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Diagnostics Companies Responding to Ebola Crisis

Could a rapid diagnostic test to identify Ebola have prevented the first cases of Ebola transmission in the United States? While it is too late to know for sure, diagnostics manufacturers, federal U.S. defense and health agencies, and regulatory bodies are aggressively working to leverage their current assets to help contain the global epidemic.

While Thomas Eric Duncan's travel history was a missed opportunity to provide earlier isolation and care, administrators at Texas Health Presbyterian Hospital in Dallas say that the lack of an accurate, rapid diagnostic hampered efforts to detect and diagnose the virus.

The current gold standard for Ebola diagnosis relies upon polymerase chain reaction (PCR) testing. There are currently no U.S. Food and Drug Administration-approved or -cleared tests for Ebola, but the FDA, as of Nov. 1, has given emergency authorization for use of five Ebola tests. The most recent emergency approvals were two assays developed by BioFire Defense (Salt Lake City; a BioMérieux company). The company's one-hour FilmArray Biothreat-E test is

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Shift Toward Comprehensive Marker Evaluation, Universal Screening for Breast Cancer Risk Assessment

Paralleling trends in other clinical areas, breast cancer risk testing is transitioning from single-marker testing to more comprehensive analysis. This broadened focus extends to both wider analysis of BRCA mutations and multigene panels, as well as the potential use of comprehensive sequencing to thoroughly assess a woman's breast cancer risk. Expansion of the scope of testing comes amid other calls to scale BRCA screening to include all women, regardless of familial cancer history, as part of routine medical care.

Previous estimates show that of the 5 percent to 10 percent of hereditary breast cancer cases, only about one-fourth involve single-gene conditions. BRCA1/2 are the most notable of the known genes conferring a higher breast and ovarian cancer risk. But there are other recognized high-risk cancer genes associated with other cancers in addition to breast and ovarian cancer, such as PTEN, p53, CDH1, and STK11. There are also moderate- to low-penetrance breast cancer genes (PALB2, CHEK2, ATM) that are being incorporated into multigene panels.

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The challenge posed with incorporation of these genes into analysis is a lack of consensus around guidelines for how to provide ongoing management for patients testing positive for these gene mutations.

Further fueling debate over the best way to assess breast cancer risk was the call this fall for preemptive, universal BRCA screening by a prominent researcher. While the downside of population-based screening is the concern that screening may increase unnecessary, invasive diagnostics and overtreatment, the hope is that expanded screening may identify women with higher genetic risk not eligible for screening based on family history.

Calls for Universal BRCA Screening

Mary-Claire King, Ph.D., from the University of Washington, Seattle, who was awarded the 2014 Lasker-Koshland Special Achievement Award in Medical Science in part for her discovery of BRCA1, says that it is time to offer universal BRCA1/2 testing to all women, and that other genes could be phased into the screening process as evidence justifies.

"To identify a woman as a [BRCA] carrier only after she develops cancer is a failure of cancer prevention," write King and colleagues in a Sept. 17 editorial published in the *Journal of the American Medical Association*. They say that genetic screening of every woman, at roughly age 30 as part of routine medical care, will better identify high-risk women who would benefit from special screening and preventive measures. They say this marked departure from current practice and U.S. Preventive Services Task Force recommendations is necessary in light of new research showing that general screening can identify many carriers not eligible for screening based on family history.

> ***"To identify a woman as a [BRCA] carrier only after she develops cancer is a failure of cancer prevention."***
> ***—Mary-Claire King, Ph.D.***

This new evidence (of which King was a co-author) was published in the Sept. 30 issue of the *Proceedings of the National Academies of Science*. The researchers identified 175 male BRCA1/2 mutation carriers and offered genetic testing to all of their female relatives. These men were viewed as a "gateway to families," since the men were not affected by breast cancer and the subsequently screened women were not identified based on a personal or family cancer history. Since the index men were all healthy, Ashkenazi Jews, screening was limited to the three loss-of-function mutations most common in this population.

The researchers found that, as expected, those with BRCA mutations had very high cancer risks. For BRCA1 mutation carriers, the combined risk of developing either breast or ovarian cancer ranged from 60 percent by age 60 to 83 percent by age 80, while for BRCA2 carriers the risk was 33 percent by age 60 and 76 percent by age 80. Importantly, the authors say that 50 percent of families identified carrying BRCA1 or BRCA2 mutations had no history of breast or ovarian cancer that would have triggered genetic screening. Yet female mutation carriers from these low-cancer-incidence families had cancer risks that were similar to female carriers from families with high cancer incidence.

The big question is, are these results applicable to more diverse populations, as in the United States? King and colleagues say yes, despite the additional number of cancer-predisposing BRCA1/2 mutations present in the U.S. population.

"Testing for BRCA1 and BRCA2 should focus solely on unambiguously loss-of-function mutations with definitive effect on cancer risk," writes King, acknowledging the challenge posed by other variants of unknown significance (VUS). "A VUS can increase confusion and compromise clinical management; for population-based screening, these variants should not be reported. . . . If any VUS ultimately proves causal for breast or ovarian cancer, it should be integrated into future testing. Meanwhile, waiting for a perfect test denies women excellent resources that are now available."

Will Sequencing-Based Screening Better Identify At-Risk Women?

As it is believed that the accuracy of breast cancer risk assessment may be improved with an examination of a larger number of genetic markers, some are wondering whether universal screening via sequencing may become an increasingly cost-effective strategy to identify and then target the women who would benefit from aggressive preventive efforts.

A study published online Oct. 23 in *Cancer Epidemiology, Biomarkers, & Prevention* found that personalized breast cancer preventive strategies based on genome sequencing will bring greater gains in disease prevention than previously projected.

"The main takeaway message is we can be more optimistic than previously predicted about the value of genomic sequencing," Alice Whittemore, Ph.D., from Stanford University, said in a statement. She tells *DTET* that comprehensive panels are currently being developed and are in the early stages of use by her colleagues in high-risk cancer clinics with the hope that improving risk assessment can lead to personalization of breast cancer prevention strategies.

The Stanford researchers developed a computational model to estimate a woman's lifetime probability of developing breast cancer. This risk score was calculated as the sum of the breast cancer-related genetic variants a woman carries multiplied by the effect of the variants. The group used published data (allele frequencies and effect size) for all 86 breast cancer susceptibility variants known at the time of the study (the number of identified variants has since increased).

They estimate that using those 86 variants, the risk score for the population as a whole is 0.35, higher than a previous study's estimated risk score of 0.07 derived using the seven loci known in 2008. The researchers say that targeting those in the top 25 percent of the risk distribution using sequencing of the currently known genetic alterations would identify approximately half of all future breast cancer cases, compared to estimates of 35 percent of future cases identified based on the 2008 variants.

"As we keep identifying additional breast cancer variants that can further explain the difference between my risk versus yours, the variance of the genetic risk score in the population will increase, and the potential utility of genomic sequencing will grow," said lead author Weiva Sieh, M.D., Ph.D., in a statement.

Takeaway: Researchers are evaluating the most cost-effective means to assess women's breast cancer risk with increasing emphasis on comprehensive mutational analysis and possibly routine, universal screening as part of wellness care.

Quest Extends BRCAvantage

At the beginning of November, Quest Diagnostics (Madison, N.J.) announced it has extended its BRCAvantage lab-developed test initially launched back in October 2013 for assessing genetic breast cancer risk. The BRCAvantage Plus now screens for mutations (point mutations, deletions, and duplications) in the BRCA1 and BRCA2 genes as well as in five additional genes: TP53, PTEN, CDH1, STK11, and PALB2.

Quest says that the test can be ordered as single-gene tests, a comprehensive panel, or with a reflex option, which tests the non-BRCA genes if BRCA1/2 results are negative.

The company cited data that the addition of the five non-BRCA genes account for an additional 3 percent to 4.5 percent of inherited breast cancers in addition to the 15 percent to 20 percent of inherited breast cancers accounted for by mutations in the BRCA1/2 genes.

▲ **Diagnostics Companies Responding to Ebola Crisis,** *from page 1*

now cleared for emergency commercial use, as is a second assay (FilmArray NGDS BT-E Assay) that can be used only by laboratories designated by the Department of Defense (DOD).

"It would have taken years to get this product approved through the traditional process," said Kirk Ririe, BioFire's CEO, in a statement. The assay is run on the FDA-approved BioFire FilmArray system. The company says there are approximately 300 of the $39,000 machines in commercial use in high- and moderate-complexity clinical laboratories. The company flew one out to Bellevue Hospital (New York City) where a U.S. doctor returning from West Africa is being treated for the virus.

The other assays approved for emergency use were developed by government agencies—the U.S. Centers for Disease Control and Prevention's (CDC's) Ebola Virus NP Real-time RT-PCR Assay and the Ebola Virus VP40 Real-time RT-PCR Assay, as well as the DOD's EZ1 Real-time RT-PCR Assay. The DOD's Ebola Zaire Target 1 real-time PCR assay—developed using Life Technologies' TaqMan series of assays, can be run on several analyzers, including Roche Diagnostics' LightCycler, the JBAIDS system by BioFire Diagnostics, and the Applied Biosystems 7500 (under the Life Technologies brand of Thermo Fisher Scientific).

While the DOD's assay is based on a single ribonucleic-based target, it is reported that the department is working on a rapid diagnostic using the xMAP Technology and instrument (Luminex; Austin, Texas). Some analysts believe the DOD has an interest in developing a high-capacity multiplexing system, likely with the ability to simultaneously test for Ebola as well as other endemic conditions in West Africa, like malaria and other hemorrhagic viruses.

Luminex had previously announced that it was supporting the U.S. Army Medical Research Institute of Infectious Diseases' (USAMRIID) diagnostics division to develop rapid diagnostics for the Ebola virus. USAMRIID is using the Luminex xMAP technology and MAGPIX instrument. Unlike the DOD's ribonucleic acid assay, this work focuses on testing serum samples for the presence of viral antigens as well as antibodies directed at these antigens. Luminex says its MAGPIX instrument has been deployed to Africa to support research efforts. Separately, with the support of a four-year National Institutes of Health (NIH) grant, Luminex, along with academic partners, is nearing the completion of development of a multiplex immunoassay

PCR-Based Ebola Diagnostic Efforts

Diagnostics companies both big and small are looking to apply their existing technologic capabilities to tackle the Ebola diagnostics challenge. Below is sampling of companies that have reported working on other PCR-based solutions:

- **Alere** (Waltham, Mass.) has begun working to add an Ebola assay for its fully automated nucleic acid Alere q testing platform, which has been launched in multiple developing countries.
- **Cepheid** (Sunnyvale, Calif.) had previously conducted work on in-field nucleic acid assays for the Ebola, Marburg, and Lassa fever viruses for the Canadian government utilizing their automated GeneXpert platform. Although the assay was never fully developed or commercialized, the company is seeking funding to revitalize this program and move toward rapid emergency regulatory clearance. The company's previous experience and the safety component of the closed GeneXpert system put the company in an advantageous position, Cepheid's CEO John Bishop said on an October earnings call. He added that with the additional funding for incremental development, the company could have a prototype available for evaluation within eight weeks.
- **Roche** (Basal, Switzerland) has a PCR-based Ebola test currently labeled for research use only. Roland Diggelman, the company's chief operating officer for the diagnostics division, said on an October earnings call that the company is in negotiations for submission for emergency use authorization by the FDA and also with the World Health Organization (WHO) for prequalification of the test. Test results take two hours and it is possible to run around 480 samples per day on a single Roche LightCycler machine.
- **PositiveID** (Delray Beach, Fla.) believes its handheld Firefly Dx real-time PCR system can deliver results in under 20 minutes at the POC. The company, in early November, signed a research and development agreement with the DOD's Special Operations Research, Development, & Acquisition Center, Science & Technology Directorate, to further develop the Firefly Dx system for use across the its mission space.

Ebola test. The company is assessing whether to seek emergency clearance status but cautions that the test has only been used in simian models to date.

"One reason I see that the country is not further along in Ebola diagnostic efforts is inadequate government funding," Amy Altman, vice president for biodefense at Luminex, tells *DTET*. She notes that given decreases to infectious disease research funding over the past 10 years and the "miniscule" size of the Ebola diagnostic market before the present outbreak, there was not a business case to justify development. "Companies involved in biothreat work were primed to respond to this Ebola outbreak because Ebola has been listed as a potential biothreat for years, but in a larger way if we are to be prepared for the next emergence of a neglected tropical infectious disease that may not necessarily be a biothreat, we will need greater federal sustainment funding."

POC Tests Also Needed

While PCR is the dominant technology for Ebola testing in laboratories, PCR-based tests are not practical in settings requiring point-of-care (POC) testing, such as in airports. Additionally, in resource-constrained areas such as in Africa, cutting the time to run a PCR test by hours does not address greater infrastructure and capacity issues such as getting the sample to the lab, which can take hours, if not days.

The Foundation for Innovative New Diagnostics (FIND) says that key to stopping virus transmission are improvements in diagnostic capabilities. The group says that most of the testing done to date in Africa is carried out in a small number of mobile laboratories or in centralized facilities. The turnaround time for testing is close to six hours for patients located in the same town as the laboratory but can be three days for patients living in neighboring towns or districts.

Given the cost and difficulty of testing (including the need for personal protective gear to collect samples), diagnostics are largely used to confirm disease in a smaller group of patients with more advanced disease. Current rates of case confirmation, FIND says, are relatively high in Guinea and Sierra Leone (75 percent to 90 percent) but are substantially lower in the hardest hit country of Liberia (30 percent), where laboratory services can't keep pace with the caseload.

In addressing these unique needs, researchers are balancing the need for rapid, POC results with the need for sensitivity. Typically, rapid POC tests have not been able to achieve the sensitivity of PCR-based tests. But in this case, accuracy is of paramount importance to stopping the spread, and false positives or false negatives could further transmit the virus with deadly impact.

Takeaway: While the combination of a lack of urgency and financial incentives may have hampered full development efforts of Ebola diagnostics in the past, U.S. diagnostics manufacturers are now scrambling with governmental partners to leverage their core technologies to develop rapid PCR tests and POC diagnostics to combat the Ebola epidemic.

POC Ebola Test Development

Several American companies are working on applying their technology to POC Ebola testing. Corgenix (Denver) has a longstanding relationship with the hemorrhagic fever group from Tulane University (under Robert Garry, Ph.D.) and the Viral Hemorrhagic Fever Consortium. The group was recently awarded a three-year, $2.9 million NIH grant to continue work on the development of a rapid, recombinant diagnostic test for Ebola. The dipstick-type test is based on lateral-flow technology.

OraSure Technologies (Bethlehem, Pa.) confirmed to analysts in mid-October that the company had initiated conversations with the FDA, WHO, and the CDC regarding applying their POC oral-fluid-based platform to the detection of Ebola. On an October earnings call, CEO Douglas Michels said the company is well positioned to extend its technology and hopes to reach key research milestones regarding adaptation of the technology to Ebola by the end of the year.

Trovagene Looks to Transform Cancer Care Using Cell-Free DNA

Antonius Schuh, Ph.D., CEO, Trovagene

Trovagene (San Diego) sees an opportunity to transform cancer care through non-invasive genomic monitoring using cell-free DNA. Unlike other technologies that rely upon blood samples, Trovagene's precision cancer monitoring technology can utilize urine samples to determine mutational status and to quantify response to treatment based upon dynamic shifts in mutational load. The platform allows for single-molecule analytical sensitivity with DNA inputs of up to 100,000 genome equivalents.

DTET recently spoke with Trovagene CEO Antonius Schuh, Ph.D., to discuss the future of genomic monitoring of cancer patients.

When looking at cell-free DNA, why is urine preferable to blood?

Our focus is really cell-free DNA and we will extract and detect cell-free DNA in any specimen we get, including plasma or blood. However, we believe there are features associated with urine that make certain applications clinically much more feasible—most importantly, monitoring.

There are monitoring applications where you need to acquire samples often. Our early clinical data indicates that you can observe changes within one week and, depending on the type of treatment, you can see indications informative of response within one day. When you are looking at higher-frequency sampling, blood becomes increasingly less feasible. Also, for all practical considerations, urine samples are not volume constrained. As a result, there is a significantly larger input of DNA, which means a higher chance of detecting low-abundant mutations. We just published a paper in *Cancer Discovery* showing with histiocytic patients (a malignancy where it is hard to obtain a usable biopsy), not only are we able to determine mutational status reliably from urine, we actually outperformed biopsies by a significant margin when it comes to successfully typing a patient's mutational status.

Trovagene has aggressive goals for publishing clinical trials in the next year. How does this contribute to the company's overall strategy?

In order to achieve reimbursement, at least two peer-reviewed publications demonstrating the utility of your approach are required by most insurance carriers to initiate technical and clinical assessment of a novel test. We are just now meeting the criteria for insurance carriers to look at our technology.

All our studies have the same objectives. There are three stages to demonstrate clinical utility and health economic impact. The first stage is simply diagnostic. Can I determine mutational status of a malignancy from urinary cell-free DNA, and how well does it correlate to results obtained from a tissue sample? This may be easy in treatment-naive late-stage cancer patients, because they typically have large amounts of circulating tumor DNA (ctDNA). But if patients are delivering plasma or urine samples six weeks after start of treatment, and if they respond well, ctDNA levels can be drastically reduced and an input-constrained sample like blood may simply not have enough ctDNA in it to reliably detect the mutation of interest.

So, again, this is the first clinical question: Can we determine mutational status reliably from cell-free urinary DNA, meaning can we save a biopsy? This would clearly be of clinical utility, and there is a strong health economic argument here because taking a urine sample is simply cheaper than taking a biopsy.

The second level of clinical utility we investigate is can we monitor for treatment response? Can we monitor quantitative changes in the mutational signal indicative of treatment response, or lack thereof? Can we observe response to treatment as fast as conventional tools, such as imaging modalities, or hopefully, significantly faster? And are we able to detect the onset of progression as fast as with standard of care, or are we able to observe progression even earlier?

A third aspect of our clinical program focuses on the emergence of resistance mutations that are relevant for a given treatment. The clinical utility is obvious. It is important to determine tumor dynamics under treatment. For us the question is, is the qualitative and quantitative mutational signal in ctDNA providing valuable information incremental to imaging? If the cell-free DNA signal is highly informative, can we then significantly reduce the number of imaging studies and repeat biopsy procedures, which would save significant expenses and radiation exposure?

Noninvasive prenatal testing (NIPT), which also uses cell-free DNA, has been hailed as a commercial success for molecular diagnostics. How does cell-free DNA monitoring for oncology monitoring compare to NIPT?

I was the CEO of Sequenom from 2000 to 2005, when we started working with Dennis Lo on cell-free DNA in pregnancies. At the time, next-generation sequencing (NGS) was commercially out of reach. Now that has dramatically changed. I see a strong parallel between both clinical applications.

There has been a convergence of three factors over the past two decades. We have learned that all cancer is caused by DNA changes, or more simplistically damage to cellular genomes. As a result, we have kicked off a massive effort to develop targeted cancer treatments that are educated by these causative genomic changes. And lastly, as stated before, NGS has become so affordable that it can be used routinely in clinical practice. I don't have hard numbers, but if you would ask how many cancer patients had their tumors sequenced in 2010 at Moores Cancer Center here in San Diego, the answer would probably be close to none. In 2014 it is likely many hundreds. Memorial Sloan-Kettering announced they plan to sequence tumor tissue from 10,000 patients this year, with estimated numbers increasing significantly over time. We predict that genomic assessment and genomic monitoring of cancer patients will become clinical mainstream fairly quickly.

Trovagene By-The-Numbers

- Number of Employees: 25
- Number of Collaborations: 12
- Number of Clinical Trials Under Way: 30
- Number of Mutations Under Research: 10

How do you predict adoption of cell-free DNA oncology monitoring will unfold?

Even in small study populations, cohorts as small as 15 or 20 patients, you can achieve high statistical significance when identifying responders and nonresponders. That is possible because you are looking at discrete yes or no answers. In theory that is what you would expect to see, much like in infectious disease, where, for example, reduced viral count indicates response to treatment. The hypothesis in oncology is if you deplete the body of tumor cells, you should see less ctDNA. It makes logical sense, but we know a lot of clean theories don't really translate in a clean manner to clinical settings. This one does. That is why we expect adoption will be fast.

It is all about the performance of your assay in a highly degraded sample. That is what cell-free DNA by definition is. If you look at currently available tests to detect KRAS

mutations, the specified analytical sensitivity is not that strong and typically reaches the low single-digit percentage range. We have demonstrated that we can achieve single-molecule sensitivity for DNA inputs of up to 100,000 genome equivalents. That's not 2 percent to 4 percent sensitivity. That is 0.002 percent or 0.001 percent, and we can do this when the target sequence that carries the mutation is as tiny as a 30-mer. A traditional assay would simply miss this fragment entirely. It would be a tiny sardine swimming through your net and you would say, "no fish in this pond."

Our early clinical data show that our superb analytical sensitivity and also quantitative performance are clinically meaningful. You would otherwise be at risk to classify patient samples as mutation negative. Mutational signals can be so faint that you simply need this level of analytical performance to see it. We believe this is clinically relevant, and our thinking as we generate data is that really urine is not just more convenient and easier to sample, but the combination of feasibility and sample size simply translates into better clinical performance.

"Mutational signals can be so faint that you simply need this level of analytical performance to see it."
—Antonius Schuh, Ph.D.

You are approaching commercialization at a time that the Food and Drug Administration (FDA) may begin regulating laboratory-developed tests. How is Trovagene preparing for that possibility?

The regulatory framework we are operating in is always on our mind. When we started building Trovagene's diagnostic laboratory operations, we considered that there will be more regulation in this space. Our CLIA lab, for example, is set up in compliance with ISO standards. That is just one component of thinking forward. As far as the proposed draft guidance for laboratory-developed tests is concerned, is there some incremental regulation necessary in the molecular diagnostics space? I'd say probably yes. I think, however, the FDA is acutely aware of the fact that we don't even know yet how this should all be regulated. The clinical science is very complex, conventional clinical validation models are not feasible, and we are moving close the sensitive area of regulating clinical practice and a physician's access to information. I believe this is the reason why the proposed transition time periods are very long, such that the clinical community and the diagnostic innovators can work through this.

Of course every diagnostic company is a little worried that they could face overreaching regulation. But assuming regulation is going to be pragmatic and focused on ensuring that clinical diagnostic data reported to a clinician is reliable, that it's good data from validated platforms, and that the clinical meaning you read into these observations is based on solid science, then we actually appreciate more regulation. Frankly, when you have excellent analytical performance and a regulatory framework that allows you to objectively differentiate yourself from competitors, that's an advantage.

In the next few years how will cancer monitoring evolve?

The new paradigm will be a mix of less imaging and more genomic monitoring. Genomic monitoring is an absolute necessity because oncology drugs are more and more directly targeting the genomic changes driving a patient's cancer. Every larger oncology hospital and large integrated health care delivery networks are thinking intensively of how they are going to integrate genomic diagnostics and monitoring into cancer care and what tools they will need to bring genomic patient management into practical care. I think this will become standard very quickly. We aren't going to wait another five years. It can be done now. Early data is very strong.

Rapid Pathogen Testing Pushing Ahead on Two Fronts

Rapid pathogen testing (RPT) has evolved dramatically in the past decade with the routine clinical use of molecular-based technologies. The simultaneous migration toward fixed-cost reimbursement for hospital-based care and the push toward more efficient outpatient care in decentralized locations are driving the diagnostics industry to confront RPT from two distinct approaches.

The need for fast results that will improve clinical care and antibiotic stewardship underlies testing in both arenas. But for hospital-based patients the need is for comprehensive information, while point-of-care (POC) settings are looking for sensitive, binary answers. In order to achieve these goals, regardless of setting, RPT combines elements of around-the-clock access to testing, rapid turnaround time, and meaningful linkage between results and treatment recommendations. Rapid and precise pathogen identification is critical for surveillance of emerging resistance and to support anti-microbial stewardship and personalized guidance of therapy and care, which in turn benefits the bottom line. The connection between RPT results, treatment decisions, and potential cost savings is most pronounced in the hospital environment.

The Value of RPT Is Quantifiable

Implementation of value-based payment programs has put financial pressure on hospitals to improve the quality and cost efficiency of care, namely by getting patients well and home faster. The conditions receiving the most attention are hospital-acquired infections and sepsis, which is one of the most expensive, avoidable complications. Hospitals are exploring investing in new care strategies and new tests that meet the objective of improving patient care, cutting lengths of stays, and lowering expenses per patient. A secondary component of this is to improve the efficacious use of antibiotics and steer usage away from broad-spectrum, empiric antibiotics, with the recognition that appropriately targeted antibiotics can also lead to shorter hospital stays and lower total costs.

"The value-based marketplace calls for system-wide thinking that considers not just the bottom line for each department, but also how changes in services, such as diagnostics, can have ripple effects across a hospital that dramatically influence value,"

Cost-Effectiveness of Multiplex Assays for RPT

A study published in the August issue of *Archives of Pathology & Laboratory Medicine* was able to quantify the savings and improvements in care associated with adoption of a rapid, multiplex molecular respiratory panel. Researchers from Children's Healthcare of Atlanta (CHOA) compared outcomes among pediatric patients admitted to the tertiary care pediatric hospital for acute respiratory tract illness before (n = 365) and after (n = 771) the adoption of the FilmArray rapid respiratory panel (RRP; BioFire Diagnostics, Salt Lake City).

Prior to RRP implementation, testing consisted of batched polymerase chain reaction (PCR) analysis for respiratory syncytial virus and influenza A and B, with additional testing for parainfluenza 1 through 3 in approximately 11 percent of patients and for human metapneumovirus in less than 1 percent of patients. The RRP test includes respiratory syncytial virus, influenza A and B, parainfluenza 1 through 4, human metapneumovirus, adenovirus, rhinovirus/enterovirus, and coronavirus NL62.

The CHOA researchers (some of whom have financial ties to BioFire Diagnostics) found that the mean time to the test result was significantly shorter with adoption of the panel (383 minutes versus 1,119 minutes). Similarly, the percentage of patients with a result in the emergency department was significantly greater (51.6 percent versus 13.4 percent). While there was no difference in whether antibiotics were prescribed, the duration of antibiotic use was significantly shorter after RRP implementation and this reduction was dependent on receiving test results within four hours. For patients with a positive test result, RRP cut length of stay by a quarter of a day and decreased antibiotics administered by a half-day. This translates to savings of $231 in hospital costs and $17 in antibiotic use per patient.

As far as the cost of testing itself, when comparing the RRP with the Focus Diagnostics Flu A/B and RSV Kit (Cypress, Calif.), the cost of the testing increased by $18 per test. But in cases when the Focus Diagnostics Flu and RSV kit was run with the Hologic Gen-Probe (Prodesse) parainfluenza 1 through 3 and human metapneumovirus, RRP cut testing costs by $178 per sample.

writes G2 in its new report *Implementing Rapid Pathogen Testing for Cost Savings and Outcomes Improvement*. "The ability of RPT to identify pathogens faster than traditional microbiological techniques creates a cascade of benefits that ultimately lead to savings for hospitals."

Improving Diagnosis Through Use of Comprehensive Multiplex Arrays

"Hospitals are under tremendous cost pressure and are looking for solutions that will better medical outcomes and save money per patient, which will positively impact the hospital's bottom line if patients are out of the ICU days earlier," Oliver Schacht, Ph.D., CEO of molecular diagnostics company Curetis (Germany), tells *DTET*.

The most straightforward use of microarrays parallels the example at CHOA. Where symptoms are clustered around a targeted group of relatively common pathogens, use of multiplex arrays can speed definitive identification of a pathogen, and ideally, its antibiotic susceptibility profile.

Curetis has two CE-marked multiplex assays for use on its rapid Unyvero PCR platform that are proving to be a valuable addition to conventional RPT. Researchers report in an abstract presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy (Washington, D.C.; Sept. 5-9) a comparison between Curetis's Unyvero P50 pneumonia cartridge and with classical microbiology culture for analysis of pneumonia patient samples. The pneumonia assay had greater than 80 percent sensitivity and 95 percent specificity for pathogen identification. More than half of the cases had polymicrobial infections, many of them involving three to six different pathogens.

"Our focus is on a key unmet medical need of providing clinical actionable information based on molecular diagnostics for severe respiratory disease and surgical site and prosthetic joint infections," says Schacht. "For critically ill patients, clinicians need to know what is causing the disease and what drug will work, and we need to give the doctors the information within a single eight-hour shift so that they can change therapy, if needed."

Curetis's i60 cartridge was developed to rapidly identify more than 90 pathogens and more than 20 resistance markers common in infections of the periprosthetic joint, diabetic foot, catheter, surgical site, skin and soft tissue, as well as cardiology-related infections. The Unyvero system can analyze native clinical sample from swabs, synovial fluid, sonication fluid, tissue, and catheters. In the trial leading to CE mark, the i60 cartridge had an overall panel sensitivity of 67 percent and panel specificity of 97.8 percent for the 81 validated analytes. From the 300-plus samples, the assay also identified 147 clinically important pathogens not found by standard microbiology culture.

Earlier this fall, Luminex (Austin, Texas) received U.S. Food and Drug Administration (FDA) clearance to add three new targets to its xTAG Gastrointestinal Pathogen Panel (GPP). The new targets include Adenovirus 40/41, Entamoeba histolytica, and Vibrio cholerae. xTAG GPP simultaneously detects 14 common viral, bacte-

rial, and parasitic causative pathogens, accounting for greater than 90 percent of the causative pathogens of infectious gastroenteritis. The company says simultaneous molecular testing on a single sample within a single laboratory shift also provides added benefits to laboratories in terms of workflow and resource utilization.

Sherry Dunbar, Ph.D., director of scientific affairs at Luminex, tells *DTET* that as laboratories are under continued pressured to deliver better results, rapid molecular diagnostics will be the "workhorse" of pathogen identification but that laboratories and diagnostics manufacturers will work to strike the balance between comprehensiveness of panels and sensitivity.

"Smart, syndromic-based panels will be moderately sized, under 50 for the most part," Dunbar says. "There will be the need to balance comprehensiveness of assays with the most common pathogens without overdoing it to the point where performance takes a hit. Multiplex still needs to be as sensitive and specific as individual tests."

Sentinel Tests

While evidence is growing to make the case regarding the clinical utility and health economics for the use of targeted panels for RPT in hospitalized patients, others in the industry are pursuing a strategy of simpler "sentry-type tests" that provide critical yes-or-no information that will in turn drive therapeutic decisions and reflex testing. While many of these tests strive to be small and cheap enough to be widely used as POC tests in the outpatient setting, some of these sentry tests will play a role in the care of critically ill patients.

Isomark (Madison, Wis.) has developed a breath test to monitor for infection. Given hospitals' incentive for early infection detection, the company is currently engaged in clinical trials of its Canary test in critically ill patients but envisions modifying the analyzer for home breath testing use.

"Our technology fits in as a sentinel," explains Joe Kremer, Isomark's CEO. "We pick up infection so early in an extremely inexpensive, very rapid, easy test. So, we are a compliment to other testing."

The test is based on technology that assesses changes in metabolism. When the body is challenged with an infection, identifiable changes occur in the way the body metabolizes energy during the acute phase response to infection. This change in metabolism can be witnessed through breath signatures—changes in the ratio of carboantes (Carbon12 to Carbon13). A special plastic collection bag captures air from a patient's blown breath or from a ventilator exhaust and can deliver results in about five minutes. The company is currently recommending six samples a day and is evaluating the economic case but is considering funding placement of the analyzer (currently it costs $100,000) in the ICU and charging per diem per patient, in the hopes that hospitals will recognize this is a small cost for catching infections early.

"Temperature and elevated white blood cell symptoms can be from trauma or infection. Right now identifying which patients have suspected infection is an extreme art, not science, so to be safe, extra diagnostics—MRIs and X-rays—are run," Kremer tells *DTET*. "Our goal is to take the subjectivity out and move care away from being reactive."

In a proof-of-concept study, the Canary detected infections in two of 32 patients before clinicians suspected an infection—in one case two days earlier and in one case one day earlier. In October the company was awarded a $1.7 million National Institutes of Health grant that will aid the company in the next phase of trials, which will culminate in an FDA submission early in 2017.

Rapid Pathogen Screening Detectors (RPS; Sarasota, Fla.) is developing POC tests to better clinical practice and patient management while improving antibiotic stewardship. The company's patented technology platforms can differentiate infectious diseases and inflammatory conditions. The company initially launched its AdenoPlus test to aid in the diagnosis of Adenoviral conjunctivitis (pink eye). The CLIA-waived, CE-marked POC test can with 90 percent sensitivity and 96 percent specificity detect Adenoviral conjunctivitis, compared against cell culture—the gold standard—as the reference method. These disposable, single-use tests lead to a more accurate diagnosis during the initial clinical exam and can aid in curtailing the use of unnecessary antibiotic prescriptions for viral conjunctivitis.

This fall, RPS announced CE mark of its FebriDx test, which can differentiate a viral or bacterial cause of acute febrile respiratory infection. The FebriDx test relies on two markers—myxovirus resistance protein A, an interferon derivative that becomes elevated in the presence of acute viral infection, and C-reactive protein (CRP), an acute-phase protein that is elevated in the presence of bacterial infection—with results at the POC in 10 minutes.

"We are taking global antibiotic stewardship and moving it to the outpatient setting to move towards guideline-based practice," says Robert Sambursky, M.D., CEO of RPS. "With FebriDx we are taking common, but clinically significant respiratory symptom presentation—a fever, cough, runny nose, or sore throat—and aiding in identifying if the condition has a viral or bacterial etiology, instead of just prescribing a Z-Pack."

U.S. Department of Health and Human Services' Biomedical Advanced Research and Development Authority (BARDA) announced grants this fall to boost influenza pandemic preparedness by increasing diagnostic capabilities in near-patient care settings such as doctors' offices, clinics, and hospitals. Alere (Waltham, Mass.) received a 3.5-year, $12.9 million contract to advance the development of a rapid, molecular, low-cost influenza diagnostic device with PCR-like performance at the POC. Alere said it will use the funding to develop the next generation of its current Alere i Influenza A & B test, which provides highly accurate, molecular results in under 15 minutes. Alere i Influenza A & B was launched in January 2014 in Europe and received U.S. clearance in June.

"Molecular testing is becoming the gold standard," says Keith Stauffer, vice president of North America Regional Marketing of Infectious Disease at Alere. "With pressure from regulatory agencies for highly accurate results, we will see more molecular formats with more access in urgent care centers, in pharmacies, grocery stores, and Walmarts."

Takeaway: RPT will continue to evolve in two distinct areas. Targeted panels will continue to gain success in cutting the time to pathogen identification and targeted treatment, ultimately also cutting cost of care, while sentinel tests will continue to develop to provide early yes-or-no infection-related answers that will direct treatment decisions and follow-on testing.

Diagnostic Yield of Exome Sequencing Consistent; Targeted Implementation Could Improve True Value

Whole-exome sequencing is permeating clinical practice, particularly for evaluation of pediatric patients with neurological conditions and developmental delay. Two recently published studies show remarkable similarity in the diagnostic yield associated with clinical exome sequencing (CES); however, questions remain regarding the contexts in which the modality would be most cost-effective to implement.

While exome-scale sequencing may reduce testing costs, compared to multiple, sequential genetic tests undertaken as part of a diagnostic odyssey, efforts are under way to define the circumstances for which CES will be economically beneficial, clinically useful, and have "personal utility" for patients and their families.

"Establishing the mechanism of an individual's disease has scientific value and satisfies intellectual curiosity, but can also have substantial personal utility for patients and their families," according to an editorial by Jonathan Berg, M.D., Ph.D., from the University of North Carolina at Chapel Hill, accompanying the two studies in the Oct. 18 issue of the *Journal of the American Medical Association*. "The personal utility—both positive and negative—afforded by such information, although potentially measurable . . . would be difficult to calculate . . . in a traditional economic sense. Because these studies focus on the molecular diagnostic yield from a clinical laboratory perspective, the effect of such diagnoses on the patients and their families are justifiably beyond their scope, but will ultimately be critical in determining how best to implement widespread clinical exome sequencing."

Berg calls the two studies "compelling" in their demonstration that CES can establish molecular diagnosis. In both cases neurological disorders or developmental delay were the most common reasons for referral and the majority of patients were pediatric. These studies begin to elucidate commonalities that may aid future efforts in defining effective implementation of CES.

Results of Sequencing at Baylor

The following is a breakdown of some of the molecular results from 2,000 cases of whole-exome sequencing at Baylor. Of the 504 cases with a molecular diagnosis:

- 708 presumptive causative variant alleles were identified.
- 53.1 percent had an autosomal dominant Mendelian disease pattern.
- 30 percent had mutations in disease genes reported since 2011.
- There were 95 medically actionable incidental findings (unrelated to the referral phenotype), including 59 patients with mutations recommended for reporting by the American College of Medical Genetics and Genomics.
- Reporting time per case review was approximately seven hours (down from 18 hours per case during the initial implementation period).

In the first study, conducted at Baylor College of Medicine (Houston), the researchers conducted analysis on the CES results of 2,000 consecutive patients (88 percent pediatric patients analyzed between June 2012 and August 2014). After excluding low-quality variants, an average of 875 variants per sample were analyzed. A molecular diagnosis was possible for one-quarter of the patients, including diagnostic mutations not previously reported (58 percent of the diagnoses). The lowest diagnostic yield was seen in the nonneurological group (20.1 percent), while the highest yield was seen in the specific neurological group (including ataxia, movement disorder, and spastic paraplegia; 36.1 percent).

"For the 25 percent of cases that received a molecular diagnosis, this information ended the diagnostic odyssey, provided more informed medical management, and allowed for precise determination of reproductive risks; however, relatively few

cases resulted in specific treatment to reverse the condition," acknowledge the authors, led by Yping Yang, Ph.D.

In the second study, a group of researchers from the University of California, Los Angeles, conducted CES on 814 consecutive patients (64 percent pediatric; 37 percent developmental delay) with undiagnosed, suspected genetic conditions (January 2012 and August 2014). The overall molecular diagnosis rate was 26 percent in this cohort. However, in this study trio-CES was performed for half of patients, in which both parents were also sequenced. The researchers found that the molecular diagnosis rate for trio-CES was significantly higher, reaching 31 percent. Among the 127 trio cases with a conclusive molecular diagnosis, half had a de novo variant, 20 percent had a compound heterozygous variant, 16 percent had a homozygous variant, and 8 percent had an X-linked hemizygous variant.

"The trio-CES test has the potential benefit of permitting more sensitive identification of de novo variants and compound heterozygotes and removing from consideration the many heterozygous rare variants observed in each exome from being considered causal in the affected individual because transmission is observed from an unaffected parent," write the authors, led by Hane Lee, Ph.D. "This has not been routinely implemented by other centers due to costs and potential concerns for incidental findings in the unaffected parents."

"Clinical genome-scale sequencing clearly has the potential to become a cost effective strategy to end an expensive and difficult diagnostic odyssey for some patients," writes Berg. "More data are needed to demonstrate whether this is broadly true and in which contexts exome sequencing will be most useful. . . . Research is required to formally establish the clinical utility of genetic testing by systematically measuring not just changes in management but also long-term outcomes."

Takeaway: CES is providing definitive molecular diagnoses for roughly one-quarter of cases. But by refining use to certain specific phenotypes and broadening testing to trios (including the parents of cases), diagnostic yield may be closer to one-third, further improving the cost-effectiveness of CES. G2

Study Finds Drug Labels Lack Evidence for Pharmacogenomic Testing

Despite the continued drive toward companion diagnostic development, as witnessed by the increasing number of diagnostic-pharmaceutical company partnerships, new research raises doubts as to whether biomarker testing recommendations are ready to be included in drug labels, given a lack of associated clinical utility data.

"Our analysis revealed deficiencies in the evidence provided in drug labels that supports the use of many pharmacogenomics biomarkers," write the authors of an Oct. 13 *JAMA Internal Medicine* study. "It may be premature to include biomarker testing recommendations in drug labels when convincing data that link testing to patient outcomes do not exist."

Biomarkers are increasingly being relied upon to predict a drug's efficacy and the likelihood of toxicity in individual patients. But the researchers say that while more than half of drug labels make clinical recommendations based on biomarker test results, less than one-sixth of drug labels contained or referenced convincing evidence of the clinical utility of biomarker testing.

The researchers utilized publicly available U.S. Food and Drug Administration databases to evaluate the evidence supporting pharmacogenomic biomarker testing in drug labels (both clinical validity and clinical utility). They examined the first available drug label that contained mention of a drug's associated biomarker.

The researchers identified 119 drug-biomarker combinations (107 drugs and 39 unique biomarkers) and found that most of these combinations (63 percent) are intended to reduce the occurrence of adverse drug events, while 37 percent related to the drugs' efficacy. Just over one-third of the labels (36.1 percent) provided convincing evidence of clinical validity, the association between the pharmacogenetic variant and drug response, while only 15.1 percent of the labels (n = 18) provided convincing evidence of clinical utility, improved clinical outcomes associated with test use. Oncology drug labels were significantly more likely to demonstrate convincing evidence of clinical utility, compared with all other biomarker-drug combinations (14 of 37 cancer drugs versus four of 82 other drugs).

"Because only one in 10 U.S. physicians reports being adequately informed about the appropriate use of pharmacogenomic biomarkers, the information and recommendations included in labels should be not only evidence based but also directly relevant to clinical decision making."
—Bo Wang, Pharm.D.

More than half of all of the labels (51.3 percent) made recommendations about how test results should impact clinical decisions, but of these labels providing recommendations, less than one-third (30.3 percent) contained convincing clinical utility data. And among the 76 labels with neither convincing clinical utility nor validity data, nearly one-third (31.6 percent) still contained clinical decision recommendations.

While acknowledging that evidence of benefit may exist but may not be adequately captured in all drug labels, Wylie Burke, M.D., Ph.D., from the University of Washington, Seattle, in an accompanying editorial called the study a "sobering" demonstration of the "limitations" of the current state of knowledge about pharmacogenetics.

"Because only one in 10 U.S. physicians reports being adequately informed about the appropriate use of pharmacogenomic biomarkers, the information and recommendations included in labels should be not only evidence based but also directly relevant to clinical decision making," write the authors, led by Bo Wang, Pharm.D., from Brigham and Women's Hospital in Boston. "We believe that testing recommendations supported by clinical validity alone adds confusion, not clarity, to the clinical decision-making process, especially if the evidence is not clearly explicated or cited alongside the guidance."

Both the study and editorial authors suggest standardization of the pharmacogenomics section for all drug labels, including a list of available pharmacogenomic tests, summaries of the associated evidence, and relevant practice guidelines, including acknowledgement if no pharmacogenetic tests meet these standards. To make the labels consistent, consensus definitions of the evidence required to establish clinical validity and utility will be needed.

Takeaway: Despite the promise of pharmacogenomic testing to support personalized medicine, there is some doubt as to whether there is currently enough widespread, high-quality evidence to support biomarker labeling on many drugs.

G2 Insider

Urine-Based Testing OK to Detect HPV . . . Urine testing is an acceptable alternative to detect cervical human papillomavirus (HPV), according to a review study published online Sept. 16 in the *British Medical Journal*. While testing first-void urine samples has the best accuracy, the authors caution that lack of standardized testing methods should be addressed prior to incorporating the method into cervical cancer screening guidelines.

While cervical cancer is largely preventable and treatable, screening for the malignancy may be limited due to the acceptability of the invasive nature of cervical cytology sampling, and access may be hampered by the need for a clinician. Urine-based testing may overcome these screening barriers. The authors say urine testing could also be used for post-vaccination HPV surveillance programs, where pelvic examination is not practical.

The U.K.-based researchers conducted a systematic literature review and meta-analysis of studies assessing urine test accuracy (HPV DNA) in sexually active women versus detection of cervical HPV DNA. Sixteen of the 21 identified articles (including 1,443 women) were included in the meta-analysis.

Most studies utilized conventional polymerase chain reaction (PCR) methods (n = 18) on first-void urine samples (n = 12), although the authors acknowledge that testing methods were not uniform. Two of the 21 studies used nested PCR and one used PCR-based DNA microarray. Three studies evaluated quantitative, real-time PCR and hybrid capture in addition to conventional PCR.

In pooled analysis, urine detection of any HPV had a sensitivity of 87 percent and specificity of 94 percent. For high-risk HPV strains, urine detection had a pooled sensitivity of 77 percent and specificity of 88 percent, while urine detection of just the high-risk strains HPV 16 and 18 had a pooled sensitivity of 73 percent and specificity of 98 percent. Sensitivity was significantly increased when urine samples were collected at first void versus at random or midstream.

"Sensitivity was moderate for detection of any HPV, high risk HPV, and HPV 16 and 18. The specificity for detection of HPV in urine was especially high for any HPV and the most oncogenic strains, HPV 16 and 18," write the authors, led by Neha Pathak, from the London School of Medicine and Dentistry. "We agree with previous reviews that heterogeneous methods of urine testing affect the interpretation of pooled accuracy measures. . . . We therefore recommend the standardization of methods for urine testing to minimize variation before incorporating urinary detection of HPV into guidelines for cervical cancer screening."

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