G2 INTELLIGENCE

Diagnostic Testing & Emerging Technologies

New Trends, Applications, and IVD Industry Analysis

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NCI Launches Sequencing-Based, Personalized Medicine Trial

The National Cancer Institute recently launched a pilot trial that has the potential to advance both personalized medicine and the use of next-generation sequencing (NGS) as a pretreatment screening tool for cancer patients with solid tumors. The prospective, randomized clinical trial, Molecular Profiling based Assignment of Cancer Therapeutics (M-PACT), will assign treatment based on sequencing-based prescreening with a custom panel.

Tumor samples from an estimated 700 patients with advanced tumors resistant to standard therapy will be analyzed to identify patients with at least one of 391 mutations in 20 genes that are known to affect the effectiveness of targeted therapies. Based on detection of mutations of interest, 180 patients will be enrolled in the trial and divided into groups based on mutations to a genetic pathway (DNA repair, PI3K, or RAS/RAF). These lesser-studied mutations were deliberately chosen, as were the therapies, including candidate drugs. Within these groups patients will be randomly assigned to receive a treatment regimen prospectively identified to target their specific mutation or to receive a treatment regimen not specific to the genetic pathway. These patients will have the option to cross over to the targeted treatment arm if their disease progresses on their initial study treatment.

Given the heterogeneity of tumors, it is not always known which mutation to target. Patients with well-established genetic markers such as BRAF mutations (in melanoma), BRCA (in breast or ovarian cancer), or EGFR (in non-small cell lung cancer) can only enroll if their cancer progressed on targeted therapy and other mutations are present. For more information on how NGS is being adopted in clinical practice, please see *Inside the Diagnostics Industry* on page 5.

Life Science Incubators Booming Nationally; Diagnostic Start-Ups Welcomed

Illumina (San Diego) launched the Illumina Accelerator Program, touted as the first-ever genomics-focused business accelerator back in mid-February. The company says its goal is to speed the time to market and lower the barriers to entry for entrepreneurs, start-ups, and early-stage companies working on "scientifically and commercially promising" next-generation sequencing applications. While the fact that the accelerator is committed entirely to genomics is a new twist, housing early-stage companies in accelerators and incubators is part of a rapidly proliferating trend in the life sciences.

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▲ **Life Science Incubators Booming Nationally,** *from page 1*

Driven by the lack of investment capital for early-stage life science companies, the desire to stimulate local economies through entrepreneurship, and the need of larger firms to scale-back internal research and development (R&D) while eyeing strategic expansion opportunities, the incubator industry has scaled up significantly since the economic downturn in 2008.

"When you look at the number of academic ideas out there and the size of investment available through venture capitalists, there is a big gap," Mostafa Ronaghi, Ph.D., Illumina's chief technology officer, told *Bloomberg*. "This is especially true in genomics, where it's more difficult for companies to get started."

Illumina's accelerator is relatively unique with its exclusive focus on genomics and its corporate backing, but like other accelerator programs, Illumina's is six months long and provides invited participants with access to technology and business guidance. Specifically, Illumina has pledged $100,000 in support through access to sequencing systems, reagents, and fully operational lab space. In addition, participants will have access to mentorship through Illumina's global customer and partner support services, including financial modeling, licensing and technology transfer support, and legal and human resource assistance.

On the financial side it has been reported that Illumina will make the investment in each company in exchange for a 10 percent share of the company's common stock. Accelerator partners Silicon Valley Bank and billionaire technology investor Yuri Milner will help with financial resources through provision of banking services, credit, and a $100,000 convertible note. Illumina says it will initially select three companies every six months with the first session beginning this August.

While Illumina is likely motivated, at least in part, by accrual to its own financial benefit if genomics companies thrive and in turn buy more Illumina products, there is a surge in life science and biotech accelerator activity across the country.

Business incubators date back to the 1950s, but there has been a recent explosion in interest. According to the National Business Incubation As-

Diagnostics Companies Currently in Incubators or Accelerators

EnterpriseWorks (Chicago; 2014)

- BioAnalytics: Focused on designing the next generation of immunoassay technology that can reduce the number of steps required for protein quantification, resulting in faster multiplexed assays.
- GlucoSentinent: Transforming the personal glucose meter into a device that is capable of quantitatively detecting other nonglucose targets.
- Oracle Biosciences: Developing a single gene-based clinical assay for diagnosis and prognosis for Basal-like breast cancer.

Harlem Biospace (New York; 2013)

- Girihlet: Developing mitochondrial DNA based biomarkers for cancer and developmental disorders based on technology developed at Mount Sinai Hospital (New York).
- Gray Box Biology: Developing a prototype medical device that can diagnose drug-resistant tuberculosis in patients in low-resource areas. The device, Bronx Box 2.0, is based on technology developed at Albert Einstein College of Medicine (New York).
- Immunovent: The Local Allergy Mucosal Brush (LAMB) test is the first local allergy test that can accurately diagnose a significant number of both airborne and food allergies that are currently undetected by traditional allergy testing methods.
- Junco Labs: Working on a handheld "lab-on-a-chip" device that can perform rapid and inexpensive diagnostic tests at the point of care.

University of Missouri Life Science Business Incubator (Columbia, Mo.; 2009)

- Columbia Diagnostics: A spinoff from University of Missouri is developing companion diagnostics for individualized cancer therapy.
- Viator Technologies: Another University of Missouri spinoff, it is building technology for early detection of circulating melanoma tumor cells.

Student Venture Incubator/Yale Entrepreneurial Institute (New Haven, Conn.; 2007)

- Ancera: Focusing on rapid cell separation and pathogen diagnostics using label- and labor-free methods to provide low-cost, high-throughput diagnostics in under 10 minutes.

sociation (NBIA), the total number of business incubators reached 1,250 incubators in the United States at the end of 2012, up from just a dozen such facilities in 1980. Life science incubators are emerging not just in established biotech clusters, such as Boston and San Diego, but also in smaller cities like Albuquerque, N.M., and New Orleans, and even in rural areas.

"The term *incubator* or *accelerator* can mean different things to different people," says Hanson Gifford III, managing partner at the Foundry, a medical device incubator in Menlo Park, Calif. "In some parts of the country it can be an economic development tool by providing some space and coffee, but we are serial entrepreneurs looking for an opportunity where there is clinical need for a dramatically better solution and where you can get from start to [Food and Drug Administration] approval or a commercialized product without spending the national debt and spending three lifetimes. Incubators are a halfway step to get companies along."

General Traits of Incubators, Accelerators

While the terms are often used interchangeably, there are some general, historical differences between incubators and accelerators.

	Incubator	Accelerator
Status	Nonprofit	For-Profit
Focus	Science-based businesses	Technology start-ups including gaming, social networks, apps, and software
Sponsors	Traditionally include universities (32 percent), economic development organizations (25 percent), and government (16 percent)	Rely more upon serial entrepreneurs and private investors
Company Selection	Competitive	Competitive
Length of Stay	Typically two years to three years	Six months

Source: Adapted from NBIA

NBIA says the most common goals of incubation programs are creating jobs, enhancing a community's entrepreneurial climate, retaining businesses in a community, and diversifying local economies. In 2011, North American incubators, the NBIA says, assisted about 49,000 start-up companies, which in turn provided full-time employment for nearly 200,000 workers and generated annual revenue of almost $15 billion. Some worry, though, that the rapid growth of science and technology incubators in recent years will outpace the ability for incubator graduates to attract investment capital or the attention of industry.

"The metrics are very easy to measure and are very straightforward. Did the company survive? Did it find funding? Or was there a transaction or exit by placing the technology with an existing company upon completion of the incubation period?" explains Wade Fallin, CEO of VentureMD, a medical device incubator in Salt Lake City. "As seed stage venture capital has dried up and large medical device companies have scaled back [their R&D] there has been very high interest in incubators, as they take away some of the investment risk."

Takeaway: Economic considerations have generated interest in incubators and accelerators as efficient means for early-stage companies to gain access to expertise and rapidly assess their business case. In an environment of little investment in early-stage companies, life science incubators are expected to continue to foster start-ups and provide a reduced risk means for investors and larger medical device companies to appraise new technology.

Economic Challenges Prevail in Development of Companion Diagnostics

While the potential benefits of utilizing genomic data to stratify patients most likely to respond to a specific treatment have been highly touted, the actual process of developing companion diagnostics (CDx) is fraught with a high degree of financial risk for diagnostic manufacturers. While some of these economic perils come from the "mismatch" between the pharmaceutical and the diagnostics industries, laboratories producing generic versions of the CDx as laboratory-developed tests (LDTs) also pose a significant economic threat to in vitro diagnostics (IVD) developers.

Despite some recent approvals by the U.S. Food and Drug Administration (FDA), only 18 CDx have been approved to date. Participants in the workshop Refining Processes for the Co-Development of Genome-Based Therapeutics and Companion Diagnostic Tests, hosted by the Institute of Medicine last year, shared concerns about the process of codevelopment of CDx, particularly the commercial aspects of such arrangements. The workshop summary, published in mid-February, highlights the challenges associated with CDx codevelopment as well as some possible solutions to accelerate progress in development of CDx.

"Without regulatory flexibility for CDx test advancements, innovation, efficiency, and patient benefit could be stifled."
—Robert McCormack, Ph.D.

"Drug development and test development are inherently difficult to coordinate," said Felix Frueh, Ph.D., then an entrepreneur-in-residence at investment firm Third Rock Ventures. "For the most part the timelines of the two businesses really do not align. The resources are completely mismatched, and the market protection between the drug and the diagnostic is entirely different."

There is "a weak business case to support investment in CDx codevelopment," writes workshop co-chair Robert McCormack, Ph.D., from Janssen Diagnostics (Raritan, N.J.), in a viewpoint published Feb. 12 in the *Journal of the American Medical Association* (*JAMA*).

Simultaneous codevelopment of CDx and the drug provides the diagnostics company some strategic advantages including the analytical and clinical validation data required for the FDA review and clinical utility data from the phase 3 clinical trial required for reimbursement determinations.

"Although the codevelopment process is lengthy and expensive for a company, the close association of the test with use of a drug and the potential for reimbursement at the time of approval are substantial incentives," writes McCormack.

However, this coupling, in reality, may not be as tight as diagnostics developers had hoped. For instance, the FDA does not recognize a test by name in its labeling. FDA's director of personalized medicine, Elizabeth Mansfield, Ph.D., told the workshop that the label in the indications, warnings, or precautions sections refers to "a type of approved or cleared IVD CDx device, but not a specific one by name." This decision takes into account the fact that better tests may be approved at a later date.

Some of the related or CDx follow-on LDTs may capitalize on newer, more efficient technology platforms, like next-generation sequencing (NGS). However, stakeholders say, the fact that regulation of CDx developed as LDTs is less stringent makes it

less costly to bring the test and is a cause for concern due to an unlevel playing field. While lack of reimbursement, and more specifically lack or reimbursement based on the informational value provided by the test, certainly contributes to the poor economic model surrounding CDx test development, IVD developers point to competition from LDTs as a serious threat to their business case.

"Current billing practices do not allow for the payer to discriminate an FDA-labeled CDx from an LDT test, so the laboratory [can receive] higher payment for a test that was less costly to develop," write McCormack and his co-authors in *JAMA*. "Thus, investment by the company that developed the CoDx is undermined before coming to market with little chance of achieving an acceptable return on investment."

There are also operational concerns regarding efficiently running CDx testing in laboratory practice. Most CDx test development focuses on single biomarkers for a single drug. Laboratory experts express concern that guidance is lacking for how to extend the use of a CDx to other cancers that express the relevant mutation and how to incorporate advancements in technological platforms (i.e., NGS) that are capable of multiplexing multiple biomarkers into a single test, often a practical consideration given that small specimen volume can be inadequate for multiple tests.

FDA-Approved Companion Diagnostics

Drug Name	Device Trade Name	Device Manufacturer
Erbitux	therascreen KRAS RGQ PCR Kit	Qiagen
Erbitux	DAKO EGFR PharmDx Kit	Dako North America
Gilotrif	therascreen EGFR RGQ PCR Kit	Qiagen
Gleevec/Glivec	DAKO C-KIT PharmDx	Dako North America
Herceptin	INFORM HER-2/NEU	Ventana Medical Systems
Herceptin	PATHVYSION HER-2 DNA Probe Kit	Abbott Molecular
Herceptin	INSITE HER-2/NEU KIT	Biogenex Laboratories
Herceptin	SPOT-LIGHT HER2 CISH Kit	Life Technologies
Herceptin	Bond Oracle Her2 IHC System	Leica Biosystems
Herceptin	HER2 CISH PharmDx Kit	Dako Denmark
Herceptin	INFORM HER2 DUAL ISH DNA Probe Cocktail	Ventana Medical Systems
Herceptin	HERCEPTEST	Dako Denmark
Herceptin	HER2 FISH PharmDx Kit	Dako Denmark
Menkinist/Tafinlar	THxID BRAF Kit	bioMerieux
Tarceva	cobas EGFR Mutation Test	Roche Molecular Systems
Xalkori	VYSIS ALK Break Apart FISH Probe Kit	Abbott Molecular
Zelboraf	cobas 4800 BRAF V600 Mutation Test	Roche Molecular Systems

Source: FDA

To help "level the playing field" many workshop participants say there is a need to require demonstration of equivalence between IVDs and LDTs. Regulatory oversight of LDTs can be achieved through strengthening of the Clinical Laboratory Improvement Amendments, risk-based FDA regulation, or creation of a single pathway for all CDx, regardless of their developer, participants proposed. While laboratories fear greater regulatory oversight of CDx created as LDTs will pose significant added expenses, others believe such mechanisms are necessary to provide "more comparable economic incentives" for IVD and LDT developers. All diagnostic developers will benefit economically, participants said, with a shift toward value-based payment for CDx focused on outcomes.

Takeaway: Codevelopment of CDx is challenged by a weak business case, particularly for IVD developers who face erosion of financial incentives due to competition from LDTs. Increased regulation of CDx LDTs has been proposed by stakeholders to level the playing field.

G2 INTELLIGENCE
Inside the Diagnostics Industry

NGS Rapidly Being Integrated Into Clinical Laboratories

Next-generation sequencing (NGS) platforms are becoming more automated, more cost-efficient, and while not quite turnkey, are reaching the point in ease of use that clinical applications of the technology are becoming mainstream. Clinical laboratories' adoption of the technology is occurring at a rate that surpasses the uptake of other molecular technologies, including polymerase chain reaction (PCR), experts say. *DTET* surveyed the NGS landscape to evaluate both the latest advances in the technology and trends in adoption by clinical laboratories.

Technological Evolution

Faster and cheaper are the two words scientists eagerly listen for when instrument manufacturers unveil their newest sequencing offerings. So far, 2014 has greeted laboratory scientists with some exciting announcements.

The first publicly released data generated from U.K.-based Oxford Nanopore Technology's much anticipated thumb drive-sized MinION sequencer was a mixed bag. A technical review of the device by collaborator David Jaffe, from the Broad Institute in Cambridge, Mass., who used it to assemble two bacterial genomes, concluded that, as promised, the nanopore-based sequencing machine allowed for much longer reads (an average length of 5.4 kilobases, but up to 10 kilobases), compared to Illumina machines, which deliver fragments hundreds of base pairs long. The technology differs substantially from other NGS platforms as it identifies DNA bases by measuring the changes in electrical conductivity DNA generates as it passes through a biological pore.

> ***"Breaking the 'sound barrier' of human genetics [by delivering a $1,000 genome] not only pushes us through a psychological milestone, it enables projects of unprecedented scale."***
>
> ***—Jay Flatley, Illumina***

However, the review, presented by Jaffe at the Advances in Genome Biology & Technology conference (AGBT; Marco Island, Fla.; Feb. 12-15), raised concerns about "systematic errors" that prevented the assemblage of the genomes with just the MinION data. Requiring assistance, some at the conference argued, defeats the point of a handheld sequencer.

The technology seemingly stalled for two years following the company's initial announcement. *Science* reports that in the interim, the company had to find a new membrane for the pore, as its original choice could not be manufactured on a large scale. The firm also shifted its focus from a large sequencer to a portable device. But following the silence, last month Oxford Nanopore not only made this initial MinION data public, but it also launched its early-access program to individual researchers interested in testing the device in their labs. Researchers must pay a $1,000 deposit, plus $250 for shipping costs.

Illumina (San Diego) also had a dramatic start to 2014 with the unveiling of two new products. The company crossed the long awaited $1,000 genome threshold with its new HiSeq X Ten system.

"With the HiSeq X Ten, we're delivering the $1,000 genome, reshaping the economics and scale of human genome sequencing, and redefining the possibilities for population-level studies in shaping the future of healthcare," said Jay Flatley,

Illumina's CEO in a statement. "The ability to explore the human genome on this scale will bring the study of cancer and complex diseases to a new level. Breaking the 'sound barrier' of human genetics not only pushes us through a psychological milestone, it enables projects of unprecedented scale."

Early adopters of the HiSeq X Ten system, which is expected to ship in the first quarter of 2014, include Macrogen, an NGS service organization in South Korea and Rockville, Md.; the Broad Institute; and the Garvan Institute of Medical Research in Australia. But while the announcement was hailed as an important milestone, this version of the $1,000 genome will remain out of reach for many clinical laboratories. The HiSeq X Ten system is available only as a combination of at least 10 HiSeq X systems, which would cost a total of at least $10 million. Few facilities have the sample volume necessary to make the investment worthwhile.

"It's a good deal if you can play in this game," Chad Nusbaum, co-director of the sequencing program at the Broad Institute, told *Nature*. "It's like the high-stakes poker table: If you're playing $200 a chip, people who can't afford those chips don't care."

A possibly more accessible entry in Illumina's sequencing portfolio is its NextSeq 500 system, which launched in January. The desktop machine can perform the most popular sequencing applications in less than a day (including one whole human genome, up to 16 exomes, up to 20 noninvasive prenatal testing samples, up to 20 transcriptomes, up to 48 gene expression samples, and up to 96 targeted panels) with a price tag of $250,000.

Novel NGS Platforms

Oxford Nanopore was not the only novel NGS platform maker garnering attention at AGBT. GenapSys (Redwood City, Calif.) unveiled plans for a DNA sequencer with the footprint of an iPad.

The company's platform uses a semiconductor chip like Life Technologies' system but differs in how the electric signal of the DNA molecule is measured. The company claims it is very easy to use and incredibly low-cost—possibly as low as a few thousand dollars to purchase—but reportedly won't be available for another year. The company's founder, Hesaam Esfandyarpour, Ph.D., tells *Forbes* the goal is to bring this technology "to the hands of the masses," with a per-run cost of a couple hundred dollars.

The company closed a $37 million series B round of financing back in November 2013 that included capital from billionaire technology investor Yuri Milner.

Clinical Adoption

Sequencing throughput once reserved for large genome sequencing centers is now capable of being performed in clinical laboratories, thanks to continued platform advancements. As the technology continues to advance and new, cheaper, and more automated benchtop—and even smaller—platforms enter the marketplace, clinical adoption of NGS is expected to proliferate. Studies of the reliability of NGS-based testing using targeted gene approaches for routine clinical care (particularly for oncology and traditional genetic diseases) are materializing in the literature. Simultaneously, early adopters of whole-genome and whole-exome sequencing are already emerging.

Gregory J. Tsongalis, Ph.D., director of molecular pathology at Dartmouth Hitchcock Medical Center (Lebanon, N.H.) points to a number of drivers that are pushing clinical laboratories toward NGS, including:

- The need to consolidate single-gene analysis into a single assay for operational efficiency;
- The cost-effectiveness of NGS compared to traditional PCR-based or other molecular methods; and

- Currently nonactionable data can be mined later as advancements in molecular understanding and therapy warrant in the future.

An additional issue is one of comprehensiveness. Unlike single-gene tests, multiplexed NGS assays ensure that all actionable tumor mutations are identified, even if the mutation occurs rarely. Using an NGS cancer hot spot panel, Tsongalis's group not only detected 100 percent of the mutations seen with PCR, but the laboratory was also able to detect two additional actionable EGFR mutations not included in their laboratory's single-gene assay.

"The current standard approach focusing on single gene and sometimes single exon analysis detects only the most commonly described mutations. Less common mutations are not tested in the single mutation, single assay model because of design, cost, sample, and time constraints," explains Tsongalis in a recent article published in the March issue of *Clinical Chemistry and Laboratory Medicine*. "There is a disconnect between appropriate personalized or precision medicine and current testing algorithms. NGS promises to bridge this gap by allowing for mutation detection in multiple exons from multiple genes in multiple patient samples, simultaneously. . . . NGS platforms offer an increased breadth of testing at a lower cost and without compromising assay performance and turn-around times."

"There is a disconnect between appropriate personalized or precision medicine and current testing algorithms. NGS promises to bridge this gap by allowing for mutation detection in multiple exons from multiple genes in multiple patient samples, simultaneously."
—Gregory Tsongalis, Ph.D.

In the recent paper, Tsongalis's group evaluated the Ion Torrent AmpliSeq Cancer Hotspot Panelv2 (CHPv2), which is capable of identifying multiple somatic mutations in 50 genes in a single assay. They show that the panel (performed on the Personal Genome Machine) is suitable for clinical testing.

The researchers compared the assay to routinely performed, standalone PCR-based methods for mutations in several genes (KRAS, V600E BRAF mutation, and the two most common EGFR activating mutations). Well-characterized cell lines, genetically engineered cell lines in fixed and embedded in paraffin, and 62 clinical samples (lung, colon, melanoma, rectal, and ovarian) that had been previously tested with the laboratory's current single-gene methods were used. Normal kidney, tonsil, and colon tissues served as controls.

The researchers demonstrated that there was 100 percent concordance in accuracy between previous PCR results and the corresponding variants identified using the Ion Torrent panel, as well as high precision. The limit of detection was 5 percent for single nucleotide variants and 20 percent for insertions and deletions. Specificity studies using normal FFPE tissue previously tested by PCR methods were also 100 percent.

Some additional practical findings from Tsongalis's group: 100-times coverage is needed to identify somatic mutation results with confidence; fine needle aspirates, biopsies, and resected surgical pathology specimens were all equally successfully analyzed; and input DNA concentrations well below those recommended by the manufacturer (as little as 1 ng of DNA isolate from FFPE) were adequate.

Furthermore, the researchers could return meaningful results through a data analysis process they called "efficient, user-friendly, and robust." The post-analytical data analysis, the authors say, is critical to ensure that accurate and actionable variants are returned to the clinicians in a comprehensible way. The variant processing pipeline they developed allows the masking of variants of limited clinical value, which can quickly decrease the number of variants returned to clinicians by an order of magnitude. Additionally, the filtered variants can be separately stored and mined when new molecular understandings become available.

Tsongalis tells *DTET* that the "take home" from his experience has been that the technology available today may not be turnkey quite yet, but laboratories also don't need significant molecular expertise to enter the NGS field. His biggest advice, though: "Don't go at it alone." A multidisciplinary approach that utilizes laboratory folks, pathologists, oncologists, genetic counselors, and information technology/bioinformatics experts is necessary to build the best system for each individual institution.

Not Just Targeted Clinical Sequencing

Even as NGS-based testing is beginning to permeate clinical laboratories, early adopters are employing whole-genome sequencing in clinical care. Children's Mercy (Kansas City, Mo.) is using several NGS-based tests in clinical practice to identify genetic diseases and for the first time, whole-genome testing is even being used in urgent scenarios to inform diagnosis and therapeutic decisions in acutely ill infants in the neonatal intensive care units (NICUs). In October 2013 Children's Mercy began employing its 50-hour STAT-Seq whole-genome analysis to test for 3,500 genetic diseases in an immediate care scenario.

"We believe that 30 percent of the babies in our NICUs are likely to benefit from next-day genome sequencing," said Stephen Kingsmore, the director of pediatric genomic medicine at Children's Mercy.

"By obtaining an interpreted genome in about two days, physicians can make practical use of diagnostic results to tailor treatments to individual infants and children."
—Stephen Kingsmore, M.B.Ch.B., Children's Mercy

STAT-Seq is being developed by the hospital's Center for Pediatric Genomic Medicine in collaboration with Illumina (using its HiSeq 2500 system) and PerkinElmer. STAT-Seq additionally uses in-house software that integrates physician-entered clinical features for individual patients and a comprehensive set of relevant diseases. This software "substantially automates identification of the DNA variations" that can explain the child's condition. Future studies are also expected to show that cutting time to diagnosis also cuts costs.

Children's Mercy received a $5 million National Institutes of Health (NIH) grant in 2013 to generate the data needed to guide the use of rapid genome sequencing in the diagnosis and treatment of acutely ill babies. These efforts will further improve the speed and cost-effectiveness of STAT-Seq, as well as evaluate the benefits, and potential harms, of rapid genome sequencing in newborns.

In addition to STAT-Seq, Children's Mercy clinically employs exome sequencing and its TaGSCAN (Targeted Gene Sequencing and Custom Analysis), a test that screens

for more than 750 diseases that are the result of a single-gene defect, including muscular dystrophy, cystic fibrosis, and polycystic kidney disease. TaGSCAN uses NGS technology along with proprietary software that allows a symptom-based analysis to diagnose genetic diseases. Unlike the rapid turnaround for STAT-Seq, TaGSCAN results are delivered in six to eight weeks and the test costs less than $3,200.

Children's Mercy is not alone. Partners Healthcare, Geisinger Health System, Scripps Health, and the Medical College of Wisconsin are all using whole-genome sequencing, while Baylor Scott & White Health, Emory Healthcare, and UCLA Health are providing clinical exome sequencing.

Partners HealthCare (Boston) is one of the first hospital systems to offer whole-genome sequencing, analysis, and interpretation in clinical care and expects to sequence the genomes from 50 patients in the next year. Partners is reportedly charging about $9,000 for an individual, including interpretation and analysis, and $18,000 analysis for sequencing for a child and both parents to better understand a child's genetic disorder. Additionally, Partners is enrolling about 200 patients and their primary-care physicians or cardiologists in an NIH-funded project to study the integration of whole-genome sequencing into clinical medicine. Including doctors in the project allows researchers to evaluate how physicians are using sequencing information in caring for their patients.

National laboratory giants Quest Diagnostics and LabCorp have both recently entered into multiyear licensing agreements with Illumina, signaling anticipated expansion of their clinical NGS testing.

"Investing in next-generation sequencing, which is increasingly used in several clinical areas as well as clinical trials, is a key element of our strategy," said Jay Wohlgemuth, M.D., Quest's senior vice president of science and innovation, in a statement at the time of the announcement.

In the agreement announced earlier this year, Quest will have broadened rights to use Illumina's sequencing and genotyping technology, including the MiSeq platform and related consumables, to develop, validate, and offer molecular laboratory-developed tests for several diseases, including several cancers and neurological and women's health disorders. LabCorp similarly will have expanded rights to use Illumina's NGS instruments to develop new diagnostic tests in genetic testing, oncology, transplant medicine, and forensics, in addition to human leukocyte antigen tests already planned to be introduced this year.

NGS Testing in Current Clinical Practice

Laboratories currently using clinical whole-genome sequencing:

- Children's Mercy (Kansas City, Mo.)
- Geisinger Health System (Danville, Pa.)
- Medical College of Wisconsin (Milwaukee)
- Partners HealthCare (Boston)
- TruGenome Clinical Sequencing (offered by Illumina [San Diego])

Laboratories currently using clinical whole-exome sequencing:

- Ambry Genetics (Aliso Viejo, Calif.)
- Baylor College of Medicine (Houston)
- Emory Healthcare (Atlanta)
- University of California, Los Angeles

Takeaway: Clinical adoption of NGS is expected to proliferate at a pace unseen with even other molecular technologies. NGS appears to have reached that inflection point where targeted sequencing assays will shortly become routine in clinical laboratories while more early adopters move into clinical whole-exome and whole-genome sequencing.

Saliva Test May ID Male Teens at Risk for Major Depression

Researchers have identified the first biomarker for identifying those adolescent boys at risk for clinical or major depression (MD). The combination of high self-reported depressive symptoms and elevated morning cortisol increase the risk of MD by up to 14 times, according to a study published online Feb. 18 in *Proceedings of the National Academy of Sciences*. Early identification of this subtype of boys may lead to earlier diagnosis and treatment as MD in childhood, teen, or young adult years substantially raises the risk of episodes of depression later in life.

In the study, self-reported depressive symptoms were measured using the 33-item version of the Moods and Feelings Questionnaire, while cortisol was measured by ELISA on 20-µl saliva samples without extraction. One cohort (n = 660) provided four early-morning samples on schooldays within a week and then again 12 months later, and depressive symptoms were measured at baseline, four months, eight months, and 12 months. The second cohort (n = 1,198) provided early-morning samples over three school days and measured depressive symptoms at baseline, 18 months, and 36 months.

The researchers utilized these repeated measures of self-reported depressive symptoms and early-morning levels of cortisol (both known correlates to MD) to categorize the young participants (12 years to 19 years of age) from both cohorts into four classes: Class 1, low morning-cortisol levels and low depressive symptoms over time (31 percent); Class 2, low levels of depressive symptoms but relatively high morning cortisol (27 percent); Class 3, high depressive symptoms and low morning cortisol (25 percent); and Class 4, high levels of both morning cortisol and depressive symptoms (17 percent).

"We provide clear cut confirmatory evidence for longitudinal stability of morning cortisol levels," write the authors, led by Matthew Owens, from the University of Cambridge in the United Kingdom. "This finding demonstrates a trait-like property, a key pre requisite for acting as a physiological biomarker."

The researchers found that Class 4 characterization (high morning cortisol and high depressive symptoms) was associated with increased levels of impaired autobiographical memory recall in both sexes but was tied to a greater likelihood of MD in boys only. Participants in Class 2 to Class 4 were more likely to have reported MD at follow-up, compared with Class 1. The odds of being diagnosed with MD increased progressively across the classes from 1.6 to 7.1 (only Class 3 and 4 were significant). For boys in Class 4 versus Class 1, the odds of being clinically depressed was 14.7.

"The clinical specificity of these findings for MD was enhanced by the fact that there was no specific association between being a member of class 4 for either sex and the presence of nondepressive psychiatric disorder or, more specifically, behavior disorder," write the authors.

Takeaway: While further validation is needed before such a test can be clinically used, the researchers are encouraged at the prospect of having an objective, quantifiable measure of depression risk, rather than an exclusive reliance on patients' self-reports.

G2 Insider

Automated CSF Cell Counters Require Updated Reference Ranges . . . Utilization of automated systems to provide cerebrospinal fluid (CSF) cell counts warrants new reference ranges, according to a study published in the January issue of *Clinical Biochemistry*. The researchers propose new CSF cell count reference ranges of < 4 cells/μL for lymphocytes, < 3 cells/μL for monocytes, and < 3 cells/μL for granulocytes. They further determined that automated systems' ability to differentiate mononuclear cells is of limited differential diagnostic utility.

CSF cell counts have traditionally been manually performed using a microscope and cell counting chamber. The downsides of this technique are that it is time-consuming, requires trained laboratory personnel to be present, and there can be high variability among trained staff. While the performance of automated systems and manual counts is similar, automated systems reduce turnaround time, lower costs, and can provide more detailed cell differentiation than manual analysis. Cells are usually classified as erythrocytes, granulocytes, and mononuclear cells using manual analysis, while automated systems can further separate mononuclear cells into lymphocytes and monocytes based on size, absorbance, and light-scattering characteristics.

The researchers used the Siemens ADVIA 2120i automated counter to establish a reference range from samples in 80 neurologically healthy volunteers (mean age 67 years) undergoing orthopedic surgery, with a sample extracted prior to spinal anesthesia. For comparison, cells were manually counted in 32 1-square-millimeter areas by two experienced laboratory technicians, with the average used for comparison. To evaluate the differential diagnostic ability of utilizing lymphocytes and monocytes cell counts, the researchers used hospital records from 175 patients with elevated CSF mononuclear pleocytosis.

Results showed that there was good correlation between automated and manual leukocyte counts for samples with erythrocyte counts < 250 cells/μL. The new suggested reference ranges were determined using the 95th percentile for the neurologically healthy volunteers.

"The objection could be raised that a patient with an automated cell count . . . would be classified as having a normal cell count using these new suggested reference ranges, despite having a total cell count of 7 cells/μL which is above the limit of 5 cells/μL of today's reference ranges," write the authors, led by Daniel Bremell, from the University of Gothenburg in Sweden. "However, we consider the probability of this being of any clinical importance as very low."

In the differential diagnosis analysis, comparisons were made between patients diagnosed with Lyme neuroborreliosis and viral infection. There were no significant differences between these two groups regarding cell counts of lymphocytes and monocytes.

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