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DIAGNOSTIC TESTING & Emerging Technologies

New Trends, Applications, and IVD Industry Analysis

January, 2015

TOP OF THE NEWS

'Solid' Year Expected for Diagnostics Industry in 2015; Concentrated VC Investments Expected 1

- Other Large Venture Capital Diagnostics Placements (p. 4)

Trend Toward Panels, NGS for Breast Cancer-Associated Testing Seen at San Antonio Breast Cancer Symposium 1

- Liquid Biopsies for Breast Cancer Monitoring (p. 9)

INSIDE THE DIAGNOSTICS INDUSTRY

With Flu Epidemic, Better Diagnostics Become Focus 5

EMERGING TESTS

Blood Markers Could Determine Necessity of CT With Mild TBI 10

Electricity-Free Amplification May Expand Molecular POC Testing 11

G2 INSIDER

Rapid Pathogen Identification Improving in Critically Ill 12

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'Solid' Year Expected for Diagnostics Industry in 2015; Concentrated VC Investments Expected

2014 was a record-setting year by many business metrics for the biotechnology industry. The volume of initial public offerings by venture-backed life science companies was high, mergers and acquisitions were robust, and private fundraising was strong, particularly for diagnostics companies positioned in the vibrant big data/digital health area.

"The unprecedented IPO and M&A activity this year will make 2014 one for the record books and unlikely to ever be surpassed," says G. Steven Burrill, founder of the bio tech investing firm Burrill & Co. and publisher of the Burrill Report. "While we expect 2015 to be another strong year for the sector with robust financial markets and dealmaking, we do expect financing activity to slow as companies put to work all of the money that's been raised. .... Attention will shift away from the promise of companies to how well they execute."

DTET examined the most significant transactions of 2014 to identify trends that will impact the financial health of the industry in the upcoming year.

Continued on page 2

Trend Toward Panels, NGS for Breast Cancer Testing Seen at San Antonio Breast Cancer Symposium

Researchers are forging ahead on utilization of markers across the spectrum of breast cancer (BC) care. At the 2014 San Antonio Breast Cancer Symposium (SABCS; Texas; Dec. 9-13, 2014), researchers presented work showing:

- genetic screening should be expanded for women with certain BC subtypes, regardless of family history;
- multi-gene panels assessing hereditary cancer risk provide incrementally more clinically relevant information than BRCA analysis alone;
- markers are improving prognosis and identification of women who, even when diagnosed with early-stage disease, are at increased risk of recurrence or metastasis and would benefit from more aggressive treatment; and
- blood-based DNA markers identified in "liquid biopsies" improve monitoring for early signs of recurrence or progression.

Continued on page 8

'Solid' Year Expected for Diagnostics Industry in 2015, *from page 1*

Initial Public Offerings

Venture-backed life science initial public offerings (IPOs) far exceeded tech IPOs in terms of quantity in 2014, with the top 20 life science exits of 2014 valued at $12.1 billion, according to data from CB Insights.

"The number of IPOs completed this year has exceeded the most optimistic expectations and has been unparalleled in the history of this industry," says Burrill. "At some point, investors will grow concerned about the quality of offerings and raise the bar to go public. For now, though, companies ... will continue to take advantage of that opportunity as long as they can."

2014 Diagnostics IPOs

Company	Date	$ Raised
T2 Biosystems (Lexington, Mass.) The T2 Magnetic Resonance platform (T2MR) enables rapid detection of pathogens with initial development efforts targeting sepsis and hemostasis.	8/7	$57 million
CareDx (Brisbane, Calif.) The company has developed diagnostic surveillance solutions for heart transplant recipients, including its AlloMap blood test to predict acute cellular rejection.	7/17	$40 million
Roka Bioscience (Warren, N.J.) Roka has an automated system for rapid molecular testing for food safety testing.	7/16	$60 million
Signal Genetics (Carlsbad, Calif.) Signal is a molecular diagnostic company focused on personalizing cancer care. Its flagship product is MyPRS (Myeloma Prognostic Risk Signature).	6/17	$8.5 million
Quotient Diagnostics (United Kingdom) Quotient develops transfusion-related diagnostic assays and reagents.	4/24	$55 million
Genomic Vision (France) Molecular Combing is the company's proprietary single DNA molecule analysis technology, which provides an easy and accurate way to visualize large DNA rearrangements and can target specific sites at any position within the genome.	4/2	$27.6 million

Source: Compiled by G2 Intelligence and Burrill Report

Mergers & Acquisitions

In addition to the strong IPO market, investor exits through mergers and acquisitions (M&A) were robust in 2014, as witnessed by high deal flow between traditional diagnostics players, as well as with players from outside the industry. According to the Burrill Report, total M&A activity in the life sciences industry (therapeutics, tools, diagnostics, and digital health companies) for the first 11 months of 2014 reached a record $340.8 billion, up from $118.3 billion for the same period a year ago, even beating the previous $189.7 billion record set in 2009.

Perhaps the most prominent pharmaceutical acquirer of diagnostics companies was **Roche** (Switzerland). Throughout 2014, Roche made a series of acquisitions aimed at solidifying its position in the sequencing market. In mid-December, it announced its acquisition of the California-based bioinformatics firm Bina Technologies. Bina's proprietary software Genomic Management Solution (Bina-GMS) enables genomic data analysis. While financial terms were not disclosed, it is expected that Bina will be integrated into the Roche's sequencing unit in early 2015. Earlier in December, Roche announced another acquisition in the next-generation sequencing (NGS) space with the purchase of non-invasive prenatal testing company Ariosa Diagnostics (Harmony Prenatal Test). The sale price was not disclosed, but Ariosa earlier in the year had priced its IPO of 3.5 million shares at $16 to $18 per share, giving

> *"It is a good cycle. It is not as much money as we were seeing in 2006 and 2007, but we are overall seeing healthy venture investment."*
>
> *—Harry Glorikian, Boston-based life sciences consultant*

the company an estimated market capitalization of $323 million. Additionally, in October, Roche acquired NGS sample-preparation technology developed by AbVitro (Boston). The two companies will partner on applying the primer extension based target enrichment technology to the development of sequencing panels capable of performing directly from blood or other biological samples. These sequencing panels could be developed in partnership on platforms by both Pacific Biosciences (the Menlo Park, Calif. company previously announced a $75 million collaborative deal with Roche) and Genia Technologies (which Roche acquired in June). Genia is developing a next generation, single-molecule, semiconductor-based sequencing platform using nanopore technology. Roche paid $125 million upfront in cash to Genia, with additional payments milestone payments of up to $225 million.

One of the highest valued deals of 2014 was **LabCorp**'s (Burlington, N.C.) acquisition of Covance. The $6.1 billion deal, announced in November, allows LabCorp to diversify its revenue with Covances' testing base in the contract clinical trial and international markets. In a smaller deal, but one more representative of deals in the industry, bio-analytical testing firm Eurofins Scientific (Luxembourg) signed a definitive agreement to purchase Boston Heart Diagnostics Corporation (Boston Heart; a portfolio company of Bain Capital Ventures) for a deal valued up to $200 million (inclusive of $60 million in milestone payments). The deal with Boston Heart, a provider of diagnostics for cardiovascular health management, strengthens Eurofins' presence in specialty clinical testing markets, including genetic testing.

Private Diagnostic Investments

Investors are "flush," Burrill says from successful exits through IPOs and M&A deals. This will likely have a cyclical effect that experts say will drive investment into the diagnostic industry in 2015.

"It is a good cycle," Harry Glorikian, a Boston-based life sciences consultant, tells *DTET*. "It is not as much money as we were seeing in 2006 and 2007, but we are overall seeing healthy venture investment."

Data from CB Insights suggests that investment in the biotech/pharmaceutical industries was poised to hit a 6-year high by the end of 2014, surpassing the $4.6 billion raised by the industries in 2013, with experts predicting a "healthy and sustainable" year for 2015 as well. However the analysts that *DTET* spoke to suggest there may be some subtle shifts underway in private diagnostic investments, including higher-dollar placements in fewer companies, as well as strong interest in diagnostics companies in the big data and digital health space.

"Instead of spreading out $2 million or $3 million investments to a broad number of companies and hoping for one winner, investors are taking a larger swing and betting on taking one company to the next level," explains Glorikian. "It gives the executive team a chance to execute, rather than perpetually fundraise."

In a further breakdown of fundraising efforts, health technology accelerator StartUp Health says that, as of mid-December, genomic companies raised $632 million and diagnostics companies raised $962 million. Virtually all analysis finds though that these sizable investments are being placed in fewer companies. Below is a sampling

of 2014 transactions that typified this trend of more concentrated investments.

- In October, health care technology company **NantHealth** (a NantWorks company; Culver City, Calif.) raised a record $320 million. The NantHealth Clinical Operating System platform combines molecular science, near real-time patient signal monitoring, computer science, and big data technology. The company's new labs are expected to be operational in early 2015.
- **Invitae** (San Francisco), also in October, closed $120 million to expand its presence in genetic testing for hereditary disorders and to accelerate the build out of its genetic information business. The company has panels for hereditary cancer, cardiology, and neurology conditions, which it says can all be delivered for $1,500 or less in three weeks.
- Also in the top private diagnostic fundraising deals of the year was **Flatiron Health** (New York), which raised $130 million back in May. The company has what it calls the oncology industry's first cloud-based data platform that aims to provide a new standard for real-time insights and intelligence both for personalized care and research. The system aggregates both clinical and financial data from EMR and billing systems in real-time.

The highest fundraising companies in 2014 all partially encompass digital health. While the term is broad and can encompass wellness apps, experts say the diagnostics industry needs to take notice as advances in digital health will quickly blur the line between technology, communications, and traditional diagnostics.

According to data from CB Insights, the digital health industry has had explosive growth since 2009, with digital health projected to exceed $4 billion in fundraising in 2014, a 987 percent increase since 2009 and a 91 percent increase over 2013. Startup Health says, more specifically, the big data and analytics segment of digital health received $1.46 billion in 90 deals before the end of 2014.

"We need to seriously consider digital health as part of the diagnostics realm," says Glorikian. "The irony is that those making the biggest investments and innovations in digital health, like Qualcomm, aren't traditional players from our world. These may not be full-blown in vitro diagnostics, but there will be a blurring of the lines. You run a diagnostic to get information and physicians will not care if that information comes from a sensor or the blood."

Takeaway: The diagnostics industry is poised for another solid year of financial transactions in 2015. Subtle trends to look for are larger, private investments concentrated in fewer companies, as well as continued interest in digital health companies that blur the line of traditional diagnostics. G2

Other Large Venture Capital Diagnostics Placements

While falling well below the $100 million mark, several other diagnostic companies closed the year with large venture capital investments.

- **CardioDx** (Redwood City, Calif.), maker of the Corus CAD gene expression test, announced mid-December it had closed a $35 million financing round after withdrawing its planned IPO earlier in the fall, citing "unattractive" market conditions. The funds will be used to broaden commercial use of the test and to further additional research efforts.
- **Assurex Health** (Mason, Ohio) closed a $30 million round of financing from new and existing investors to support the clinical adoption and development of the firm's GeneSight neuropsychiatric pharmacogenomic tests. The company had announced earlier in the year that the Department of Veterans Affairs and Medicare both decided to the cover the test.
- Cancer diagnostics firm **Helomics** (Pittsburgh; formerly called Precision Therapeutics) announced the close of a $60 million round in November that it said will be used to expand its reach of personalized medicine services in genomics, proteomics, bioinformatics, and cellular analysis. The company offers a personalized reporting system for tumor profiling and predictive analytics to aid physicians in determining the most appropriate treatment.

Inside The Diagnostics Industry

With Flu Epidemic, Better Diagnostics Become Focus

While Ebola was the infectious disease grabbing most of the headlines in the last year, experts say it is actually the flu that should have us most worried domestically. In the closing days of 2014, the U.S. Centers for Disease Control and Prevention (CDC) officially declared that the flu reached epidemic status in the United States, with 6.8 percent of all deaths observed through the agency's 122 Cities Mortality Reporting System attributed to pneumonia and flu. Widespread activity was found in 36 states. While epidemic status is reached every year, what infectious disease experts find troubling is the trend toward earlier reports of flu intensity each of the past few years.

Differentiating the cause of the infection (bacterial or viral) can impact treatment decisions - whether ensuring appropriate antibiotic stewardship or providing the option of using antiviral therapy.

The high flu activity seen this season is the result of a confluence of factors: an early start to the flu season (the peak has typically occurred in February); relatively low vaccination rates (less than 50 percent of the population, despite recommendations for universal vaccination in everyone over six months of age); and a mismatch between this year's flu vaccine and the most prevalent circulating virus, influenza A (H3N2). This strain also tends to cause more severe illness than other variants. The last influenza A (H3N2)-predominant season in the United States was in 2012-2013.

Key to stopping the spread of any infectious disease outbreak is accurate diagnosis. Flu testing volumes can vary dramatically year to year and testing this flu season is expected to be in high demand. Driving the increased volume is, of course, high flu activity, but experts say that increased awareness of flu-like symptoms in light of the Ebola threat will further drive sick patients to seek care and testing.

Even before the beginning of the current flu season, experts began to reexamine the adequacy of current flu testing methods, raising concerns over the low sensitivity of rapid flu tests. In light of the bad flu outlook for the current season, *DTET* examined trends in flu testing and emerging tests that will shape testing in future seasons.

Differentiating Flu

Early symptoms of the flu are often clinically indistinguishable from other viral and bacterial causes of upper respiratory infections. Differentiating the cause of the infection (bacterial or viral) can impact treatment decisions - whether ensuring appropriate antibiotic stewardship or providing the option of using antiviral therapy. While the majority of patients recover from the flu without treatment, the growing number of antibiotic resistant pathogens is causing alarm and efforts are underway internationally to rein in inappropriate antibiotic use.

Acute respiratory infections are by far the most common reason for prescribing an antibiotic in primary care, even though the majority of these infections are caused by viruses, which will not respond to antibiotic treatment. Researchers recently found, though, that use of a point-of-care biomarker test in the primary care setting can cut inappropriate antibiotic use. In a meta-analysis, published Nov. 6, 2014 in the *Cochrane Library*, Danish researchers assessed six randomized trials (involving 3,284 predominantly adult patients) of a point-of-care test for C-reactive protein (CRP). Although, CRP is a non-specific test of inflammation, it can indicate a serious bacterial infection.

The researchers found that 631 out of the 1,685 people tested were prescribed antibiotics, compared to 785 out of the 1,599 people not tested. The authors caution that differences in

Inside The Diagnostics Industry

study design precluded a precise effect estimate in the reduction of antibiotic use, but they did find that cuts in antibiotic use did not affect patient-reported outcomes, including recovery from and duration of illness, or patient satisfaction with their care.

While the results do show that rapid, point-of-care tests can positively guide the appropriate use of therapies, there has been increasing concern in the United States regarding the accuracy of rapid influenza diagnostic tests (RIDTs).

Rapid Flu Tests

The use of commercial RIDTs has increased substantially in recent years, with a dozen products currently on the market, according to the U.S. Food and Drug Administration (FDA). These tests can be performed in a doctor's office or clinic and conveniently provide results within 15 minutes. They rely on enzyme immunoassay technology to detect antigens to the seasonal flu virus in the patient sample, but RIDTs vary in terms of sensitivity and specificity. According to data collected by the CDC, when compared with reference standards for flu testing (viral culture or reverse transcriptase polymerase chain reaction (RT-PCR)), RIDT sensitivities range from 50 percent to 70 percent, while specificities are greater than 90 percent, yielding more false negative results than false positive results, especially during peak influenza activity. Experts say though, that there can be serious consequences in some individuals whose cases of flu are missed by these tests.

To address these sensitivity concerns, the FDA is proposing to reclassify RIDTs from class I devices to class II with special controls. The flu viruses' ability to frequently mutate and the new strains, the FDA says, "potentially affect the performance of these devices." By reclassifying the tests, the FDA can require additional ongoing regulation. The FDA's Microbiology Advisory Panel made four recommendations, laid out in the proposed rule published in the Federal Register in May 2014. These recommendations include: setting minimum sensitivity criteria; identifying the best "comparator" test to determine RIDT accuracy of RIDTs (would likely include newer molecular-based assays); annual reassessments of device accuracy based on the latest influenza viruses in circulation; and subject the device immediately to novel flu strains in emergency situations (i.e., the 2009 H1N1 pandemic).

Flu Testing Methods	Test Time	CLIA-Waived
Viral cell culture*	Conventional - 3 days to 10 days Rapid - 1 day to 3 days	No
Immunofluorescence antibody staining (not recommended)	1 hour to 4 hours	No
Molecular assays* (including RT-PCR)	1 hour to 6 hours	No
Rapid diagnostic tests (antigen testing)	15 minutes or less	Yes

*Reference standard for laboratory confirmation. Source: Adapted from the CDC

Emerging Technologies

Back in June, Alere (Waltham, Mass.) received the FDA's first clearance of a rapid molecular flu test, delivering results within 15 minutes. The Alere i Influenza A & B test uses isothermal nucleic acid amplification for viral detection, eliminating the need for thermal cycling and sample purification steps, enabling highly accurate, molecular results in less than 15 minutes. In trials, the test delivered PCR-comparable results. The Alere i Influenza A & B test received the FDA's first CLIA waiver for a nucleic acid-based flu diagnostic. The company's Strep A test for the Alere i platform is under FDA review, while a respiratory syncytial virus (RSV) assay is in development.

Inside The Diagnostics Industry

Alere feels very well positioned to capitalize on shifting flu testing needs. "Knowing now matters," the company's vision to provide reliable, actionable results when and where they are needed most, encapsulates many of the driving forces reshaping flu testing, Keith Stauffer, vice president of marketing for rapid diagnostics at Alere, tells *DTET*. Rapid molecular flu testing addresses many of the factors reshaping flu testing, including the FDA's potential reclassification of rapid tests, the need to initiate antiviral therapy within 48 hours of symptom onset, and calls for better antibiotic stewardship. But, Stauffer cautions that "the shift towards molecular will be an evolution, not a revolution."

The need for rapid results closer to the patient is also driving development of a novel, low-cost POC testing platform at OJ Bio (United Kingdom). While the immunochemistry platform is capable of detecting a broad range of pathogen targets, the company is initially focusing on flu and respiratory virus detection.

The wireless diagnostic system is a joint venture between Japan Radio Company and Orla Protein Technologies. The biosensor at the core of the device combines a surface acoustic wave (SAW) electronic chip coated with disease-specific biocapture surface coating. The biochip is held in a disposable cartridge and used in conjunction with a low-cost, handheld reader. The company says that the presence of a disease antigen causes a shift in the phase angle of the SAW passing across the chip surface and this is translated into an electronic signal. Bluetooth connection of the reading device to special diagnostic software enables the test results to be displayed within seconds.

The three-channel chip is capable of assessing multiple analytes on one cartridge. The company has validated the chip for Flu A/B and RSV, which delivered performance characteristics comparable to PCR, in a study of retrospectively collected samples. Dale Athey, Ph.D., managing director at OJ Bio, tells *DTET* that the device brings better performance than lateral flow devices (as well the value-added capability of providing quantitative results). Given the electronics component, it will not be as low of a cost test as lateral flow, but the company is aiming for a $30 device with per test costs ranging from $2 to $10 depending on the application. OJ Bio is now in final product development of a single cassette and plans to commence prospective clinical trials in 2015 and bring the device to market within the next two years.

2014 FDA Approved Flu Tests

Company	Test Name	Date
Focus Diagnostics (Quest)	Simplexa Flu A/B & RSV Direct	12/5
Cepheid	XPERT FLU/RSV XC Assay	11/22
Quidel	Parainfluenza Multiplex Nucleic Acid Assay	10/9
CDC	Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping	8/1
Alere	ALERE I Influenza A & B	6/13
Meridian Bioscience	TRU FLU, a rapid, qualitative, lateral flow immunochromatographic assay for detecting both influenza A and influenza B	1/3

"In the United States the whole mix of telephone, electronics, and life science companies is exploding right now from the testing view," says Athey. "There is strong interest on the consumer-side and those on the clinical-side to wake up to this idea that people want to understand their own health and desire the technology to do this themselves."

Takeaway: Companies are developing innovative solutions to address evolving flu testing needs, with the goal of improving sensitivity and bringing quick results to care providers closer to the point of care.

Trend Toward Panels, NGS for Breast Cancer-Associated Testing, *Continued from bottom of p.1*

Two trends underlying these advances in understanding breast cancer risk assessment and prognosis that will impact future BC-associated testing are the shift to panel-based testing and the employment of next-generation sequencing (NGS) technology. A year and a half after the Supreme Court struck down Myriad's exclusive patent on the BRCA gene, it is evident that many laboratories are pushing ahead with multi-gene panels to assess hereditary risk of BC, even while researchers continue to grapple with the clinical significance of lesser-studied variants and work to identify improved parameters for selection of patients for genetic testing.

Expansion of Hereditary Screening

Patients with triple-negative BC (TNBC) should receive BRCA1/2 testing, regardless of age at diagnosis or family history of cancer, according to a study published online Dec. 1, 2014 in the *Journal of Clinical Oncology* and presented at SABCS.

The Mayo Clinic researchers assessed the frequency of mutations in 122 DNA repair genes, including 17 BC predisposition genes using sequencing in 1,824 patients with TNBC, unselected for family history of breast or ovarian cancer. The researchers found that 271 deleterious mutations were identified in 14.6 percent of all patients. BRCA 1/2 accounted for 57 percent and 18 percent of deleterious mutations, respectively, while 25 percent of the deleterious mutations were found in 12 of the 15 other predisposition genes. The authors say that the prevalence of mutations in the non-BRCA1/2 predisposition genes was stable across all age groups and reported cancer family histories, consistent with lower penetrance of disease for mutations in many of these genes.

"Because a relatively high proportion (7.5 percent) of patients with TNBC with no family history and diagnosed between age 50 and 60 years had mutations, perhaps testing ... all patients irrespective of age or family history, should be considered," writes lead author Fergus Couch, Ph.D., from the Mayo Clinic (Rochester, Minn.).

Couch and colleagues caution that while expanding screening beyond existing guidelines (such as the United Kingdom's National Institute for Clinical Excellence) will identify more carriers, the clinical utility of lower penetrance mutations remains debatable. Clear clinical management guidelines for BRCA1/2 mutation carriers have been developed over the two decades since the genes were identified, but management guidelines for the other predisposition genes are lacking, making the results from broader hereditary gene panels "controversial."

But, other abstracts presented at SABCS are demonstrating emerging evidence of the value of this additional clinical information, in spite of the higher associated rates of variants of unknown significance.

Multi-Gene Hereditary Cancer Risk Panels

Multi-gene hereditary cancer tests deliver comparable performance to traditional BRCA genetic testing, with potentially additional clinically beneficial information. The 29-gene panel from Invitae (San Francisco) increased the yield of findings with potential clinical impact for almost 8 percent of patients, over BRCA testing alone.

In addition to guideline-directed BRCA testing, 821 patients were also tested using a panel of 29 known cancer risk-associated genes that assessed sequence

Liquid Biopsies for Breast Cancer Monitoring

Researchers from Dartmouth-Hitchcock Medical Center (Lebanon, N.H.), Thomas Jefferson University (Philadelphia), Erasmus Medical Center (Netherlands), and the Translational Genomics Research Institute (Phoenix) presented studies at SABCS highlighting the potential of circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) as a liquid biopsy for ongoing molecular monitoring of BC.

"We believe our ability to assess cancer DNA in a non-invasive liquid-biopsy sample to monitor the genomic alterations as they occur in response to therapy or simply over the course of the disease will transform the way cancer is treated, monitored, and managed," said Steven Shak, M.D., executive vice president of research and development at Genomic Health, which expects to have a commercially available liquid biopsy-based test in 2016.

Biocept (San Diego) presented data showing the ability of its CTC capture method to identify how a patient's breast cancer can evolve over time and necessitate alterations in the optimal course of treatment. In the study, conducted at Farber Cancer Center (Boston), 22 percent of 311 patients with disease progression, who were previously HER2 negative based on solid tumor biopsy, were subsequently identified as HER2 positive (and eligible for anti-HER2 therapy) using CTC analysis.

Separately, researchers in partnership with Guardant Health (Redwood, Calif.) isolated and analyzed ctDNA for 54 mutations in 35 patients with inflammatory BC (IBC), who failed standard treatment. Based on testing using Guardant360, 94 percent of patients with stage III or IV tumors had identifiable ctDNA alterations (TP53, PIK3CA, ERBB2, NOTCH1, and ALK). The genomic information obtained from ctDNA, NGS (12 patients had NGS analysis of tissue biopsy), or both, was used to select treatments in 11 cases (31 percent), with some evidence of improved outcomes from ctDNA-associated treatment changes.

A number of ongoing studies are trying to further ascertain: the extent of genetic heterogeneity between primary and metastatic tumors and how reflective plasma DNA markers are of these mutations; the role of ctDNA/CTCs in predicting specific treatment response; and ultimately, the association between ctDNA/CTC levels with patient outcomes.

changes and deletions/duplications. The researchers (some of whom report financial ties to Invitae) found that 13.8 percent of the patients carried BRCA1 or BRCA2 mutations, with greater than 99 percent concordance between the traditional and panel results. Among BRCA-negative patients, 7.6 percent carried mutations in other cancer risk genes that confer a moderately increased risk for breast and ovarian cancer, as well as genes involved in Lynch syndrome (which is not uniformly accepted to be associated with breast and ovarian cancer).

The authors, led by Leif Ellisen, M.D., from Massachusetts General Hospital (Boston), say that the panel identified nearly 40 percent more patients with a deleterious mutation, compared to results reported with BRCA testing alone. Nearly 80 percent of these non-BRCA mutations are not incidental findings, the authors say, and 70 percent of these mutations would "potentially warrant a change" in care for the patient or his or her family, including additional testing or screening and in a few cases, surgical interventions.

Stephen Lincoln, Ph.D., from Invitae, in a second abstract says that while concordance is high between the panel and traditional BRCA testing, the details of interpretation are "hampered by the limited reporting of proprietary data by some established laboratories." Lincoln says that efforts to establish large public databases, like ClinVar, "will promote greater transparency and accountability and thus can help improve access to high quality care for hereditary conditions."

Prognosis Predicting Panels

There is also a growing body of evidence that multi-gene panels, coupled with proprietary algorithms, can positively guide treatment decisions by identifying patients at greatest risk of recurrence that would benefit from more aggressive treatment regimens as well as those at low risk of recurrence who could be spared excessive treatment.

In what is one of the first prospective trials utilizing a predictive genetic panel, Genomic Health (Redwood City, Calif.) presented early results from a European study supporting the use of Oncotype DX to guide treatment decisions. The Oncotype DX breast cancer test, which analyzes the RNA expression of a panel of 21 genes (16 cancer genes and five reference genes) from a tumor sample using reverse transcriptase-polymerase chain reaction, was used to identify higher-risk patients who would benefit from the treatment. In the study of 3,198 patients, most patients qualified for chemotherapy under traditional parameters. However, patients with Oncotype DX Recurrence Scores of 12 or higher were randomized to one of two different chemotherapy regimens, while patients with Recurrence Scores of 11 or less (low score) were offered hormonal therapy alone. After

nearly three years of follow-up, patients with low Recurrence Score had very high survival rates without evidence of recurrence (98.3 percent), despite having node-positive disease or high-risk node-negative disease by traditional parameters. Researchers will continue to follow patients to assess longer-term outcomes.

Takeaway: While the search is on for additional markers for early-stage diagnosis of BC and those predictive of metastasis, progress is being made in the use of panels to identify BC patients who would benefit from more aggressive treatment. Additionally, panels are being employed for assessment of risk of hereditary BCs, despite limits in understanding the clinical significance of low penetrance variants. G2

Blood Markers Could Determine Necessity of CT With Mild TBI

A blood test may be able to diagnose patients with a concussion or traumatic brain injury (TBI), cutting the need for a head CT, according to a study published Nov. 15, 2014 in the *Journal of Neurotrauma*. Glial fibrillary acidic protein (GFAP) seems to be a brain-specific marker of trauma and out performed S100β in predicting intracranial lesions on CT, particularly in patients with non-head-related fractures.

Lead author Linda Papa, M.D., tells *DTET* that GFAP, a relatively new marker associated with TBI, is a protein that "spills out" of glial cells in the brain into cerebrospinal fluid and blood with brain injury. By contrast, Papa says S100β (a major low-affinity calcium binding protein in astrocytes) is the most well-studied biomarker for TBI, but is not sufficiently brain-specific and rises in patients with non-head bone injuries.

"GFAP is loyal to the brain even in the face of other fractures," says Papa, from Orlando Regional Medical Center and the University of Central Florida.

Papa and colleagues assessed the ability of GFAP and S100β to predict the presence of traumatic intracranial lesions on CT scan (n=262 patients) in a convenience sample of adult trauma patients with and without mild or moderate TBI (without 47 percent). Serum samples were obtained within four hours of injury. Samples were analyzed in batches using sandwich enzyme-linked immunosorbent assays.

Both GFAP and S100β rapidly appeared in serum post-injury, with levels detectible within an hour of injury. S100β appeared most elevated within the first two hours, while GFAP levels remained steady over four hours. The researchers found that levels of both markers were significantly higher in those with lesions on CTs. But GFAP levels were significantly higher in those with intracranial lesions, while S100β was unable to discriminate between intracranial and extracranial lesions on CT. In the presence of fractures, GFAP had a specificity of 55 percent, compared to 5 percent for S100β.

The potential market for a diagnostic test is "large," Papa says with two percent of all emergency room visits due to TBI. Additionally, such a test, if adapted for the point-of care, could be used on the field or locker room in sports settings. Papa says she is currently working with the U.S. Food and Drug Administration and several companies interested in commercializing a TBI test. In addition to reducing the use of CT, especially in young patients, the use of markers may provide a more sensitive diagnosis than can be made on CT, enabling earlier diagnosis and treatment.

Several study authors report financial ties to Banyan Biomarkers (Alachua, Fla.), which is working to develop a point-of-care test to diagnose TBI.

Takeaway: The use of biomarkers may improve definitive TBI diagnosis and could potentially reduce the need for head CT and associated radiation exposure, particularly in young patients. G2

Electricity-Free Amplification May Expand Molecular POC Testing

The need for molecular point-of-care (POC) tests is perhaps greatest in low-resource settings, where logistical constraints, including unreliable electricity make most current molecular testing technologies unfeasible. Electricity-free, non-instrumented nucleic acid amplification (NINA) is nearing a reality with the development of a platform by researchers at the nonprofit PATH (Seattle), according to a study published online Nov. 26, 2014 in *Plos ONE*.

The group further showed that the heater can be paired with complementary, instrument-free technologies, such as a biplexed loop-mediated isothermal amplification (LAMP) assay and visual endpoint detection with nucleic acid lateral flow (NALF) and applied to the detection of HIV. The authors say that improvements over previous design iterations bring the technology from the "proof-of-concept stage to an optimized, robust alpha prototype."

By bringing molecular testing for infectious diseases closer to the site of patient care researchers hope to overcome challenges with patient follow-up while improving upon the sensitivity of over-the-counter antibody-based tests by enabling detection of infections in the very early stages of disease.

The electricity-free, self-contained NINA system uses an inexpensive insulated thermos where the source of heat is a small-scale chemical reaction, rather than electrical power. In the latest iteration, the researchers utilize magnesium iron alloy for the exothermic reaction due to its high energy density and low cost ($.06 per test for heater reaction materials). The researchers demonstrate that the heater design has a thermal standard deviation less than 0.5 degrees C at operating temperature, which can range from an ambient temperature of 16 degrees C to 30 degrees C.

While the platform design is pathogen-agnostic, the researchers demonstrated the utility of the electricity-free molecular amplification and visual detection system using HIV-1 detection as a model analyte. A biplexed LAMP assay detected HIV-1 infection (and ß-actin for internal amplification control) with processed sample to result in less than 80 minutes. One outstanding need the authors hope to address is the need for "appropriate" sample preparation methods. The complete system, the authors say, will enable infectious disease case detection and surveillance well beyond centralized laboratories, at lower levels of the health care system.

Senior author Paul LaBarre, a senior technical officer PATH, tells *DTET* that the team will next focus on integrating the amplification hardware and the lateral flow detection hardware into a single disposable device. Additionally, they are working with collaborators to "optimize" resilient polymerase enzymes that enable reverse transcriptase amplification of unpurified samples.

LaBarre says that the commercialization timeline will be dictated, in part, by the need for additional funds. The general distribution plan will be disease specific, he says, with non-exclusive licenses for multiple isothermal methods and for multiple diseases likely. While the initial focus is sub-Saharan Africa and Southeast Asia, LaBarre envisions one day having a CLIA-waived molecular test available in local pharmacies or grocery stores.

Takeaway: By coupling new electricity-free nucleic acid amplification methods with complimentary, instrument-free assays and visual detection endpoints, point-of-care molecular testing for infectious diseases will be feasible in low resource settings.

G2 INSIDER

Rapid Pathogen Identification Improving in Critically Ill

There has been improvement in rapid diagnostic techniques for identifying infections in critically ill patients over the last decade, according to a review study published Nov. 28, 2014, in *BioMed Central Infectious Diseases*. However, there are still pressing improvements needed to expedite diagnosis, especially among drug resistant strains and to widen the spectrum of identifiable pathogens and the sample types tests can be run on.

Given the well-documented findings that infections in ICU patients are often deadly "rapid etiologic microbiological diagnosis is mandatory," the authors write. The Spanish researchers conducted a systematic literature search of peer-reviewed publications published between 1995 and 2014 to evaluate the evolution of diagnostics for common ICU infections, including bloodstream infection (BSI), and ventilator-associated pneumonia (VAP).

Diagnosis of sepsis remains a "major challenge," the authors say with no specific marker available to determine a true diagnosis of sepsis. While time-consuming blood cultures are still considered the gold standard for diagnosis, they are currently being used in conjunction with molecular tests. The researchers found that molecular technologies are improving diagnosis and impacting clinical decision-making, with results ideally produced within 6 hours, but still have some notable shortcomings, including the lack of an appropriate gold standard, as well as the need for some expertise.

"Although there are still some unresolved limitations of the use of molecular techniques for a rapid diagnosis of infection in the ICU patient, this approach holds much promise for the future," writes co-author Almudena Burillo.

Suggested areas of future improvement of molecular tests include the need to improve sensitivity to detect clinically relevant low bacterial loads and fastidious microorganisms and to distinguish between living and dead bacteria. For identification of isolated colonies, matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry can be run directly on positive blood culture broths in under one hour and is now replacing biochemical and gene sequencing methods for organism identification.

For VAP bacterial identification and antibiotic susceptibility testing can still take two days to four days. Molecular techniques are needed, the authors say, that can detect multiple microorganisms or resistance mechanisms directly on clinical samples before cultures are available. By directly subjecting clinical samples to PCR (GeneXpert, Cepheid) the authors have shown "high diagnostic efficiency" and can shorten the time to adequate antibiotic treatment, although the kit has not received regulatory approval for this purpose. A definitive marker for VAP diagnosis is also lacking, although evidence is demonstrating that procalcitonin may be a good prognostic marker, with elevated levels indicating a more severe clinical course.

Company References

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 Diagnostic Testing and Emerging Technologies (ISSN 2330-5177) is published by G2 Intelligence, Plain Language Media, LLC, 15 Shaw Street, New London, CT, 06320. Phone: 1-888-729-2315 • Fax: 1-855-649-1623. Web site: www.G2Intelligence.com.

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