same day—just a few hours after the tests are performed. This makes it imperative that we optimize turnaround time (TAT) for whole blood tacrolimus testing. Favorable TAT not only contributes to patient satisfaction and the efficiency of post-transplant care but also serves as a quality metric for our laboratory's operational performance. Given these factors we used Lean techniques to streamline our workflow and TAT for tacrolimus measurements.

UCLA uses Abbott Architect analyzer for our whole blood tacrolimus testing. Prior to being analyzed, tacrolimus must be extracted from red blood cells (RBCs). This requires exposing the RBCs to a lysing reagent and centrifuging them. We previously identified this extraction step as the bottleneck in our testing workflow and published about a batched extraction method that uses metal batch racks. This method, however, is not applicable for small and medium-sized laboratories that do not process a large number of specimens and rely on a manual extraction method using a standard centrifuge. This led us to our Lean analysis, which sought to determine the optimal batch size for the manual extraction method that minimizes TAT for whole blood tacrolimus testing.

A NEW PROTOCOL

Our whole blood tacrolimus measurement occurs in three steps. First, we expose whole blood specimens to Architect tacrolimus whole blood precipitation reagent. We then manually vortex and centrifuge the treated samples. Finally, we place the samples in a standard rack and load them into the Architect. The average time per rack in the analyzer is 23 minutes.

Prior to this new protocol, each performing technician would determine at his or her discretion the batch size for the manual extraction phase. For example, if our laboratory received 36 patient specimens, a technician could either perform manual extraction on all 36 specimens at the same time or split them into smaller batches. We hypothesized that batch size variability was the bottleneck in the preanalytical phase. Based on this hypothesis, for 2 months and four different batch sizes—random (technician dependent), 15, 20, and 21 specimens—we recorded TAT, defined as the time between our lab's receipt of specimens to when we verified results. We capped the sample size at 21 because the Xsystems centrifuge, commonly used for tacrolimus testing, has a maximum sample capacity of 21 tubes. Whenever we received more samples than the selected batch size, our laboratory technicians were



Dr. Tim Evans / Science Source

instructed to perform the extraction step for the remaining specimens at hand once the first batch was loaded into the Architect for analysis.

During the 2-month period we collected 1,361 data points, excluding from our analysis two obvious outliers (3 and 994 minutes). Our mean TAT at baseline was 103 minutes versus 100, 83, and 77 minutes post-intervention for the 15-, 20-, and 21-sample groups, respectively. We compared the means by Student's t-test with a p-value <0.05 to indicate statistical significance. The mean TAT for the baseline group (103 minutes) was significantly higher than that for the 20-, and 21-sample groups (83 minutes, $p<0.0001$; 77 minutes, $p<0.0001$). However, there was no statistical difference between the baseline and 15-sample groups (100 minutes, $p=0.49$).

Laboratory TAT is an important metric that measures the quality and performance of a clinical laboratory. Aside from serving as a quality indicator, achieving short TAT is critical to the success of UCLA's current patient care model in which provider visits occur just hours from when we collect patients' blood samples to determine their tacrolimus levels.

NO MORE WAITING

We demonstrated that standardizing batch sizes during the manual extraction phase of our testing significantly reduced our overall TAT. One possible reason for this reduction comes from improved time management. Working on extraction while the first sample batch is being analyzed increases the instrument utilization rate and improves TAT. This method eliminates waiting, which is one of the seven wastes of Lean principle.

Based on this finding, we recommend that labs performing whole blood tacrolimus testing maximize the number of samples loaded into a single rack and perform extraction on the remaining samples while their instrument is analyzing the first rack. This study illustrates an excellent use case for Lean, which enables laboratories to identify and resolve obstacles in specimen processing by standardizing and devising novel strategies for process improvement.

The authors gratefully acknowledge the participation of Nathan Okawa, ASCP, and Vincent Buggs, MS, in designing, planning, and analyzing this quality improvement initiative and in reviewing this article.

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The Sample

Sepsis Accounts for 20% of Deaths Globally, Double Prior Estimates

The global burden of sepsis is roughly double that of conventional estimates, amounting to an estimated 48.7 million cases and 22 million deaths worldwide in 2017, or about 20% of all deaths (Lancet 2020;395:200-11). Sepsis also disproportionately affects low- and middle-income countries, where 85% of incident cases occurred in 2017. Even though the estimated incidence of sepsis fell by 37% and mortality decreased by 52.8% between 1990 and 2017, sepsis incidence and deaths varied significantly across regions, most impacting Southeast Asia, sub-Saharan Africa, Oceania, and other parts of Asia.

Women are more likely to experience sepsis than men, with an estimated age-standardized incidence in 2017 of 716.5 cases per 100,000 versus 642.8 cases per 100,000 respectively. Children also bear the brunt of sepsis, with more than 40% of all cases occurring in those younger than age 5. The leading underlying cause of sepsis-related death in every year from 1990 to 2017 was lower respiratory infection. Diarrheal diseases and neonatal disorders were the other two most common underlying causes of sepsis-related death in 2017.

Researchers developed these updated sepsis figures by accessing data from the Global Burden of Disease, Injuries, and Risk Factors Study 2017, which contains more than 1 billion datapoints for 282 underlying causes of death in 195 countries. Prior estimates considered only hospital administrative databases (thereby missing individuals never admitted to hospitals) and national or subnational locations from only selected middle- or high-income countries.

The findings "highlight the need for greater prevention and treatment of sepsis," according to the investigators.

ONE-QUARTER OF THOSE WITH ATHEROSCLEROTIC CARDIOVASCULAR DISEASE AND ELEVATED TRIGLYCERIDES MIGHT BENEFIT FROM EMERGING THERAPIES

Up to 25% of individuals with atherosclerotic cardiovascular disease (ASCVD) and low-density lipoprotein cholesterol (LDL-C) controlled by statin medications but who still have elevated triglyceride (TG) levels might be eligible for and benefit from emerging therapies such as ethyl eicosapentanoic acid (EPA) (Eur Heart J 2020;41:86-94).

This finding comes from CANHEART, an observational cohort study of adults in Ontario, Canada, based on a dataset that links 17 different individual-level data sources. Of 2.4 million people in CANHEART with recorded lipid panel results, the researchers identified 196,717 who had established ASCVD. The investigators sought to assess the prevalence of hypertriglyceridemia and ASCVD events in this population, estimate the percentage who might qualify for emerging therapies, and see how well this population's experience tracked with the REDUCE-IT trial. REDUCE-IT found that subjects with ASCVD and plasma TG ≥135-499 mg/dL who were on statin therapy and daily EPA therapy had 25% lower risk of major ASCVD outcomes compared with those who took placebo.

CANHEART researchers observed 24,097 composite ASCVD events over a mean of 2.9 years' follow-up. They found an increasing adjusted hazard ratio for composite ASCVD based on TG level, ranging from 1.0 for TG <89 mg/dL to 1.52 for TG ≥354 mg/dL.

While these findings suggest the potential of EPA therapy to lower risk of ASCVD events, an accompanying editorial cautions that the estimate of 25% of eligible individuals deriving benefit from this approach is optimistic given the "broad definition" of hypertriglyceridemia the investigators used (Eur Heart J 2020;41:95-8). The more usual threshold, >200 mg/dL, "would identify substantially fewer candidates for this intervention." The editorialist also suggested that because TG affects

zeljkosantrac / E+ / Getty Images

the "whole spectrum" of apolipoprotein B-containing lipoproteins, "we should not expect interpretation of the relationship between lipid reduction and decreased ASCVD risk to be as straightforward as it has been for [LDL-C]."

4 BLOOD BIOMARKERS SHOW POTENTIAL IN IDENTIFYING STUDENT ATHLETES WITH SPORT-RELATED CONCUSSION

A quartet of candidate blood biomarkers has a "modest ability" in the acute post-injury period to differentiate collegiate athletes with sport-related concussion (SRC) from control group student athletes (JAMA Netw Open 2020;3:e1919771). This finding supports the use of these analytes as research tools to better understand the underlying mechanisms of concussion and eventually, if optimized and validated, as clinical measurements, according to the researchers.

This multicenter, prospective, case-control study of 264 student athletes with concussion, 138 contact sport control athletes, and 102 noncontact sport control athletes was conducted by the National Collegiate Athletic Association and the U.S. Department of Defense Concussion Assessment, Research, and Education Consortium. Participants had preseason baseline measurements taken, and in the acute post-injury period, 24-48 hours after injury, at the point of being asymptomatic, and 7 days after return to play. The researchers matched athletes with concussion against nonconcussed contact sport athletes based on sports-related and concussion history criteria.

Athletes with concussion versus controls had, at the point of acute post-injury in comparison with preseason baseline levels, significantly elevated concentrations of glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1), and total tau but not neurofilament light chain (NF-L). GFAP levels remained elevated in comparison with baseline levels at all time points. The area under the receiver operating characteristic for GFAP, UCH-L1, NF-L, and total tau in differentiating athletes with concussion from sports-playing controls in the post-injury period was 0.72.

Athletes with concussion who experienced loss of consciousness or post-traumatic amnesia had significantly higher levels of GFAP than their concussed peers who did not lose consciousness or have amnesia.

The authors of a related commentary suggested that the authors would have advanced the understanding of these analytes' clinical relevancy in sports concussion if they had reported absolute and relative changes in the biomarkers between baseline and the acute post-injury period (JAMA Netw Open 2020;3:e1919799). They also noted that the median time for acquiring acute phase samples was 3.42 hours after injury, generally too late to have much diagnostic importance.

Parkinson's Disease Playbook

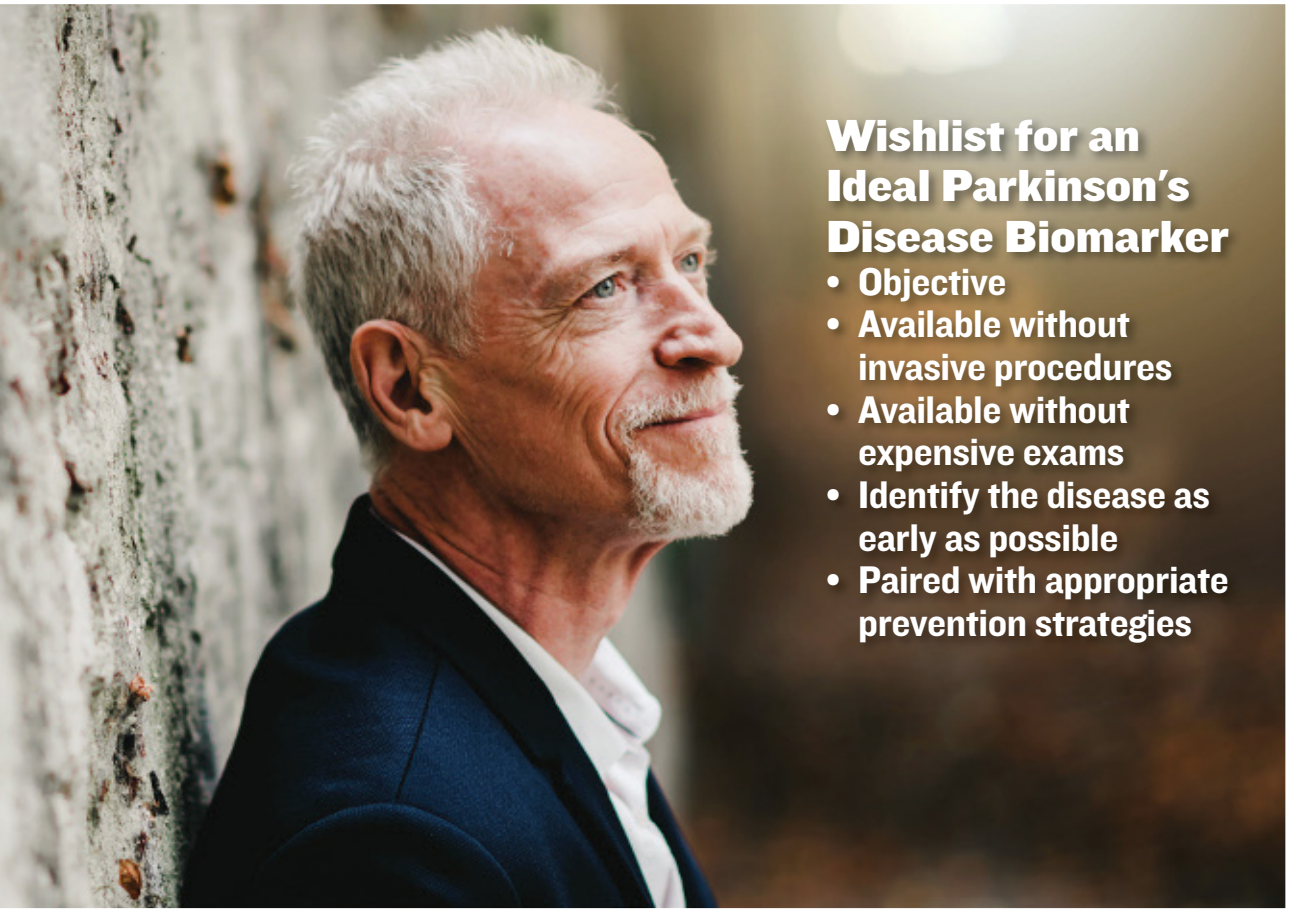
PROTEINS, EPIGENETIC MARKERS TARGETED AS POTENTIAL EARLY DIAGNOSTICS, PROGRESSION MONITORS

BY DEBORAH LEVENSON

kupicoo / E+ / Getty Images

Parkinson's disease (PD), a movement disorder and leading cause of disability worldwide, causes tremor, slowness, stiffness, and walking and balance problems, plus other issues such as constipation, depression, and memory loss. Most PD patients are diagnosed after age 60, and many develop cognitive changes, including dementia. Currently, diagnosing PD is difficult because patients often present at advanced stages, with vastly different symptoms. With no definitive tests, clinicians rely on clinical exams, imaging, and assays that rule out other diseases. Meanwhile, treatment is limited to alleviating symptoms. Researchers are searching for reliable biomarkers to help clinicians diagnose PD, track its progression, and develop effective targeted therapies.

An ideal biomarker would be objective, available without invasive procedures or expensive exams, and identify PD as early as possible—ideally before symptoms develop—and paired with appropriate prevention strategies, said Mark Frasier, PhD, senior vice president of research programs at The Michael J. Fox Foundation for Parkinson's Research (MJFF). MJFF's $900 million in research funding includes sponsorship of the Parkinson's Progression Markers Initiative (PPMI), an international study that aims to identify and validate clinical, imaging, genetic, and protein PD biomarkers. PPMI makes its data available to the research community in real time and takes applications for biosample use.



An Uncertain Pathophysiology

PD's cause remains unknown. Historically, scientists have seen PD as the result of lost dopamine neurons in brain regions that control movement, Frasier said. But in the last 15 years, research has pointed to misfolded alpha-synuclein proteins (α-syn)—which clump and form Lewy bodies in the brain—as a culprit. Patients with Alzheimer's disease (AD) and other neurologic conditions also have Lewy bodies.

More recent evidence suggests that PD may begin outside of the brain, perhaps in the gut, Frasier added. It might travel along neurons to the brain (Neuron 2019;103:627–41). This theory jives with the digestive problems that many PD patients notice up to 10 years before they develop tremors, researchers have noted.

On the Horizon

In recent years, the PD research field "has transformed from one with no biomarker activity to one with many emerging biomarkers that might translate into clinical labs," Frasier said. The MJFF research portfolio includes support of studies into 100 biomarker candidates, half of which are molecular. Frasier identified several as especially intriguing.

The first is the radiopharmaceutical imaging agent DaTscan, approved by the Food and Drug Administration in 2018. DaTscan helps provide images that show evidence of degeneration in the brain's nigrostriatal region, based on a reduced density of dopamine transporters. Frasier said DaTscan could have sufficient sensitivity to show if an intervention slows cell loss. But it cannot be used on its own and isn't useful for all patients, according to MJFF.

Studies show that α-syn consistently occurs at lower concentrations in PD patients' cerebrospinal fluid (CSF), compared with controls, Frasier noted. Most recently, PPMI data have shown that α-syn that originates in the brain and is present in CSF decreases early in PD, before motor symptoms develop (Mov Disord 2019;34:1354-64). Response of α-syn in blood plasma

thodonal / iStock

to peripheral changes in PD patients has also attracted interest. A recent proof-of-concept study shows that α-syn in blood cells reliably differentiated between Parkinson's patients with motor symptoms and healthy controls (Ann Clin Transl Neurol 2019;6:2426-36).

Neurofilament light chain (NfL) is an attractive biomarker because it's found in blood plasma, so testing is noninvasive. This protein marks neuronal cell loss, which is a feature of PD and other diseases. NfL appears to be changed in PD patients, compared with controls, Frasier noted. Research has focused mostly on NfL in CSF and has noted a good correlation between NfL levels in mouse CSF and serum (Neuron 2016;91:494–6). A recent study in humans suggests that an NfL blood test might track PD progression (Neurology 2019;93:e1104–11).

Accessible in urine, the lipid bis(monoacylglycero)phosphate (BMP) increases with lysosomal dysfunction in PD and other neurodegenerative diseases. Research has shown an association between BMP and *LRRK2* mutations, a common cause of inherited PD (Mov Disord 2019; doi:10.1002/mds.27818). More phase 1 trials are looking at BMP.

The Role of Epigenetics

Research has also found epigenetic changes—especially abnormal DNA methylation—in blood samples from PD patients. These DNA methylation patterns might provide insights into the molecular mechanisms driving PD and serve as biomarkers, said Travis Dunckley, PhD, and Paula Desplats, PhD. Many studies show that neurotoxins—especially those in pesticides—interfere with epigenetic processes in PD (Acta Neuropathol 2016;132:515–30). "Genetic studies don't explain most of PD risk, so the rest of it is probably environmental, including these types of toxins," said Dunckley, an assistant research professor at the Arizona State University-Banner Neurodegenerative Disease Research Center in Tempe.

To determine whether DNA methylation signatures in blood might be useful PD biomarkers, Dunckley and Desplats, an associate professor of neurosciences and pathology at University of California San Diego School of Medicine, are studying 2,500 samples from the PPMI cohort that were collected longitudinally over 3 years. The researchers will analyze methylation profiles from patients with unknown causes of PD, PD patients and asymptomatic participants with *LRRK2* mutations, at-risk people with sleep disorders and smell loss, and healthy controls.

The researchers intend to describe participants' methylation profiles for use in future research, to determine if genetic variants modify profiles and assess whether methylation influences when people with sleep and smell problems develop PD. They would also like to find 10-12 methylation markers that could be used as part of a lab panel, said Desplats.

> ABOUT 90% OF PARKINSON'S DISEASE PATIENTS HAVE ALZHEIMER'S DISEASE PATHOLOGY. "THIS FINDING ISN'T WIDELY KNOWN OR APPRECIATED ESPECIALLY BY DRUG COMPANIES."
>
> —JOHN TROJANOWSKI, MD, PHD

A Link to Alzheimer's Disease

PPMI Steering Committee members Leslie Shaw, PhD, and John Trojanowski, MD, PhD, both professors of pathology and laboratory medicine at the University of Pennsylvania Perelman School of Medicine, emphasized a connection between PD and AD. Cognitive impairment is very common in PD patients, and about two-thirds eventually develop AD. Trojanowski's non-PPMI research also suggests that AD biomarkers may be useful in assessing prognosis for PD and in developing potential treatments for the disease (Lancet Neurol 2017;16:55-65).

Unpublished findings from neuropathology exams on deceased patients who participated in PPMI and the Alzheimer's Disease Neuroimaging Initiative (ADNI) show that about 90% of PD patients have AD pathology, Trojanowski added. "This finding isn't widely known or appreciated, especially among drug companies," he said.

Trojanowski and Shaw have shown that types of tau proteins (especially tau $A\beta_{42}$) that are part of Lewy body protein tangle are also seen in AD patients and have potential as PD prognostic markers. Low levels of tau and $A\beta_{42}$ are correlated with severe motor function loss in PD patients, compared with controls (JAMA Neurol 2013;70:1277-87; Acta Neuropathol 2016;131:935–49).

Trojanowski and Shaw also noted the importance of PPMI's and ADNI's longitudinal designs, focus on consistent exams, and collection of data over a long period of time. It takes several years for many participants to show changes, but their experiences will lay the groundwork for studies of new medicines. Ideally, PD biomarker studies would last 10-15 years so that researchers can see whether markers detect disease early enough for interventions to have meaningful effects, said Shaw and Trojanowski.

Visions of the Future

Because PD has a varied presentation, researchers and clinicians need information about several aspects of its pathophysiology. Probably several biomarkers will be useful and necessary, said Ronit Sharon, PhD, an associate professor of biochemistry and molecular biology at Hebrew University-Hadassah Medical School in Israel, who is developing an α-syn blood test.

Dunckley thinks PD might have dozens of risk genes and envisions a future test that would detect causative mutations in several, provide information about gene expression measured in RNA (which PPMI sequences), and produce an epigenetics profile to establish an overall PD molecular risk profile.

Frasier is optimistic about this possibility. "Molecular profiling technologies have advanced such in the last five years that they are cost-effective and generate incredible amounts of molecular data," he said. PPMI samples are enabling whole genome sequencing, RNA, and proteomic data to help distinguish specific PD subtypes, he noted.

These methods have allowed cancer researchers to identify subtypes based on molecular signature and led to targeted therapies, said Frasier. He's excited to see how the PPMI data drive similar advances in PD.

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NEXT-GENERATION SEQUENCING

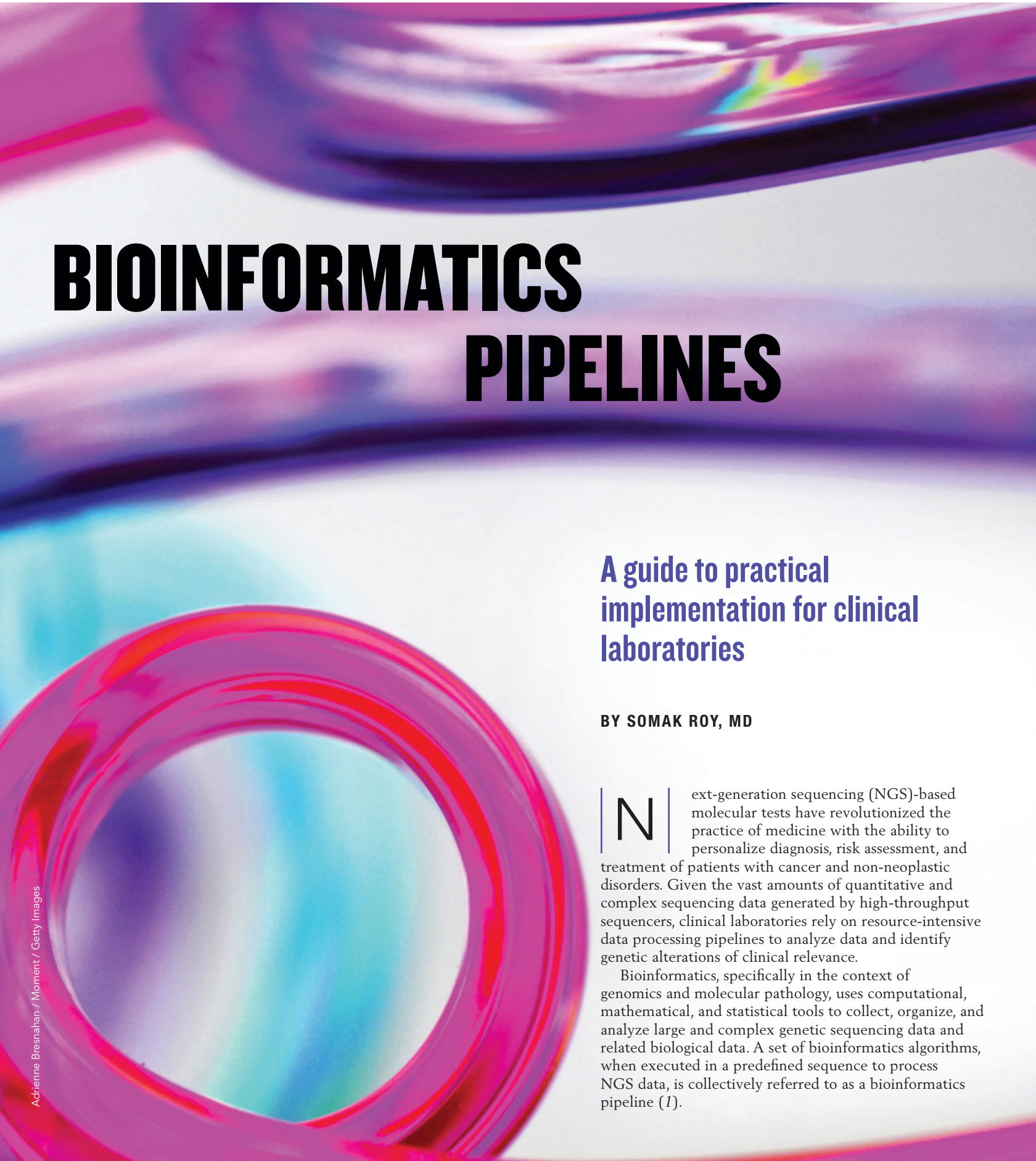
BIOINFORMATICS PIPELINES

A guide to practical implementation for clinical laboratories

BY SOMAK ROY, MD

Next-generation sequencing (NGS)-based molecular tests have revolutionized the practice of medicine with the ability to personalize diagnosis, risk assessment, and treatment of patients with cancer and non-neoplastic disorders. Given the vast amounts of quantitative and complex sequencing data generated by high-throughput sequencers, clinical laboratories rely on resource-intensive data processing pipelines to analyze data and identify genetic alterations of clinical relevance.

Bioinformatics, specifically in the context of genomics and molecular pathology, uses computational, mathematical, and statistical tools to collect, organize, and analyze large and complex genetic sequencing data and related biological data. A set of bioinformatics algorithms, when executed in a predefined sequence to process NGS data, is collectively referred to as a bioinformatics pipeline (*1*).



Adrienne Bresnahan / Moment / Getty Images

Clinical molecular laboratories performing NGS-based assays have as an implementation choice one or more bioinformatics pipelines, either custom-developed by the laboratory or provided by the sequencing platform or a third-party vendor. A bioinformatics pipeline typically depends on the availability of several resources, including adequate storage, computer units, network connectivity, and appropriate software execution environment. Ensuring consistent, on-demand access to these resources presents several challenges in clinical laboratories.

This article will discuss some important practical considerations for laboratory directors and bioinformatics personnel when developing NGS-based bioinformatics resources for a clinical laboratory. This short review is not a comprehensive guide for all aspects of bioinformatics resource development. Readers should consult the references for additional details.

The Bioinformatics Workflow in Clinical Laboratories

An emerging sub-specialty in laboratory medicine, clinical bioinformatics focuses on the application of bioinformatics principles, methods, and software tools to analyze, integrate, and understand biologic and healthcare data in a clinical setting (*2*). Clinical bioinformatics has several applications in a clinical molecular laboratory offering NGS-based testing.

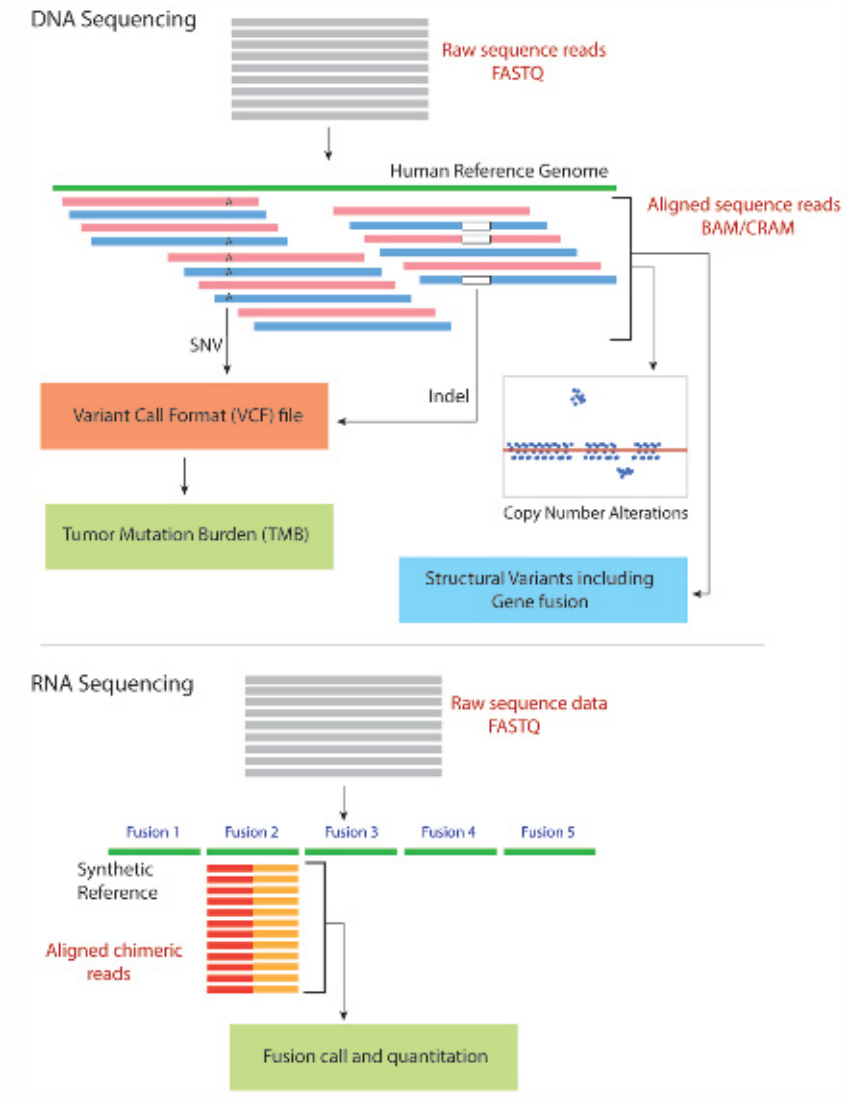
NGS generates several million to billion short-read sequences of the DNA and RNA isolated from a sample. In contrast to traditional Sanger sequencing, with read lengths of 500-900 base pairs (bp), short reads of NGS range in size from 75 to 300 bp depending on the application and sequencing chemistry. Newer NGS technologies such as those from PacBio, Nanopore, and 10x Genomics enable longer read sequences in excess of 10 kilobases (*3*).

NGS also sequences RNA molecules by converting them to complementary DNA (cDNA) molecules using reverse-transcriptase polymerase chain reaction. In addition to the sequence itself and unlike Sanger sequencing, the high-throughput nature of NGS provides quantitative information (depth of coverage) due to the high level of sequence redundancy at a locus. This property of NGS data enables laboratories to identify a vast repertoire of genetic alterations from a single NGS run on a sample using different bioinformatics algorithms (*4*) (Figure 1).

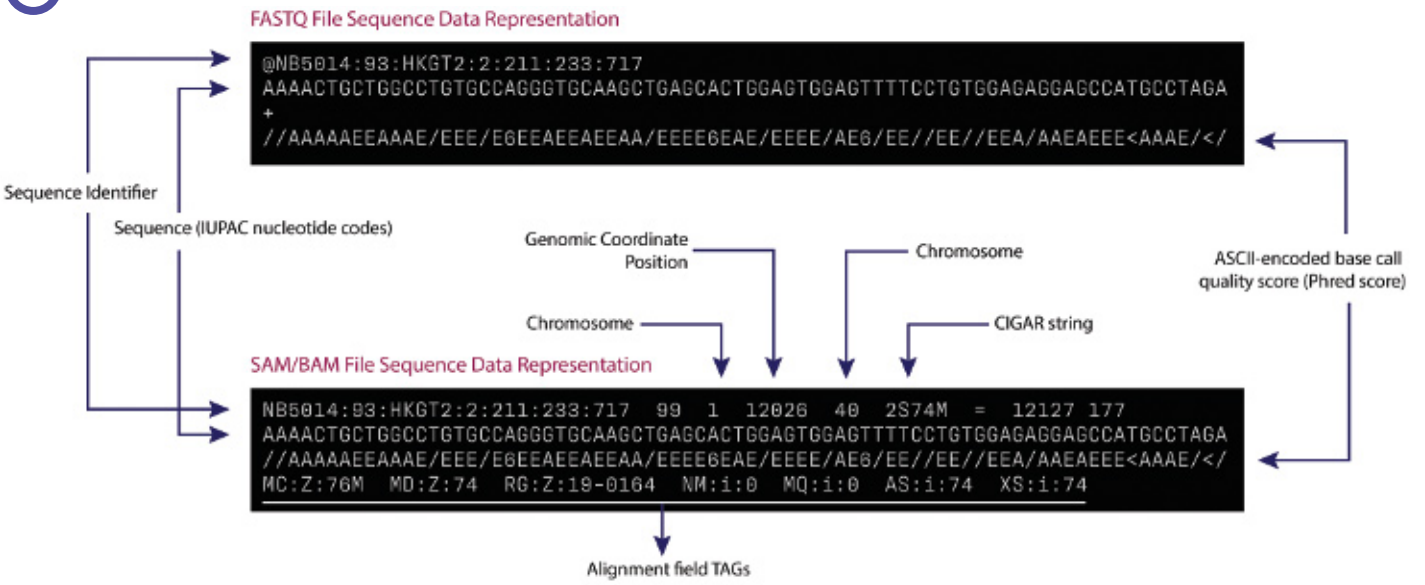
The bioinformatics pipeline for a typical DNA sequencing strategy involves aligning the raw sequence reads from a FASTQ or unaligned BAM (uBAM) file against the human reference genome. The FASTQ and uBAM file formats store short sequences as plain text with metadata about each short sequence such as base quality score and read identifiers (Figure 2a).

The sequence alignment process assigns a genome positional context to the short reads in the reference genome and generates several metadata fields, including alignment characteristics (matches, mismatches, and gaps) in Concise Idiosyncratic Gapped Alignment Report format. The aligned sequences and the related metadata are stored in a Sequence Alignment

F1 NGS Sequencing Data



F2 Sequence Data Format Examples (FASTQ and BAM)



Mapping (SAM/BAM) (Figure 2b) or CRAM file format (*5*). Downstream algorithms consume the BAM file to identify a range of genetic alterations, including single nucleotide variants, insertions and deletions (indels), and tumor mutation burden (*4*,*6*).

Laboratories commonly estimate copy number alterations (CNA) from aligned sequencing reads by using the depth of coverage approach. More extensive and specific DNA sequencing strategies also enable identification of large structural variants (SV), including gene fusions, and microsatellite instability (*6*,*7*). In addition, a split-read alignment strategy identifies gene fusions from genomic DNA sequencing (*7*).

For RNA-based gene fusion detection using NGS, the bioinformatics process typically involves aligning the cDNA sequences to an artificially constructed genome containing a list of known fusion sequences. The total number of reads from the sample that align to one of the known fusion sequences can be counted to identify and quantify the gene fusion (Figure 1) (*8*).

The results of variant identification are stored in one of the variant call formats (VCF), including genome VCF, generic feature format, and others. These formats allow encoding quantitative information about the variant, such as variant allele fraction, depth of coverage at the variant position, and genotype quality. Given the more complex representation of CNA and large SV, including gene fusions, there is ongoing work on using alternative file formats to represent such data types appropriately (*9*).

Finally, the downstream bioinformatics analysis for DNA sequence variants involves queries across multiple genomic databases to extract meaningful information about gene and variant nomenclature, variant prevalence, functional impact, and assertion of clinical significance. A user interface renders and visualizes annotated DNA sequence variants, CNA, SV, and other genetic alterations (*4*,6). Such a user interface allows trained molecular pathologists and practitioners to interpret the clinical significance of the genetic alterations and release a comprehensive molecular report.

Additional important applications of bioinformatics in molecular laboratory operations include quality control monitoring of sequencing data across runs, identification of background sequencing noise to reduce false-positive results, validation of upgrades to the bioinformatics pipeline, and the development and validation of novel algorithms for sequence data processing and variant interpretation.

Implementing a Bioinformatics Pipeline

In order to have high confidence in the performance of NGS results, laboratories must perform a thorough validation as described in practice guidelines (*1*). Subsequent updates to the bioinformatics pipeline should undergo appropriate revalidation and systematic version control (See Box p. 16).

A bioinformatics pipeline and the related software interoperate closely with other devices, such as laboratory instruments, sequencing platforms, high-performance computing clusters (HPC), persistent storage resources, and other software such as laboratory information systems and electronic medical records. It is essential that the pipeline validation include such interface functions.

During the validation and implementation of bioinformatics resources in a clinical laboratory, it is crucial to ensure compliance with Federal, state and local regulations as well as specific accreditation requirements (e.g. CAP laboratory accreditation). This is particularly important for safeguarding protected health information (PHI) (*4*).

Detection, accurate representation, and the nomenclature of sequence variants can be challenging depending upon the variant type, sequence context, and other factors. This makes it crucial that labs understand and evaluate the region of the genome sequenced by the NGS assay for accurate clinical reporting. Appropriate automation of bioinformatics resource development and deployment in clinical production contributes to optimized test turnaround time, better productivity of the bioinformatics team, and maintainable infrastructure (*10,11*).

Mastering a Team Approach

Laboratory directors need to consider a multidisciplinary approach when developing bioinformatics resources. Key stakeholders should include clinical, laboratory, and hospital informatics teams, cloud and/or system architects, molecular pathologists, laboratory personnel, and the laboratory quality assurance team.

Building a robust bioinformatics infrastructure undeniably requires staff with expertise and training in bioinformatics and software engineering, strategic planning, and phased implementation, including validation and version control before clinical testing is performed. Additionally, validation automation and use of container technology can be incorporated during development or phased to a later stage based on the size of the bioinformatics team and availability of laboratory resources.

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ELEMENTS OF A BIOINFORMATICS PIPELINE IN A CLINICAL LABORATORY

Bioinformatics Pipeline Implementation

Validation

The most critical requirement for implementing a bioinformatics pipeline is a proper, systematic clinical validation in the context of the entire next-generation sequencing (NGS) assay (*1,12*). Laboratories should determine a pipeline's performance characteristics based on the types of variants the NGS test intends to detect and should consider the sample matrix, such as fresh tissue, peripheral blood, or formalin-fixed paraffin-embedded tissue.

A clinical laboratory, with the assistance of a bioinformatics professional or team, reviews, understands, and documents each component of the pipeline, the data dependencies, input/output constraints, and develops mechanisms to alert for unexpected errors. Command-line parameters for each component of the pipeline and their settings should be documented and locked before validating the pipeline along with an appropriate minimum number of variants, based on desired confidence and reliability, for each variant type that will be part of the validation cohort (*1,12*).

Version Control

Laboratories can enforce version control using software frameworks such as git, mercurial, and source control, among others. These tools enable not only systematic management of the pipeline source code but also collaborative development by a team of bioinformatics and software engineers. Version control of the pipeline should include semantic versioning of the deployed instance of a pipeline as a whole. Every deployment, including an update to the production pipeline, should be semantically versioned (e.g., v1.2.2 to v1.8.1).

Laboratories also should document the versions of the individual components of the pipeline. If a laboratory develops and manages one or more pipeline components, it should follow the same version control principles as the entire pipeline. Since pipeline upgrades often significantly change the NGS test results (e.g. ability to detect new variant types, change in report format) and the clinical report content, it is a good practice to communicate such changes to clinical teams and clients.

Reporting NGS Test Results

Variant Nomenclature

Variant nomenclature is an essential part of a clinical report and represents the fundamental element of a molecular test result. The Human Genome Variation Society (HGVS) variant nomenclature system is the de-facto representation of sequence variants in a clinical report, which is universally accepted as a standard by laboratory accreditation agencies and understood by molecular professionals, clinicians, and medical genetics professionals (*13*). The synthesis of this nomenclature for

variants identified by NGS testing requires a complex process of conversion of the coordinate system from the reference genome to specific complementary DNA and protein transcripts. The alignment of the transcripts to the forward or the reverse genomic DNA strands and the HGVS 3'rule for variants in repeat sequence regions add additional complexity to the process. Several annotation tools—both open source and commercial—can generate HGVS nomenclature. However, since they might render inconsistent HGVS nomenclature, laboratories need to optimize and validate them for clinical use.

Variant Identification and Manipulation

The ability to detect sequence variants determines the performance characteristics for both a bioinformatics pipeline and an overall NGS assay. Several aspects of the pipeline can impact performance characteristics and affect the sensitivity of variant detection.

Estimating a pipeline's false-negative rate accurately can be challenging. Identifying phased variants is one of the challenges. For example, *EGFR* inframe mutations in exon 19, which render tumors sensitive to tyrosine kinase inhibitors, are often identified as multiple variants that can be a variable combination of single nucleotide variants and insertions and deletions (Figure 3, online). Such horizontally complex variants represent a haplotype, in which the individual variants (primitives) are in-phase, i.e., present on the same contiguous sequencing reads (*1*). The correct identification of complex variants is vital for accurate clinical reporting and follow-up molecular testing on tumor relapse, including minimal residual disease testing. A limited number of variant calling algorithms are haplotype-aware, so laboratories should carefully review their variant calling algorithms during validation. VarGrouper is a relatively recent software tool that was developed to primarily address the limitation of variant calling algorithms without haplotype-aware variant detection features (*14*).

Another contributor to the pool of false-negative (missed) variants is the process of in-silico masking of targeted NGS panels. Clinical laboratories commonly mask a portion of an NGS panel to report a subset of genes on the panel. This cost-effective approach is optimal for assay maintenance. Masking involves using tools that intersect a variant call file with a browser extensible data (BED) file that defines the regions of interest of the smaller reportable panel. The typical behavior of these algorithms results in an empty but perfectly valid variant call format (VCF) file in the absence of an input BED file. Consequently, for clinical testing, accidentally missing a BED file due to inconsistent transfer from development to the production environment may produce false-negative results and significantly impact patient care. Laboratories should design their pipelines to handle such intrinsic algorithm behavior and alert end-users to unexpected results in clinical testing.

Making a Scalable and Efficient Pipeline

Software Containers

The multiple components of a bioinformatics pipeline frequently have dependencies on different software runtimes and in some instances, different versions of the same software. This results in a complex software ecosystem with unnecessary maintenance overhead, lack of portability, inconsistencies between development and production environments, and increased chance of errors. The integration of the pipeline with other software systems can also be challenging.

Software container technology has revolutionized the practice of software development and deployment across the globe. Containers are a standard unit of software that enables the packaging of software and its dependencies to be run on different computers and operating systems with virtually no configuration changes. Unlike virtual machines, containers are a lightweight Linux operating system process that isolates the software running inside the container from all other running applications on the computer. This allows portability across different IT platforms in healthcare systems and the cloud and avoids software conflicts.

Since containers are typically small software units, they can be instantiated very quickly to execute a specific task. A recent study demonstrated the distinct advantage of using containers for the bioinformatics pipeline such that NGS data analyzed on various IT infrastructures and with different workflow managers produced the same results (*15*). Containers also help implement version control.

Containers are available as different open-source projects such as Docker, Singularity, Rkt, and LXC. Docker container is the most widely used of the general-purpose application containers. Singularity containers are designed specifically for bioinformatics applications on high-performance computing cluster systems.

Containers also provide a framework in which each step of the pipeline is provisioned into a container or application service. This enables an individual component of the pipeline to be updated in isolation without impacting other components.

Similarly, different pipeline components can be horizontally scaled to remove performance bottlenecks. A sophisticated software application that is deployed using several containers is typically managed in a production environment using container orchestration platforms such as Kubernetes, Mesos, Docker Swarm, and cloud vendor-specific frameworks.

Automation

Automation helps manage bioinformatics resources and workflows and streamlines day-to-day bioinformatics operations. The lifecycle of pipeline development, from testing and deployment to production infrastructure, is a complicated task. A laboratory must revalidate any upgrades to its pipeline to prevent unintended effects on test results. This manual testing and validation is time-consuming and, in some instances, inconsistent. The advantages of automation include more thorough and consistent enforcement of validation policies, regular testing and validation of pipeline upgrades, standardized version control, codebase integration, and proper documentation of audit trails for regulatory compliance.

An important use case of automation is the real-time monitoring of deployed bioinformatics pipelines in production. Edge-case scenarios related to the nature of sequencing data or unexpected changes in the deployment environment can significantly, often silently, impact NGS test results. In such scenarios, continuous monitoring and automated alert mechanisms are critical to avert unanticipated downtimes and erroneous test results. However, these advantages of automation come with a burden: time for initial setup and the learning curve of the bioinformatics team with automation tools.

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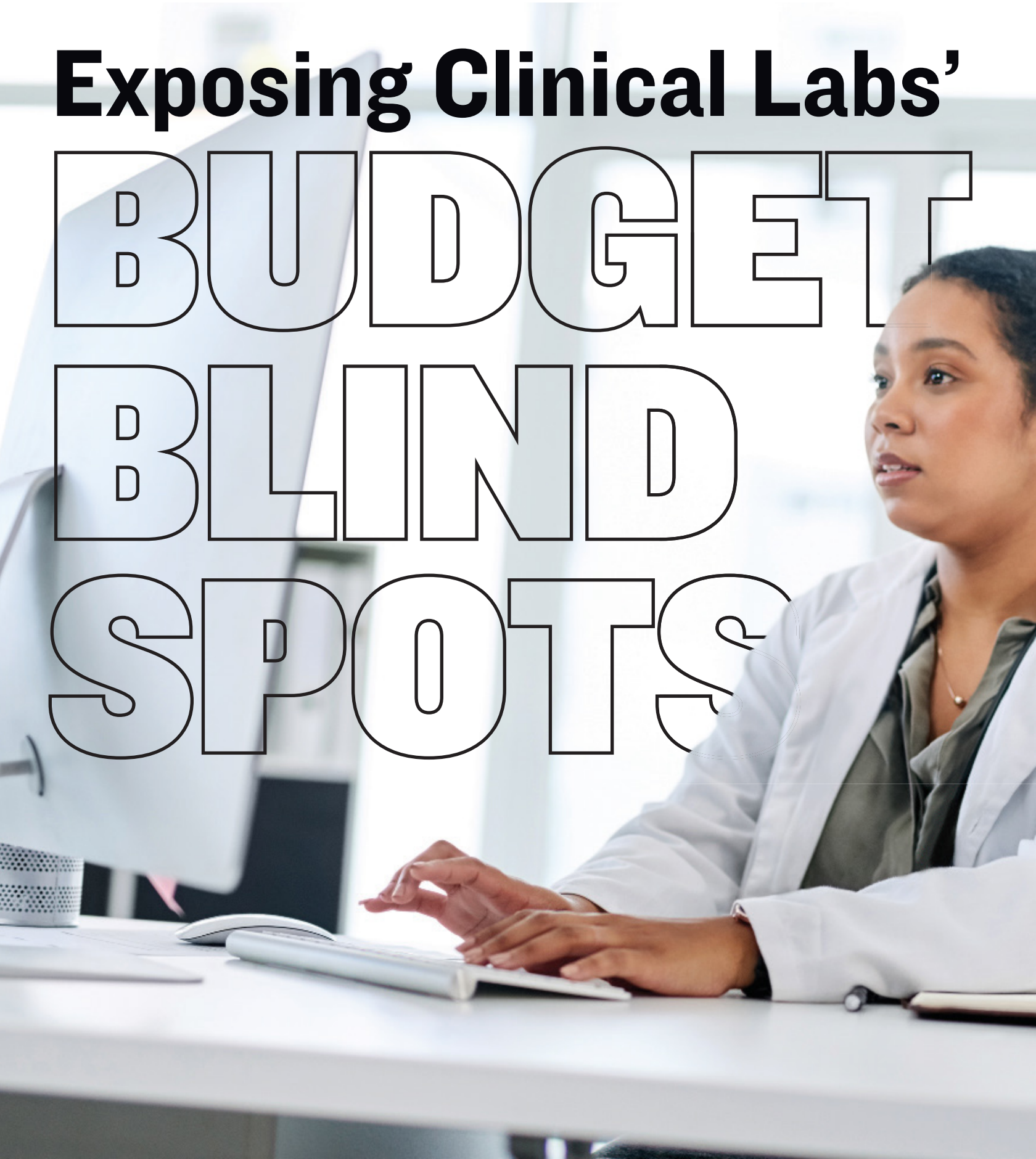

Even non-financial managers need to build their business acumen

BY KIMBERLY SCOTT

As clinical laboratories face continued reimbursement pressures from both government and private payers, laboratory leaders of all stripes have ample incentives to become even more financially savvy. Gone are the days when a manager could simply focus on performing tests and leave concerns about money to those higher up in the organization.

Hospital laboratories have traditionally been considered cost centers, but when outreach comes into play, the equation changes and a lab should be evaluated as a profit center, according to Thomas Joseph, MBA, MT(ASCP), president and CEO at Visiun, a laboratory analytics company in Ann Arbor, Michigan.

"You have to look at both incremental (or differential) revenues and expenses, but a lot of outreach directors don't because outreach revenues are often not easily obtained," Joseph said. "Lab directors need to know how to do the proper financial analysis on their own because hospitals tend to apply fully loaded costs, which will overstate expenses and make any outreach program look unprofitable."

Jane Hermansen, MBA, MT(ASCP), director of the outreach consulting program for Mayo Clinic Laboratories in Rochester, Minnesota, concurred, noting that while hospital lab directors know how many tests are run and what is being charged, they rarely know what revenue is collected.

Why should a lab director or manager know their lab's financials? Because doing so is one way to demonstrate the value of the laboratory to the hospital's executive leaders, Hermansen said. At a time when hospitals increasingly sell or outsource outreach programs, value is a key metric for the C-suite. In fact, a survey conducted by the Advisory Board in 2019 found that, for the first time, healthcare executives say their top priority is not controlling costs but growing revenues.

"The four commonly used criteria for measuring success are reduction of unit costs, increased productivity, increased net revenues, and profitability," Joseph explained. "Many managers rely on the first three measures alone, but the first three don't really matter. Profitability is the only reliable indicator."

Hermansen cautioned that for the profitability calculation to be meaningful, the allocation and input must be accurate. If a service unrelated to a laboratory is allocated to that lab (such as parking lot construction expenses), any profitability numbers will be way off.

Determining whether a laboratory outreach program is profitable comes down to its balance sheet. Lab leaders need to understand both costs—variable or fixed, direct or indirect—and revenues. Variable costs are those that vary with the number of tests performed, such as reagent costs. Fixed costs are those that do not change with an increase or decrease in the number of tests performed, such as instrument cost and space. Direct costs are those that can be fully attributed to the performance of a test, such as staff time, while indirect costs are not directly related to test production, such as overhead or institutional allocations. Marketing program expenses and outreach-related courier expenses should also be allocated to the outreach program.

"Fixed and indirect costs for the hospital laboratory will exist with or without outreach and should not be fully loaded onto the outreach program," Hermansen said. "It's important for a lab director to know how costs are allocated."

Directors and managers also need to have a firm handle on profitability. In simple terms, revenue minus costs equals profit (or loss), but determining the profitability of hospital outreach testing can be more complex. To deal with these billing and reporting complexities, some hospital labs

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"You need to consider the cost savings of the money you don't have to spend to fix a problem. It's about error-proofing the process, which leads right back to Lean and Six Sigma."
– PATRICK MAUL, MBA, MT(ASCP)

Moyo Studio / E+ / Getty Images

outsource their outreach billing operations. Alternatively, some are bringing in business analysts to focus on the financial side of the lab.

"Many hospital laboratories are now using the dyad leadership model, where they have both medical (technical) and business leadership for their lab," Hermansen said. Equipped with meaningful and relevant data, lab directors will be able to take key steps to make their operations profitable.

Reduce Revenue Rejections and Delays

Rejected laboratory claims can cause lengthy delays in payment or in lack of payment altogether, resulting in write-offs or bad debt. Hermansen advised that laboratory directors and managers work with both accounts receivable and billing departments to determine which claims are being rejected, including payer types and the reason for rejections.

"Some hospitals have automatic write-off policies in place. They might not even bother to collect a bill if they feel it is too small," Hermansen said. "The lab might be really busy doing lots of tests and generating lots of costs, but if the hospital writes off many of those claims, the laboratory won't be profitable."

Once the reasons for rejections are known, lab leaders can focus on reducing them. This might mean working with ordering clients to ensure they are supplying the appropriate diagnosis codes with their test requests, working with payers to understand why they are rejecting claims, or working internally to apply modifiers to specific tests prior to submitting claims for payment.

Working with a hospital's finance department to lower the organization's write-off threshold is also critical to ensuring that the hospital is collecting everything it can, according to Hermansen. A laboratory with a write-off threshold of $250 will collect very little in lab claims while a write-off limit of $10 or even $5 will result in an increase in collections and revenues. Knowing the hospital laboratory's bad debt rate and days sales outstanding is the first step to improving collections and increasing revenue, Hermansen added.

Expand Laboratory Services

Hospital-based laboratories typically send between 3%–5% of their tests to reference laboratories. But when volumes exceed a certain threshold for a particular test, it might make sense to add those tests to a lab's internal testing menu, Hermansen noted.

The evaluation about whether to do so should include immediate revenue opportunities, potential growth in volumes from existing laboratory outreach customers, and the financial impact of not having to purchase given tests from another laboratory. Such a review should recognize other benefits, such as improved turnaround time related to performing a test on-site versus transporting it to a reference lab. Speedier results might provide a quicker diagnosis, allowing for a patient's earlier discharge and reducing costs related to his or her hospital stay.

Adding new, specialized testing to the menu may also make sense if there is a need. Laboratories should periodically review their test menus to see if any assays should be removed or added.

Increasing volume can also lower overall cost per test because fixed costs—such as capital, labor, and information technology—are spread out over a greater number of tests, noted Hermansen. If a laboratory is not operating at full capacity, the lab director should be looking to increase both outreach and in-reach volumes. "If local physician practices are a part of your system, they should be using your lab," she said.

Managing Costs

Two of the largest expenses in a laboratory are labor and supplies. Investing in more efficient instruments or installing automated equipment helps reduce the cost

of manual labor and allows a lab to leverage technology to make the best use of staff. But to determine whether automation or new equipment makes financial sense, laboratory leaders need to calculate a return on investment, which essentially is net income generated by the equipment divided by cost of the investment.

Patrick Maul, MBA, MT(ASCP), a laboratory consultant and Lean Six Sigma Black Belt, advised looking at both the macroeconomics and microeconomics of financial decisions—in other words, understanding the total cost of use of an item in a lab versus the cost of the individual item.

"You can buy something less expensive, but you might end up using far more of it because of the defect rate in that item," he explained. "For example, you can buy cheaper microscope slides, but if they stick together and your techs have to spend time separating them and cleaning them before use, the total cost of use, factoring in labor, is much higher. You are better off buying a more expensive slide because the total cost ends up being less."

Understanding the cost of defects and errors is a critical part of controlling costs in clinical laboratories, he emphasized. "You need to consider the cost savings of the money you don't have to spend to fix a problem," said Maul. "It's about error-proofing the process, which leads right back to Lean and Six Sigma."

Beyond Dollars and Cents

Considering cost and financial performance should never usurp the importance of performing the right test at the right time, Hermansen said. "We have a responsibility to ensure that our testing is used correctly and managed efficiently."

All the experts *CLN* consulted agreed that to ensure that a laboratory contributes to the goals of the larger organization, lab leaders need to focus on effectively managing costs, maximizing revenue opportunities, and demonstrating added value. Honing their financial acumen will go a long way toward ensuring the laboratory is seen as a profit, not a cost, center.

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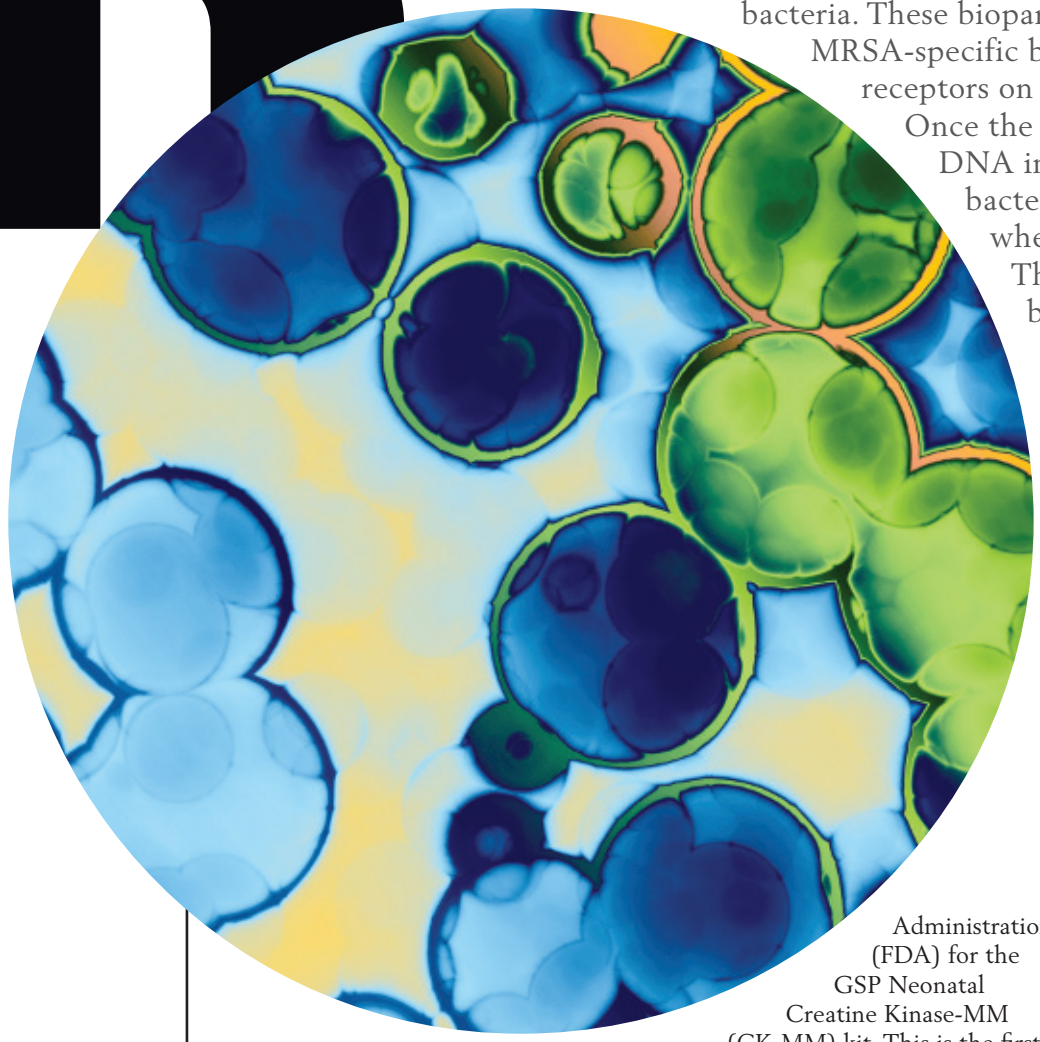
Regulatory Roundup

FDA Authorizes Roche's Novel Diagnostic Test for Detecting MRSA Bacteria

The Food and Drug Administration has granted de novo marketing authorization to Roche for the cobas vivoDx MRSA diagnostic test, which detects methicillin-resistant Staphylococcus aureus (MRSA) bacterial colonization. This test identifies patients who require enhanced precautions for infection control such as isolation and additional decolonization efforts. It detects MRSA from nasal swab samples in as little as 5 hours using Roche's Smarticles technology, which uses bioparticles to deliver DNA encoded with a bioluminescent reporter gene into MRSA bacteria. These bioparticles have a shell derived from MRSA-specific bacteriophages that binds to receptors on the surface of target bacteria. Once the bioparticles bind to and inject DNA into their targets, live MRSA bacteria then go on to produce light when the proper substrate is added. This enables detection of viable bacteria directly from clinical samples.

In performance studies, this method correctly identified MRSA in approximately 90% of samples in which MRSA was present and correctly identified no MRSA in 98.6% of samples that did not have MRSA present.



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PERKINELMER GETS FDA NOD FOR DUCHENNE MUSCULAR DYSTROPHY NEWBORN SCREENING TEST

PerkinElmer has received de novo marketing authorization from the Food and Drug Administration (FDA) for the GSP Neonatal Creatine Kinase-MM (CK-MM) kit. This is the first test to earn FDA authorization that aids in newborn screening for the genetic disorder Duchenne muscular dystrophy (DMD). The kit measures concentrations of the protein CK-MM from dried blood samples collected via heel-stick from newborns 24 to 48 hours after birth. If a patient's CK-MM levels were found to be elevated, this could indicate the presence of DMD, and labs would then confirm this finding with further testing such as muscle biopsies, genetic, and other laboratory tests. To evaluate the GSP Neonatal Creatine Kinase-MM kit, FDA reviewed data from a clinical study of 3,041 newborns whose dried blood samples were tested for protein levels that are associated with DMD. In the study, the kit accurately identified the four screened newborns who had DMD-causing genetic mutations.

FDA APPROVES MYRIAD'S BRACANALYSIS TEST AS A CO-DIAGNOSTIC FOR PANCREATIC CANCER

The Food and Drug Administration (FDA)

has approved Myriad Genetics' BRACAnalysis CDx for use as a companion diagnostic to identify patients with metastatic pancreatic cancer who have a germline *BRCA* mutation and are candidates for treatment with Lynparza (olaparib). BRACAnalysis CDx detects and classifies oncogenic variants in the protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes, while Lynparza is a poly (ADP-ribose) polymerase inhibitor marketed by AstraZeneca and Merck. Previously, FDA approved BRACAnalysis CDx to identify candidates for Lynparza treatment who have either advanced ovarian cancer; recurrent platinum-sensitive germline *BRCA*m ovarian cancer; or germline *BRCA*m metastatic breast cancer that has been treated with chemotherapy. As part of Myriad's ongoing collaboration with AstraZeneca, the company also recently submitted a new supplementary premarket approval application to FDA for BRACAnalysis CDx as a companion diagnostic to Lynparza for men with metastatic castration-resistant prostate cancer.

CAREDX RECEIVES CE MARK FOR CFDNA ORGAN TRANSPLANT MONITORING TEST

CE mark approval has been given to CareDx for its AlloSeq cfDNA kit, which aids with organ transplant surveillance by quantifying donor-derived cell-free DNA (dd-cfDNA) in transplant recipients. When graft injury occurs, dd-cfDNA increases in the blood, and studies have shown that this is a more accurate indicator of active transplant rejection than serum creatinine. The AlloSeq cfDNA works by first preparing a sequencing library. For each sample, the test performs a single multiplex polymerase chain reaction (PCR) that involves simultaneous locus-specific amplification of the targeted genes of interest and index PCR. Next, samples are pooled, purified, and quantified for sequencing on an Illumina MiSeq instrument. The AlloSeq cfDNA software then analyzes the results, using the fraction of donor-specific nucleotides at single nucleotide polymorphism loci to determine the patient's levels of dd-cfDNA. Altogether, this process takes 24 hours. Separate genotyping of the donor or recipient is typically not required but may be advised depending on the use case.

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CE MARK GRANTED TO BAEBIES FOR PEDIATRIC ENZYME DEFICIENCY POC TEST

Baebies has earned the CE mark for Finder, a point-of-care testing platform that tests for glucose-6-phosphate dehydrogenase (G6PD) deficiency, which causes a spectrum of diseases including neonatal hyperbilirubinemia, acute hemolysis, and chronic hemolysis. Finder is designed for use in a variety of settings, from neonatal intensive care units to birthing centers. The platform uses digital microfluidic technology, which enables testing using a low volume (50 µL) of whole blood, and it has a turnaround time of approximately 15 minutes. It also involves only one step for the user—loading the sample—and features a cartridge with all necessary reagents onboard, including those for sample preparation, as well as a tablet for user interface. In the U.S., Finder is currently undergoing a clinical trial, which Baebies plans to complete in early 2020 for Food and Drug Administration 510(k) submission.

FDA CLEARS APPLIED BIOCODE'S SYNDROMIC RESPIRATORY PATHOGEN PANEL

The Food and Drug Administration has given 510(k) clearance to Applied BioCode for its BioCode Respiratory Pathogen Panel (RPP) for use on the BioCode MDx-3000 system. The BioCode RPP tests nasopharyngeal swabs for the most common viruses and bacteria, including influenza A and subtypes H1, H1N1 2009pdm and H3; influenza B; respiratory syncytial virus A/B; parainfluenza virus types 1, 2, 3, and 4; human metapneumovirus A/B; adenovirus; rhinovirus/enterovirus; coronavirus (229E, OC43, HKU1, and NL63); *Mycoplasma pneumoniae*; *Chlamydia pneumoniae*; and *Bordetella pertussis*. The BioCode MDx-3000 system on which the test runs combines the amplification, hybridization and target capture, and detection steps for the RPP assay, and processes up to 188 samples in an 8-hour shift. The system is based on Applied BioCode's Barcoded Magnetic Beads technology, which uses biocompatible polymeric magnetic beads to digitally barcode biomarkers. The BioCode MDx-3000 also offers flexible ordering and reporting capabilities to accommodate variations in test ordering patterns and potential changes in reimbursement.

Industry Playbook

Veracyte Expands Reach With NanoString Deal

Aiming to expand its global molecular diagnostics business, Veracyte inked a $50 million deal with NanoString Technologies for access to the NanoString nCounter Analysis System, which analyzes RNA, DNA, or protein targets in various sample types. Prior to the collaboration, Veracyte had been focused on advancing the diagnosis of thyroid cancer, lung cancer, and idiopathic pulmonary fibrosis through genomic testing. By obtaining access to the nCounter platform, the company is now in the position to provide genomic tests to hospitals and laboratories worldwide. "Veracyte is the ideal company to help ensure that our nCounter-based diagnostic platform can benefit as many patients around the world as possible," said Brad Gray, president and CEO of NanoString.

The companies plan to begin running an extensive list of genomic tests on the nCounter platform in 2021. As part of the agreement, Veracyte also acquired access to NanoString's Prosigna Breast Cancer prognostic assay and LymphMark lymphoma subtyping assay, which is still in development. As of now, Veracyte hopes to utilize the nCounter system to distribute tests internationally beginning with its Envisia classifier for idiopathic pulmonary fibrosis diagnosis and its nasal swab classifier for lung cancer diagnosis. NanoString also plans to merge key team members with Veracyte.

ROCHE, ILLUMINA PARTNER ON ONCOLOGY NGS

Roche and Illumina have entered into a 15-year partnership in the hopes of broadening the adoption of next-generation sequencing (NGS)-based testing in oncology. The companies are bullish on their ability to improve the diagnosis and treatment of cancer by combining Illumina's expertise in NGS with Roche's focus on clinical oncology.

The settled agreement specifies terms for both in vitro diagnostic (IVD) and companion diagnostic (CDx) products. Roche will obtain full access to Illumina's NextSeq 550Dx System, as well as all other diagnostic sequencing systems, to develop, manufacture, and commercialize Avenio IVD tests for tissue and blood. In return, both companies will collaborate to develop and pursue CDx claims on Illumina's pan-cancer assay TruSight Oncology 500 for Roche's existing and future targeted oncology therapies on the NextSeq 550Dx System. "This collaboration is uniquely positioned to improve medical value and clinical decision-making globally and will provide more patients with access to NGS to characterize their disease and identify the right treatment for them," said Thomas Schinecker, PhD, CEO of Roche.

The partnership comes after Illumina and Pacific Biosciences (PacBio) announced termination of their 2018 agreement to enhance Illumina's NGS technology. Under that agreement, Illumina was set to acquire PacBio for $1.2 billion to merge both companies' specializations in DNA sequencing. However, both the Federal Trade Commission and the United Kingdom's Competition and Markets Authority had reservations about the agreement, stating that the deal would cause Illumina to dominate the field of NGS technology and diminish competition. Both companies acknowledged that ending the agreement was the best decision for their employees and shareholders. As part of the original agreement, Illumina will have to pay PacBio a $98 million termination fee.

SYSMEX, OPTIM ANNOUNCE DIGITAL MEDICINE JOINT VENTURE

In a bid to accelerate the commercialization of digital medicine, Sysmex and OPTiM announced a joint venture to develop diagnostic technology solutions through the use of artificial intelligence (AI). Specifically, the agreement will merge Sysmex's skills in healthcare global sales and services with OPTiM's AI technologies, such as OPTiM Cloud IoT OS.

The collaboration aims to improve the use of AI for image processing of genetic data acquired from Sysmex analyzers. The companies also plan to work closely with pharmaceutical companies and medical device manufacturers to include additional applications. This initiative comes at a time when digital medicine platforms are crucial for managing patient testing and diagnosis and treatment of diseases, the companies said. The newly agreed upon business plan follows a partnership between the two companies that was established in February 2019.

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AACC ANNOUNCES **2021 CANDIDATES**

Anthony A. Killeen, MD, PhD, AACC secretary, has announced the slate of candidates selected by the Nominating Committee for the 2021 AACC elections. The Nominating Committee identifies a single candidate for each open officer and Board position and more candidates than open positions for the Nominating Committee. The online election process will run May 1-31, 2020, and the membership will have the opportunity to vote for or against each candidate on the single-candidate slate and to elect three members to the Nominating Committee through a plurality vote.

The candidates and positions are:

PRESIDENT-ELECT

Stephen R. Master, MD, PhD, FAACC, chief, division of laboratory medicine, Children's Hospital of Philadelphia; medical director, Michael Palmieri Laboratory for Metabolic and Advanced Diagnostics; and associate professor of pathology and laboratory medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia.

SECRETARY

Paul Jannetto, PhD, DABCC, FAACC, consultant and laboratory director, Mayo Clinic, Rochester, Minnesota.

BOARD OF DIRECTORS

Christopher McCudden, Phd, DABCC, FAACC, NRCC vice chair, department of pathology and laboratory medicine, University of Ottawa; deputy chief medical and scientific officer, Eastern Ontario Regional Laboratory Association; clinical biochemist, department of pathology and laboratory medicine, The Ottawa Hospital; and associate professor, department of pathology and laboratory medicine, University of Ottawa, Ottawa, Ontario, Canada.

Christine Schmotzer, MD, chief, division of clinical pathology, University Hospitals Cleveland Medical Center; co-director, University Hospitals Diagnostic Institute; and medical director, UH Cleveland Medical Center and UHLSF Laboratories, Cleveland.

NOMINATING COMMITTEE

Lorin Bachmann, PhD, DABCC, associate professor, department of pathology, and co-director, clinical chemistry and point-of-care testing, Virginia Commonwealth University, Richmond, Virginia.

Allison Chambliss, PhD, DABCC, FAACC, assistant professor of clinical pathology, University of Southern California, Los Angeles.

Mari DeMarco, PhD, DABCC, FAACC, FCACB, clinical chemist and clinical associate professor, St. Paul's Hospital, Vancouver, British Columbia, Canada.

Jane Dickerson, PhD, director, chemistry lab; director, reference lab services; medical director, North Clinic; and clinical director, Patient-Centered Laboratory Utilization Guidance Services, Seattle Children's Hospital, Seattle.

Corinne Fantz, PhD, DABCC, director, medical and scientific affairs, point-of-care testing, Roche Diagnostics, Indianapolis.

T. Scott Isbell, PhD, DABCC, FAACC, associate professor/ medical director of clinical chemistry and point-of-care testing, St. Louis University School of Medicine, St. Louis.

Nader Rifai, PhD, director of clinical chemistry, Boston Children's Hospital, Boston.

VERAVAS, TYMORE COLLABORATE ON ALZHEIMER'S EARLY DETECTION

Diagnostic companies Veravas and Tymora Analytical Operations are combining technologies to improve neuroscience applications in the in vitro diagnostics market. The companies plan to focus on Alzheimer's disease (AD) early detection biomarkers. As the number of patients diagnosed with AD rises, research has shown the need for a more rapid assay as an alternative to the current extensive procedures used to detect the illness.

The collaboration will merge Veravas' proprietary sample preparation technology with Tymora's extracellular vesicle enrichment technology to develop a diagnostic test for early detection of AD. If successful, it would mark the first effective screening test from human plasma for AD and could replace the need for the cerebrospinal fluid neuromarker testing that is currently used for diagnosis and treatment, according to the companies. "This new partnership allows us both to expand our resources to favorably impact assay development and performance," said Anton Iliuk, PhD, Tymora's president and chief technology officer.

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Ask The Expert

Genetic Testing for Primary Immunodeficiency Disorders

EXPERT
Ann Moyer, MD, PhD

Why is it important to establish a molecular diagnosis in primary immunodeficiency disorders?

A: This information allows healthcare providers to better inform affected individuals and families about prognosis and optimal surveillance strategies, and may also guide therapy. For example, patients with severe combined immunodeficiency might have similar phenotypes even though they have variants in different genes. Knowing which gene is affected helps determine whether the best treatment option might be enzyme replacement therapy, gene therapy, or a stem cell transplant.

In addition, once the molecular diagnosis is established, healthcare providers can better define the risk of recurrence and decide whether to screen unaffected family members for carrier status, to identify a suitable stem cell donor, or for other purposes.

How has the diagnostic approach to immunodeficiency disorders changed in recent years?

Historically, when a patient presented with a clinical history suspicious for a primary immunodeficiency, the diagnostic process began with a basic immunological evaluation and subsequent functional studies to identify the specific immunological defect. Then, after narrowing the differential diagnosis or making a clinical diagnosis, labs might have performed genetic studies, if available, to establish a molecular diagnosis.

However, the clinical and genetic heterogeneity of some primary immunodeficiency disorders can make this approach challenging. The phenotypic spectrum is quite broad for some disorders, resulting in the same molecular defect presenting quite differently among individuals. In addition, given the rare nature of some of these disorders, a patient might present with a phenotype that isn't yet fully characterized. These scenarios can make it difficult to select for sequencing the gene(s) most likely to cause disease. If a lab sequences multiple individual genes separately, testing can also be cost prohibitive when the immunologic work-up does not narrow the diagnosis to one or a few genes.

With advances in technology, genetic testing has drastically decreased in cost and become more widely available. In particular, the introduction of massively parallel or next-generation sequencing (NGS) has given labs an economically feasible way to test many genes simultaneously, which contrasts with traditional Sanger sequencing approaches that typically test only one or a few genes at a time. These advances, now available in clinical settings, have led to an alternate "genotype-first" approach to the diagnosis of immunodeficiency disorders. Additionally, widespread genetic testing has resulted in a rapid increase in the identification of monogenic immunodeficiency disorders.

Which NGS test approach is most appropriate for immunodeficiency disorders—a targeted panel or exome sequencing?

There are pros and cons to both approaches. Targeted panels test an expertly curated list of genes associated with the disease of interest and can include variants in the exons and known variants located in noncoding regions. These panels may also involve complementary methods for genes difficult to test by NGS. However, it's challenging for labs to keep targeted panels updated as additional genes become associated with disease. Exome sequencing, which tends to be more expensive and have a longer turnaround time, might not include important noncoding variants or key genes that are not amenable to NGS. However, exome sequencing allows for the discovery of additional disease-associated genes and might be more suitable for individuals with a nonspecific phenotype who might otherwise require multiple targeted panels.

Are there limitations to genetic diagnosis?

Despite advances in testing, labs still aren't able to reach a genetic diagnosis for all patients. This can be due to a gene that has not yet been associated with disease, oligogenic or polygenic inheritance, epigenetics, or other variables. Healthcare providers should therefore continue to integrate genetic information with clinical history and functional studies when making a diagnosis. This requires close collaboration between clinicians and laboratory professionals.

Ann Moyer, MD, PhD, is a co-director of the Personalized Genomics Laboratory at Mayo Clinic in Rochester, Minnesota.
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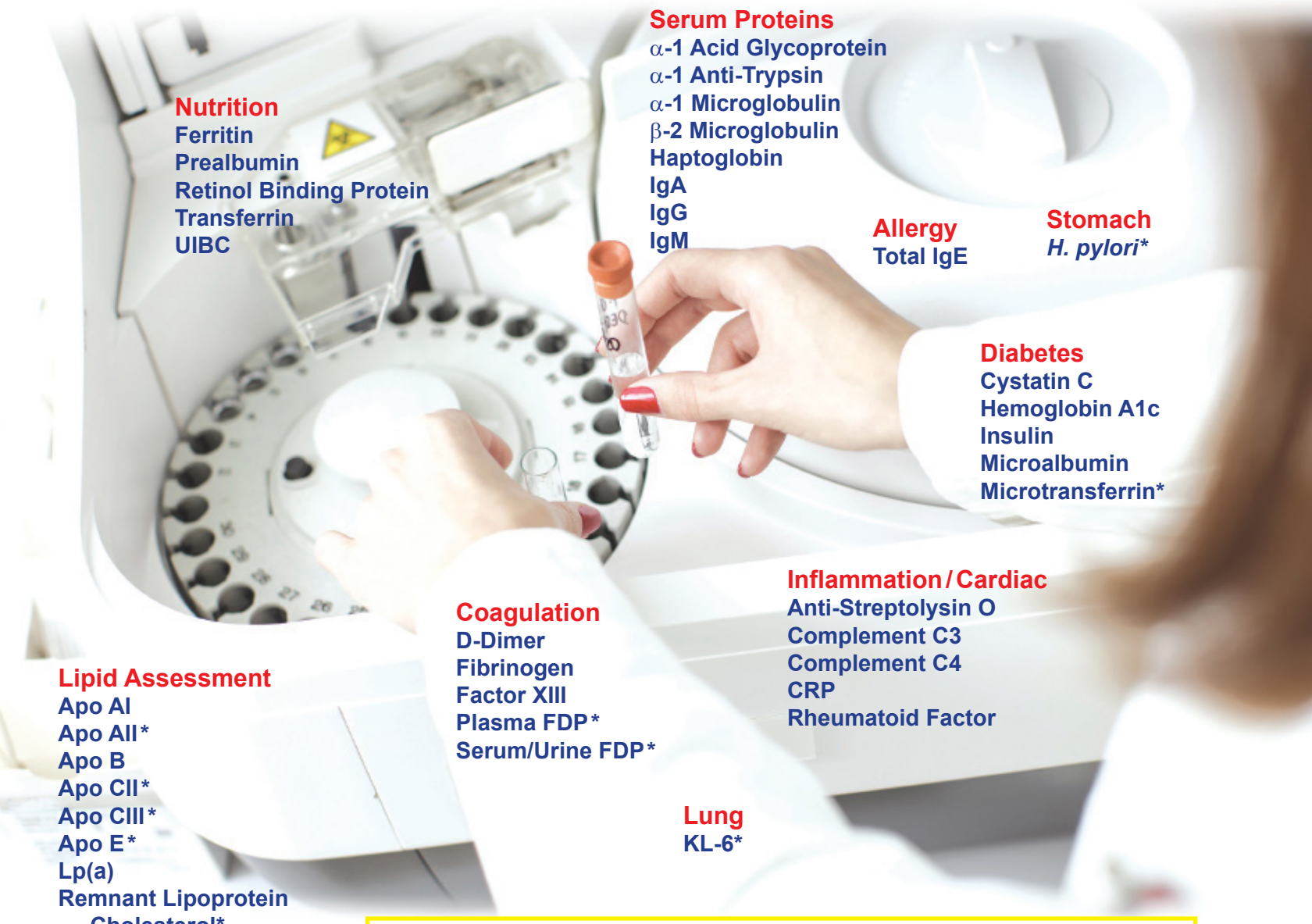

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